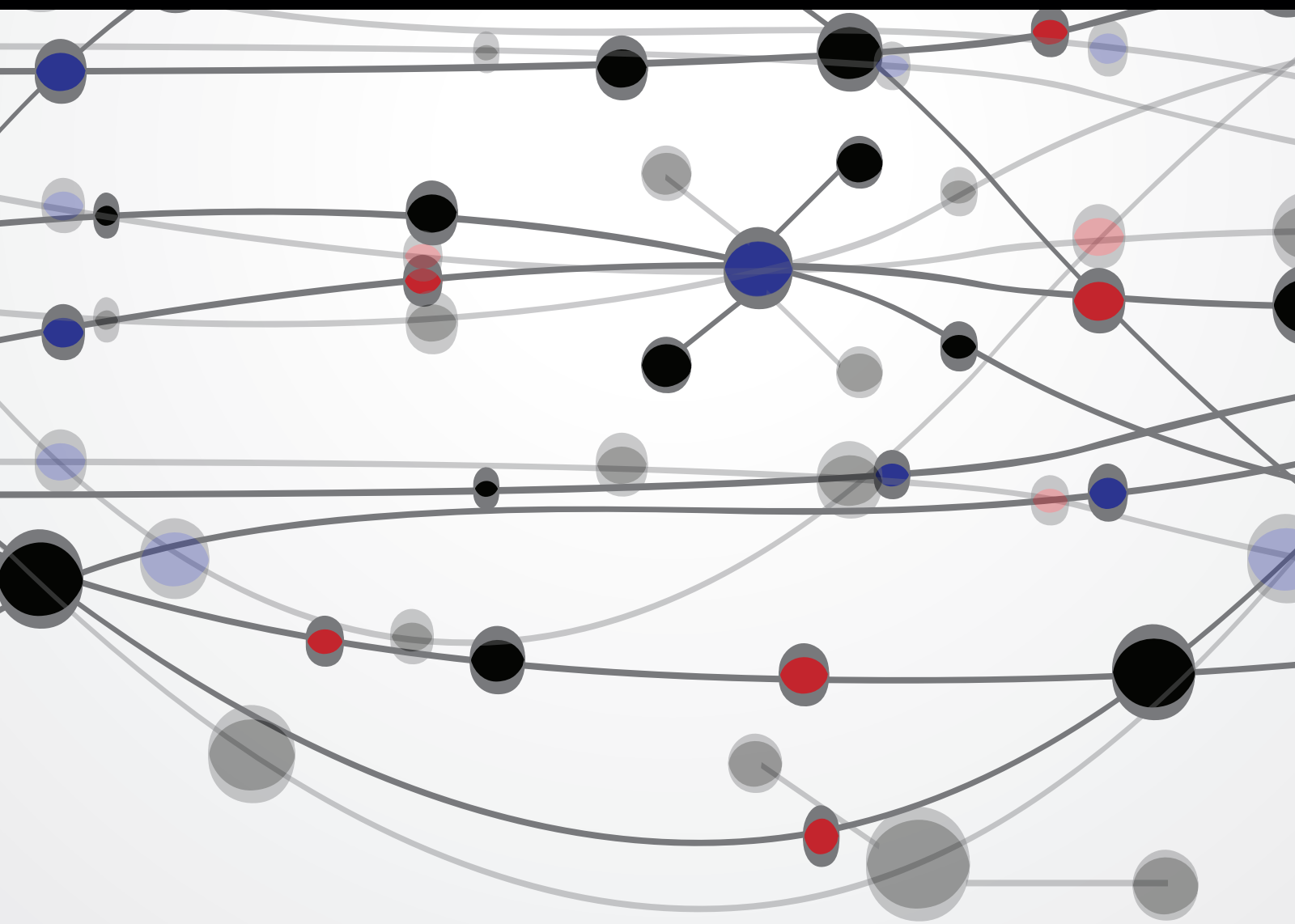
The Scientific World Journal

Emerging Advancements in Canine and Feline Metabolism and Nutrition

Guest Editors: Anna K. Shoveller, Maria R. C. De Godoy, Jennifer Larsen, and Elizabeth Flickinger



Emerging Advancements in Canine and Feline Metabolism and Nutrition

The Scientific World Journal

Emerging Advancements in Canine and Feline Metabolism and Nutrition

Guest Editors: Anna K. Shoveller, Maria R. C. De Godoy, Jennifer Larsen, and Elizabeth Flickinger

Contents

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 9023781, 2 pages
http://dx.doi.org/10.1155/2016/9023781

Editorial

Emerging Advancements in Canine and Feline Metabolism and Nutrition

Anna K. Shoveller,[1] Maria R. C. De Godoy,[2] Jennifer Larsen,[3] and Elizabeth Flickinger[4]

[1] *University of Guelph, Guelph, ON, Canada*
[2] *University of Illinois, Champaign, IL, USA*
[3] *University of California, Davis, CA, USA*
[4] *Kent Pet Group, Muscatine, IA, USA*

Correspondence should be addressed to Anna K. Shoveller; ashovell@uoguelph.ca

Received 1 December 2016; Accepted 4 December 2016

The pet industry continues to grow in both developed and developing countries. According to the 2015-2016 APPA National Pet Owners Survey, 65% of US households own a pet, which encompasses 85.8 million pet cats and 77.8 million pet dogs [1]. In Europe alone, there are another estimated 81 million dogs and 63 million cats, and this market is expected to have a compound annual growth rate of 4.4%, as compared to a 3.5% projected growth rate in the USA [2]. Much of this growth has been stimulated by the increasing appreciation of the value of pets to human health and well-being through both physical and emotional effects. Studies indicate associations between pet ownership and(or) animal-assisted therapy and numerous aspects of positive health outcomes, ranging from improved cardiovascular health to enhanced mental well-being [3, 4]. Humans, reciprocally, are engaged in gaining a deeper understanding of nutrient requirements and the effects of diet and care practices on the health, metabolism, and behavior of cats and dogs of all ages, breeds, and lifestyles. This special issue adds to the primary literature concerning canine and feline metabolism, nutrition, and behavior.

Vitamin A has long been known to be essential for life for all vertebrates, and the review by A. S. Green and A. J. Fascetti included in this issue provides a concise summary of the research available to date across multiple species. As the authors point out, we still have much to learn about the metabolism of the most important vitamin A precursor, β-carotene. Dogs can meet their entire vitamin A requirement through β-carotene [5]. And although cats require preformed vitamin A, they can efficiently absorb β-carotene, as evidenced by elevated plasma concentrations after supplementation [6]. Furthermore, β-carotene may play additional roles in health, including immune response, gap junction communication, and other cellular functions [7], but more research is needed to further elucidate these effects.

In addition to companions, dogs long have been employed for work and admired for their athleticism. Ultra-marathon sled dog races are an excellent model for studying metabolism under extreme energy demands. The original research conducted by M. W. Brunke et al. included in this issue reports how concentrations of insulin-like growth factor-1 and its binding proteins fluctuate in response to exercise and negative energy balance. These data give important evidence on how to better fuel these athletes and highlight the need for further research into the dietary needs of canine athletes in order to optimize health and well-being as well as maximize performance.

Most dogs and cats, however, have a sedentary lifestyle, which is correlated with the rise in pet obesity [8]. The study by S. A. Sapowicz et al. underscores how pervasive obesity is regardless of socioeconomic status and how the role of nutrition in promoting healthy weight is poorly understood by pet owners. Dietary approaches to combat obesity commonly include feeding low fat, high fiber, and(or) low carbohydrate diets, as well as using nutraceutical additives [9]. One such nutraceutical, L-carnitine, was studied by M. A. Gooding

et al. in the diet of cats. The results included in this issue suggest that higher doses of L-carnitine may attenuate body fat accumulation when cats are free fed.

In addition to nutrition and feeding practices, understanding pet owners' management practices is vital for promoting pet welfare. In particular, research in cat behavior is lacking. This issue adds two original works conducted by J. L. Stella and C. C. Croney. Behavioral problems are a leading reason for cat relinquishment in the USA [10]. An underlying factor may well be that cats' needs are inadequately met in the home environment. These papers outline the key considerations for cat environmental management and reveal that a sizeable proportion of homes do not implement best practices for cat care.

Clearly, more research is needed in canine and feline nutrition, metabolism, and behavior. While much work has been done to elucidate nutrient requirements of adult dogs and cats at maintenance, a paucity of information exists with regard to the role of nutrient precursors or requirements in exercising animals. In addition, the pet obesity epidemic has continued to grow despite research in this area, so novel approaches are needed. Furthermore, nutrition can greatly impact health, but, for a truly holistic approach, it is necessary to also provide excellent animal care in an appropriate environment. A deeper understanding of companion animal nutrition, metabolism, and behavior will allow improvements in diet formulation and management practices and will ultimately lead to a better quality of life and perhaps even longer life for dogs and cats. Ultimately, improvements in the life of pets will have beneficial effects on the families that these pets are part of.

Anna K. Shoveller
Maria R. C. De Godoy
Jennifer Larsen
Elizabeth Flickinger

References

[1] APPMA, 2015-2016 National Pet Owners Survey. Greenwich, UK: American Pet Products Manufacturers Association, 2016, http://www.americanpetproducts.org/pubs_survey.asp.

[2] L. Beaton, "US market dominates global pet food structure," *Petfood Industry*, vol. 58, no. 6, pp. 20–27, 2016.

[3] R. L. Matchock, "Pet ownership and physical health," *Current Opinion in Psychiatry*, vol. 28, no. 5, pp. 386–392, 2015.

[4] S. B. Barker and A. R. Wolen, "The benefits of human-companion animal interaction: a review," *Journal of Veterinary Medical Education*, vol. 35, no. 4, pp. 487–495, 2008.

[5] R. G. Turner, "Effect of prolonged feeding of raw carrots on vitamin A content of liver and kidneys in the dog," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 31, no. 7, pp. 866–868, 1934.

[6] F. J. Schweigert, J. Raila, B. Wichert, and E. Kienzle, "Cats absorb β-carotene, but it is not converted to vitamin A," *The Journal of Nutrition*, vol. 132, no. 6, pp. 1610S–1612S, 2002.

[7] B. P. Chew and J. S. Park, "Carotenoid action on the immune response," *The Journal of Nutrition*, vol. 134, no. 1, pp. 257S–261S, 2004.

[8] A. J. German, "The growing problem of obesity in dogs and cats," *The Journal of Nutrition*, vol. 136, no. 7, pp. 1940S–1946S, 2006.

[9] W. J. Burkholder and J. E. Bauer, "Foods and techniques for managing obesity in companion animals," *Journal of the American Veterinary Medical Association*, vol. 212, no. 5, pp. 658–662, 1998.

[10] G. J. Patronek, L. Glickman, A. M. Beck, G. McGabe, and C. Ecker, "Risk factors for the relinquishment of cats to an animal shelter," *Journal of the American Medical Association*, vol. 209, pp. 582–588, 1996.

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 7108374, 13 pages
http://dx.doi.org/10.1155/2016/7108374

Research Article

Management Practices of Cats Owned by Faculty, Staff, and Students at Two Midwest Veterinary Schools

Judith L. Stella and Candace C. Croney

Center for Animal Welfare Science, Purdue University, West Lafayette, IN, USA

Correspondence should be addressed to Candace C. Croney; ccroney@purdue.edu

Received 30 May 2016; Revised 26 September 2016; Accepted 13 November 2016

Academic Editor: Jennifer Larsen

Understanding cat owners' housing, care, and management practices is important for promoting cat welfare. A survey study was conducted on the housing and management practices used for cats by students, faculty, and staff of The Ohio State University and Purdue University veterinary colleges. Subjects were 138 cat-owner dyads. Most cats (74%) were housed strictly indoors in keeping with common US veterinary recommendations. However, many did not implement best practices outlined for behavior and other welfare needs of indoor cats. The percentage of respondents placing resources where cats could be disrupted while using them was 31%, 53%, and 30% for resting areas, food/water dishes, and litter boxes, respectively. Many cats were not provided a litter box in a private area (35%), in multiple areas of the house (51%), or that was regularly washed (73%). Horizontal scratching opportunities were not provided to 38% of cats; 32% were not provided toys that mimic prey and 91% of cats were fed a diet consisting of >75% dry food. These findings suggest a need for more concerted efforts to educate owners about meeting their cats' welfare needs so as to attenuate risks and improve cat physical and behavioral welfare outcomes.

1. Introduction

In the US, an estimated 74 to 85 million domestic cats (*Felis silvestris catus*) are thought to be kept as pets, making them the most commonly maintained companion animal [1, 2]. How we house, manage, and care for cats may impact owner attachment and cat health and behavior. These factors also influence outcomes such as whether cats remain in their homes or are ultimately abandoned or relinquished to shelters. An estimated 3.4 million cats of which approximately 1.4 million are euthanized are admitted to US animal shelters annually [3]. Behavioral reasons are among the most common causes for cat relinquishment. Based on a survey of cat owners, Salman et al. [4] reported that at least one behavioral reason was given for 28% of cat relinquishments. The most common included house-soiling (43.2%), problems between pets (18.9%), aggression toward people (14.6%), unfriendliness (5.4%), fearfulness (3.8%), and destructive behavior (12.4%). A significant association was found between relinquishment for such reasons and owning another household pet, suggesting that cats living in single animal households had a lower risk of relinquishment for behavioral reasons [5, 6]. Thus, the ways in which cats are housed (indoor or outdoor) and the social environments they experience may significantly impact their behavior, which in turn may have consequences for their well-being.

In regard to cat housing, it is now common practice for US veterinarians to advise clients to keep their cats indoors. The American Association of Feline Practitioners Statement on Confinement of Owned Indoor Cats-December 2007 (http://www.catvets.com/guidelines/position-statements/confinement-indoor-cats) takes the position that "Veterinarians are encouraged to educate clients and the public concerning the dangers associated with allowing cats' free-roam access to the outdoors… Additionally, these cats have an increased potential to be exposed to feline-specific and zoonotic diseases…."

Although indoor confinement reduces the risk of some health problems of cats, it also increases risk in other areas. For example, to optimize behavioral well-being, cats maintained indoors require attention to aspects of their environments that many owners may not be prepared to meet. According to the American Association of Feline Practitioners (AAFP) and the International Society of Feline Medicine

(ISFM) recommendations for indoor-housed cats (referred to as the five pillars of a healthy feline environment) [7] include provision of (1) a safe place, (2) multiple and separated key environmental resources (food, water, toileting areas, scratching areas, play areas, and resting or sleeping areas), (3) opportunity for play and predatory behavior, (4) positive, consistent, and predictable human-cat social interaction, and (5) an environment that respects the importance of the cat's sense of smell (e.g., not removing scent marks, minimal use of harsh chemicals and perfumes, and use of synthetic pheromones).

In addition, indoor housing may be associated with certain physical health risks. Epidemiological studies have found that indoor housing is associated with increased risk (odds ratio) for a variety of common cat diseases, including odontoclastic resorptive lesions (~4.5), obesity (1.6–15.8), type 2 diabetes mellitus (1.4–4.6) [8], hyperthyroidism (4–11.2), and behavioral disorders [8, 9].

Given the popularity of the cat as an indoor-housed companion animal, understanding the extent to which owners provide care, management, and housing that meets their needs may help to promote their well-being and avoid adverse health and welfare outcomes for them. This pilot study therefore aimed to obtain information on the housing and management practices (including resources provided and feeding practices) used for companion cats by a cohort of veterinary professionals at two Midwestern veterinary colleges.

2. Materials and Methods

2.1. Survey. This data was collected as part of a larger project assessing cats' responses to environmental factors where cats were temporarily housed in cages for two or three days to simulate conditions and acclimation that might be experienced at a shelter [10]. The studies were conducted at The Ohio State University (OSU) and Purdue University (PU) Colleges of Veterinary Medicine. The questionnaire was intended to gather information on the housing environment, resources available to the cat, and frequency of certain behaviors including sickness and abnormal behaviors (the Appendix). Close-ended questions were used to identify cat signalment (age, breed, sex, neuter status, and declaw status), source of the cats (how they were obtained), housing environment, and feeding practices. Frequency of sickness and abnormal behaviors was reported on a scale of 0–5 where 0 = never, 1 = once, 2 = at least once/year, 3 = at least once/month, 4 = at least once/week, and 5 = at least once/day. Information about resources provided to cats in the home (e.g., toys and beds) was collected as yes, no, do not know, or not applicable.

2.2. Procedure. A sample of 130 cat-owner dyads (OSU, N = 74; PU, N = 56) from 81 households (OSU, N = 44; PU, N = 36) participated in the study. Owners were recruited from the faculty, staff, and students of The Ohio State University (OSU) and Purdue University (PU) veterinary colleges via email. Questionnaires were completed electronically. The protocols for the studies were reviewed and approved by the Institutional Review Board, the Animal Care and Use Committee, and the clinical trials committees of both The Ohio State University and Purdue University.

2.3. Statistical Analysis. Descriptive statistics were used and reported from each institution separately and as the pooled total. Analysis was performed using STATA IC 11 (StataCorp LP, College Station, TX, USA) statistical software.

3. Results

3.1. Signalment and Sourcing (Table 1). All 130 cats in this study were neutered (female: N = 61; male: N = 69), 31% were declawed, and 95% were nonpedigreed. Ages ranged from 0.75 to 13 years (mean = 4.7 years). The average length of ownership was 4.0 years (range 0.3–12.75). The majority of cats were obtained from rescue or shelter (38%) or as a stray adult or kitten (44%). The remaining cats were obtained from friends (16%), were offspring of another owned cat (1%), or were gifts (1%). None were obtained from a breeder or pet store.

3.2. Housing Environment. The mean number of cats in the household was 2.1 with the maximum being 6 cats. The mean number of dogs in the household was 1.2 ranging from 0 to 8 dogs and the mean number of other animals in the house was 0.7 (SD 1.3) ranging from 0 to 7 animals (Table 2). Multicat households were more common than single-cat households (64% versus 36%). Of the multicat households, 81% consisted of unrelated individuals (OSU 84%; PU 80%).

Seventy-four percent of the cats were housed strictly indoors (OSU 84%; PU 61%) (Table 3). Sixty percent of households were attached or single houses with the remainder being apartments. The majority of owners (95%) fed diets consisting of 25% or less of wet food (Table 3).

The number of hours each day the owners spent in sight of their cat ranged from 1 to 24 (median 7.5). Time spent petting their cat each day ranged from 1 to 120 minutes (median 30) and reported time spent playing with their cat ranged from 0 to 90 minutes per day (median 20) (Table 4).

3.3. Sickness and Abnormal Behaviors. Owner-reported sickness and abnormal behaviors were dichotomized and reported as infrequent (once a year or less) or frequent (once a month or more often) (Table 5). The most commonly reported behaviors seen at least once per month were excessive appetite (34%), vomiting (25%), and nervous (22%) and aggressive (13%) behavior.

3.4. Resources. A summary of owner-reported resources is shown in Table 6. Owners reported that resting areas (31%), food/water bowls (53%), and litter boxes (30%) were located in areas where another animal could surprise them when they were in use. Twenty-one percent of cats did not have their own food bowl and 60% did not have their own water bowl. Thirty-five percent of cats did not have their own litter box in a private area of the house, 51% did not have litter boxes located in multiple areas of the house, and 73% were not washed regularly (at least monthly). Horizontal scratching opportunities were not provided to 38% of cats while vertical

Table 1: Cat signalment and demographic information of the subjects.

Characteristic	OSU (N = 74)		PU (N = 56)		Total (N = 130)	
	N	%	N	%	N	%
Gender						
Female	33	45	28	50	61	47
Male	41	55	28	50	69	53
Breed						
Nonpedigree	70	95	54	96	127	95
Other	4	5	2	4	7	5
Declawed						
Yes	17	23	23	41	40	31
No	57	77	33	59	90	69
Source						
Shelter/rescue	32	43	17	30	49	38
Stray/orphan	30	41	27	48	57	44
Friend	10	14	11	20	21	16
Offspring of owned cat	1	1	0	0	1	1
Gift	1	1	1	2	2	1
Breeder	0	0	0	0	0	0
Pet store	0	0	0	0	0	0

OSU = The Ohio State University; PU = Purdue University.

Table 2: Other animals in the home-the number of cats, dogs, and other pets in the home.

		OSU (N = 44)		PU (N = 36)		Total (N = 81)	
		N	%	N	%	N	%
Cats							
Single cat	1	13	30	16	44.5	29	36
Multicat	2	15	34	8	22	23	28
	3	7	16	9	25	16	20
	4	3	7	2	6	5	6
	5	0	0	0	0	0	0
	6	2	5	1	3	3	4
Dogs	0	20	45	9	25	29	36
	1	12	27	10	28	22	27
	2	6	14	12	33	18	22
	3	3	7	4	11	7	9
	4	2	5	1	3	3	4
	>5	1	2	0	0	1	1
Other	0	28	64	22	61	50	62
	1	7	16	5	14	12	15
	2	6	14	5	14	11	14
	>2	0	0	4	11	4	5

OSU = The Ohio State University; PU = Purdue University.
N = the number of cats and % = the percentage of the respondent population.

scratching opportunities were not provided to 22% of cats. Sixty percent of cats were not provided with chewing items such as cat grass, 32% were not provided toys to chase that mimic prey, and 78% did not have toys rotated to provide novelty.

4. Discussion

The results of this survey showed that, consistent with common US veterinary recommendations, most cats were confined to the indoors exclusively (74%); many lived in

Table 3: Housing and diet.

	OSU (N = 74)		PU (N = 56)		Total (N = 130)	
	N	%	N	%	N	%
Hours/day spent indoors						
0–6	0	0	1	2	1	1
6–12	2	2.5	7	12	9	7
12–18	3	4	3	5	6	4
18–24	7	9.5	11	20	18	14
All	62	84	34	61	96	74
% of diet fed as wet food						
0	38	57	31	50	69	54
25	29	32	21	43	50	37
50	4	7	3	5	7	6
75	1	1	0	0	1	1
100	2	3	1	2	3	2

OSU = The Ohio State University; PU = Purdue University.
N = the number of cats and % = the percentage of the respondent population.

Table 4: Owner-cat interactions.

	OSU (N = 74)			PU (N = 56)			Total (N = 130)		
	Median	25% IQ	75% IQ	Median	25% IQ	75% IQ	Median	25% IQ	75% IQ
hr/day in sight of cat	7.5	5	12	5	2	6	6	5	10
min/day spent petting cat	30	20	49	30	15	39	30	15	40
min/day spent playing with cat	20	10	30	15	10	20	15	10	30

OSU = The Ohio State University; PU = Purdue University.
Median and 25th and 75th interquartile ranges are presented.

Table 5: Sickness and abnormal behavior.

	OSU		PU		Total		Total %
Behavior	IF	F	IF	F	IF	F	% F
Have excessive appetite	49	25	35	21	88	46	*34%*
Have little appetite	71	3	54	1	125	4	3%
Vomit (hair, food, and bile)	57	17	40	16	97	33	*25%*
Have diarrhea	73	1	55	1	128	2	2%
Have constipation	73	0	55	1	128	1	1%
Defecate outside of litter pan	70	4	52	4	122	8	6%
Strain/frequent attempts to urinate	73	1	56	0	129	1	1%
Urinate outside of litter pan	72	2	51	5	123	7	5%
Have blood in urine	74	0	55	0	129	0	0%
Spray urine	74	0	52	4	126	4	3%
Groom excessively	69	5	53	3	122	8	6%
Have excessive hair loss	73	1	54	2	127	3	2%
Scratch themselves excessively	72	2	54	2	126	4	3%
Have discharge from the eyes	64	10	53	3	117	13	10%
Seem nervous (anxious, fearful)	57	17	44	12	101	29	*22%*
Seem aggressive	64	10	48	7	112	17	*13%*

OSU = The Ohio State University; PU = Purdue University.
IF = infrequent, once a year or less; F = frequent, monthly or more often.

Table 6: Owner-reported resources provided to cats (%).

	OSU (N = 74)				Purdue (N = 56)				Total (N = 130)			
	No	Yes	DK	N/A	No	Yes	DK	N/A	No	Yes	DK	N/A
Does each cat have its own resting area in a convenient location that provides some privacy?	8	91	1	0	5	92	0	3	7	92	1	2
Are resting areas located such that another animal cannot sneak up on the cat while it rests?	34	65	1	0	22	61	6	11	28	63	4	6
Are resting areas located away from appliances or air ducts (machinery) that could come on unexpectedly while the cat rests?	7	91	1	1	11	89	0	0	9	90	1	1
Does each cat have the opportunity to move to a warmer or cooler area if it chooses to?	3	96	1	0	0	100	0	0	2	98	1	0
Does each cat have its own food bowl?	19	80	1	0	23	74	0	3	21	77	1	2
Does each cat have its own water bowl?	58	39	1	1	58	39	0	3	58	39	1	2
Are the bowls located in a convenient location that provides some privacy while it eats or drinks?	19	76	5	0	17	83	0	0	18	80	3	0
Are bowls located such that another animal cannot sneak up on this cat while it eats or drinks?	49	45	4	3	47	39	3	11	48	42	4	7
Are bowls located away from machinery that could come on unexpectedly?	11	88	1	0	14	86	0	0	13	87	1	0
Does each cat have its own box in a convenient, well-ventilated location that still gives the cat some privacy while using it (1 litter box per cat + 1)?	39	60	1	0	28	64	2	6	34	62	2	3
Are boxes located in more than one area of the house?	30	34	3	34	47	39	3	11	39	37	3	23
Are boxes located so another animal cannot sneak up on the cat during use?	31	62	3	4	22	64	0	14	27	63	2	9
Are boxes located away from machinery that could come on unexpectedly during use?	14	85	1	0	14	83	3	0	14	84	2	0
Are boxes washed regularly (at least monthly) with a mild detergent (like dishwashing liquid), rather than strongly scented cleaners?	68	32	0	0	75	19	3	3	72	26	2	2

Table 6: Continued.

	OSU (N = 74)				Purdue (N = 56)				Total (N = 130)			
	No	Yes	DK	N/A	No	Yes	DK	N/A	No	Yes	DK	N/A
Does each cat have the opportunity to engage in play with other animals or the owner if it chooses to on a daily basis?	1	96	3	0	5	92	0	3	3	94	2	2
Does each cat have the option to disengage from other animals or people in the household at all times?	8	90	1	0	3	94	0	3	6	92	1	2
Are horizontal scratching posts provided?	20	78	1	0	56	44	0	0	38	61	1	0
Are vertical scratching posts provided?	8	92	0	0	36	64	0	0	22	78	0	0
Are chew items (e.g., cat-safe grasses) provided?	49	49	3	0	67	28	5	0	58	39	4	0
Does each cat have toys to chase that mimic quickly moving prey?	32	67	0	1	31	69	0	0	32	68	0	1
Does each cat have toys that can be picked up, carried, and tossed in the air?	4	96	0	0	11	89	0	0	8	93	0	0
Are toys rotated on a regular basis (at least weekly) to provide novelty?	68	32	0	0	86	11	3	0	77	22	2	0

OSU = The Ohio State University; PU = Purdue University.
DK = do not know; N/A = not applicable.

multicat households (64%), of which 81% lived with unrelated conspecifics. Ninety-five percent of the owned cats in this cohort were nonpedigreed and 98% had been acquired from a shelter, as a stray, or from a friend. None were purchased from a breeder or pet store which is less than has been previously reported (2% and 3%, resp.) [2]. Our respondents' cats were therefore primarily self-bred. This is not surprising given that of the "domesticated" species commonly kept in the US cats maintain the unique characteristic of owned populations interbreeding freely with feral populations rather than having mating strictly controlled by humans. Nonpedigree cats typically select their own mates and readily interbreed both with free-living feral domestic cats and with the wild progenitor species *F. silvestris,* where they coexist [11]. It is important to understand domestication when assessing the needs of cats. Evidence suggests that many of the differences between domestic and wild populations result from quantitative changes in the thresholds for performing a behavior rather than in qualitative changes in the behavior itself [12]. The cat's behavioral organization has been shaped by evolution to use information obtained from the environment to react to an event or to interact with an environmental feature to form rules of response for similar events or stimuli. The extent to which these "decision rules" of the ancestral species become altered by domestication may influence the negative subjective experiences (suffering) of an animal especially when there is a mismatch between an animal's current environment and the environment in which its decision rules evolved [13]. In this sense, we may consider domestic cats as similar to zoo animals, with the proximity of conspecifics and other animals, combined with limited resources and opportunities to express species-typical behavior potentially influencing cats' perceptions of control.

Additionally, cats have evolved as solitary hunters with typical social groups consisting of related females and their offspring. Adult males live on the periphery of a group of females with adolescent males, and often females, dispersing from their natal territory [14]. Yet the domestic cat, while less gregarious than other domestic species, does exhibit great plasticity in social behavior, with sociability appearing to be influenced by early experience and socialization [15]. While indoor living in itself and sharing of homes with other cats do not pose de facto feline welfare problems, living confined in close proximity to unrelated conspecifics may be stressful to some individual cats, particularly if they are not provided with adequate resources. Therefore, attention to the quality of the indoor housing environment is of critical importance to companion cat health and welfare.

Contrary to our expectations, however (given the owners' affiliations with veterinary medical colleges), the indoor housing environments provided to many of the cats were lacking in many of the resources most feline experts agree are essential for good welfare. At a minimum, these include individual resting, feeding, and elimination areas that are private and relatively free of unpredictable noise and interruption from other animals in the household. As noted

previously, environmental enrichment that provides outlets for typical feline behaviors, such as novel toys that allow cats to "hunt," daily play sessions with owners, scratching, and climbing opportunities, is also recommended to promote cat health and well-being [16]. Yet few owners reported routinely providing such resources to indoor-housed cats. Similar results have been reported for members of the general public elsewhere. For example, using owner self-reports to explore the living conditions of indoor-housed cats, Heidenberger [17] found that 24% of cats did not have their own food bowls and 51% had to share the litter pan with other cats.

An interesting finding was that 91% of cats were fed a diet consisting of 25% or less of wet food with 54% receiving no wet food at all. A review of the literature by Zoran and Buffington [18] found evidence suggesting factors relating to diet, including form (wet versus dry), composition (high carbohydrate versus high protein), and presentation (in a bowl or in a puzzle), are important environmental factors for domestic cats confined indoors [18]. Plantinga et al. [19] estimated the nutrient intake of feral cats based on data of food consumption patterns in order to understand the nutrient profile to which the cat has been evolutionarily adapted. The calculated diet was 69.5% water with daily energy intake from protein 52%, from fat 46%, and from NFE only 2%, supporting the fact that cats are truly obligate carnivores. In a pair of studies Hewson-Hughes et al. [20, 21] have indicated that providing wet food as well as dry may be beneficial to cat well-being. They demonstrated that cats will regulate their macronutrient intake to reach a "target" intake of total energy comprised of 52% protein, 36% fat, and 12% carbohydrate [20]. Importantly, this "target" could only be met by provision of wet foods in addition to dry and the cats consistently consumed more wet food (85% of total food intake) than dry when offered both [21]. More research is needed to determine if the shift from an obligatory meat-based natural diet to a meat-based and grain-based pet food rich in carbohydrates places the cat's metabolism under stress and possibly has unwanted negative health effects in the long run. Unfortunately, only limited conclusions can be drawn from this study as detailed information pertaining to diet was not collected which should be addressed in future studies.

Thirty-four percent of owners reported that their cats had excessive appetite at least once a month. This is in contrast to studies of cats housed in laboratories and in housing mimicking shelter environments, where *decreased* appetite was the most common abnormal behavior reported [10, 22, 23]. One explanation is that free-fed cats living in multicat households may have decreased appetite that goes unnoticed if changes in food intake are of short duration. Conversely, cats may use begging for food or overeating as an attention seeking behavior or in response to environmental stressors or negative emotional states [24].

A limitation of the current study is that body condition score was not recorded for the cats recruited from OSU. The mean body condition score of the cats recruited from PU ($N = 56$) was 6 ranging from 4 to 9 (using a 1–9 scale, 5 being ideal) indicating that the majority of cats were overweight or obese. It is important for future studies to assess if a correlation between excessive appetite and obesity exists. Obesity is now the most common nutritional disorder in cats in the United States. Studies in several countries have reported up to 63% of the cat population to be overweight or obese [25–29]. A recent report from the 2015 National Pet Obesity Awareness Day Survey conducted by the Association for Pet Obesity Prevention (APOP) noted that 58.2% or roughly 42 million cats in the US were found to be overweight or obese by their veterinarians [30]. Many factors contribute to this problem, including gender and hormonal changes due to neutering, age, inactivity, boredom due to indoor confinement, overfeeding, and feeding style (meal feeding versus free choice) [9, 18, 31]. Further, many cat health issues are associated with obesity, including type-2 diabetes mellitus, joint disease and lameness, development of feline lower urinary tract disease, idiopathic hepatic lipoidosis, and nonallergic skin condition [9]. Thus, the importance of owner attention to cats' diets, appetitive behaviors, and their implications for feline health cannot be overstated.

Another finding of particular interest was that owners reported that the most common sickness or abnormal behaviors in their cats were excessive appetite (34%), vomiting (25%), and nervous (22%) and aggressive (13%) behavior. Similar behaviors have been reported in other studies. For example, Morgan and Houpt [32] found the most common behavior problems to be scratching furniture (60%), eating houseplants (42%), conspecific aggression (36%), food stealing (25%), hissing/aggression to people (17%), house-soiling (16%), excessive vocalizations (16%), fabric chewing (7%), and "shyness" (4%). Later, Heidenberger [17] reported the most frequent behavior problems cited by cat owners to include anxiety (16.7%), scratching furniture (15.2%), feeding problems (10.9%), aggression (10.5%), and inappropriate urination (8.2%) and defecation in the house (5.1%). Many of these behaviors have been observed in response to inappropriate environments under laboratory conditions as well [22].

In summary, the current findings indicate that in our study population several owner oversights occurred relative to meeting best practices for indoor-housed cats as dictated by recent scientific findings and feline medicine practitioners' recommendations. However, the limitations of our study constrain drawing of broad conclusions, particularly as they apply to the general cat owning public. Cat-owner demographic information, such as age, level of education, and specific knowledge about cats and their physical and behavioral needs, was not obtained, which would be necessary to understand potential relationships between these factors and the housing and management practices adopted for cats. Additionally, owners' rationales for their housing and management choices were not investigated in this pilot study. Further, our sample is by no means representative of the US public, being limited to a very small pool of cat owners who were already participating in a larger study, which introduced sampling bias. Finally, validation studies of the survey tool should be conducted. Future studies should consider and address these factors to provide greater insight and more robust data.

It was anticipated that given their affiliation with veterinary medical schools this study population was likely to be

familiar with recommended cat housing and management practices and, thus, would provide preliminary insight on how well best practices for meeting cats' needs were translated to and applied by those with relatively easy access to current information and education on the subject.

Nevertheless, the finding that faculty, staff, and students affiliated with both veterinary medical colleges did not appear to closely follow or implement many of the recommendations for optimal feline care suggests that there are significant gaps in translation of current findings about best cat care practices and their potential impacts on cat quality of life. Greater attention should be paid to developing more effective strategies for engaging and educating owners about meeting cats' comprehensive welfare needs.

5. Conclusion

Understanding cat owners' housing, care, and management practices is an important step toward promoting cat welfare. The results of this study indicate that even cat owners affiliated with veterinary medical colleges may not be fully aware of or implementing many best practices outlined for the welfare of cats housed indoors. This suggests a need for continuing education and "marketing" of messages about cats and their welfare needs so that all veterinary practitioners have the available tools, knowledge, and comfort to advise their cat owning clients. Ultimately, improved application and transfer of information to cat owners of current scientific information on the housing and handling is necessary to better meet cats' needs, promote the human-animal bond, and reduce risk of cat abandonment and relinquishment to shelters.

Appendix

Questionnaire (Adapted from [33])

Cat and Client History Form

Owner name: —

Cat's name: —

Date: —

Contact Information:

Phone #: —
E-mail: —

Breed: —

Date of Birth: —

Weight: — lb/kg

Sex: (circle one)

FI
FS
MI
MN

Declawed?

No: —
Yes: —
If yes,

Front: —
All: —

Owned How Long?

— Years, — months

Total Cats: —

Total Dogs: —

Other Pets: —

Other people: —

Housing: Apartment:

☐ studio,
☐ 1-2 bedrooms,
☐ 3 or more bedrooms,
☐ attached house/twin duplex,
☐ attached house, 3 or more units,
☐ single house,
☐ other —

Previous Illnesses or Surgeries: —

Directions. For items below, please use the following choices to describe how many times you have seen your pet experience the symptom, adding *comments/explanation* – as appropriate *Score* =

0 = I have *NEVER* seen it

1 = I have seen it at least *ONCE*

2 = I see it at least *ONCE per YEAR*

3 = I see it at least *ONCE per MONTH*

4 = I see it at least *ONCE per WEEK*

5 = I see it *DAILY*

How often does your cat:

Have excessive appetite

Score: —
Comments/explanation: —

Have little appetite

Score: —
Comments/explanation: —

Vomit (food, hair, bile, other)

Score: —
Comments/explanation: —

Have diarrhea

Score: —
Comments/explanation: —

Have constipation

Score: —
Comments/explanation: —

Defecate outside the litter box

Score: —
Comments/explanation: —

Strain or have frequent attempts to urinate

Score: —
Comments/explanation: —

Urinate outside the litter box

Score: —
Comments/explanation: —

Have blood in the urine

Score: —
Comments/explanation: —

Spray urine

Score: —
Comments/explanation: —

Grooms excessively

Score: —
Comments/explanation: —

Have excessive hair loss

Score: —
Comments/explanation: —

Scratch excessively

Score: —
Comments/explanation: —

Have discharge from eyes

Score: —
Comments/explanation: —

Seem nervous (anxious)

Score: —
Comments/explanation: —

Seem fearful

Score: —
Comments/explanation: —

Seem Aggressive

Score: —
Comments/explanation: —

Seem "needy" of contact or attention

Score: —
Comments/explanation: —

Please check the box that best applies to your cat

Diet: wet food (name —)

☐ None
☐ 25%
☐ 50%
☐ 75%
☐ 100%

Diet: dry food (name —)

☐ None
☐ 25%
☐ 50%
☐ 75%
☐ 100%

Litter type: (clumping, clay, recycled paper, etc)

—

How many hours each day, on average, does your cat spend indoors? (check one)

☐ 0–6
☐ 6–12
☐ 12–18
☐ 18–24
☐ Indoor Only

If you have more than one cat, what is their relationship?

☐ Not Related
☐ Littermate
☐ Sibling
☐ Parent-Offspring
☐ Single Cat Household
☐ Other

Where did you obtain your cat (source)?

☐ Shelter

☐ Offspring from a pet I already own(ed)
☐ Purchased from a friend
☐ Purchased from a breeder
☐ Purchased from a pet shop
☐ Gift
☐ Stray/orphan
☐ Other

Client Resource Checklist. The following questions ask about your cat's resources because we want to learn more about your cat's environment. Please ✓ DK if you don't know, NA if a question does not apply to your home, or Yes or No after each question.

Space

(1) Does each cat have its own resting area in a convenient location that provides some privacy?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(2) Are resting areas are located such that another animal cannot sneak up on the cat while it rests?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(3) Are resting areas are located away from appliances or air ducts (machinery) that could come on unexpectedly while the cat rests?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(7) Does each cat have the opportunity to move to a warmer or cooler area if it chooses to?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(8) Is a radio or TV left playing when the cat is home alone?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

Food and Water

(9) Does each cat have its own food bowl?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(10) Does each cat have its own water bowl?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(11) Are the bowls located in a convenient location that provides some privacy while it eats or drinks?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(12) Are bowls located such that another animal cannot sneak upon this cat while it eats or drinks?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(14) Are bowls located away from machinery that could come on unexpectedly?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

Litter Boxes

(15) Does each cat have its own box in a convenient, well-ventilated location that still gives the cat some privacy while using it (1 litter box per cat + 1)?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(16) Are boxes located in more than one area of the house?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(17) Are boxes located so another animal cannot sneak up on the cat during use?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(18) Are boxes located away from machinery that could come on unexpectedly during use?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(20) Are boxes washed regularly (at least monthly) with a mild detergent (like dishwashing liquid), rather than strongly scented cleaners?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(22) Is the brand or type of litter purchased changed infrequently (less than monthly)?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

Social Contact

(24) Does each cat have the opportunity to engage in play with other animals or the owner if it chooses to on a daily basis?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(25) Does each cat have the option to disengage from other animals or people in the household at all times?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(26) Do any cats interact with outdoor cats through windows?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(27) How many hours a day are you in sight of your cat?

☐ DK
— (h/day)
Other/Comments: —

(28) How many minutes a do you spend petting your cat?

☐ DK
— (min/day)
Other/Comments: —

(29) How many minutes a do you spend playing with your cat?

☐ DK
— (min/day)
Other/Comments: —

Body Care and Activity

(30) Are horizontal scratching posts provided?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(31) Are vertical scratching posts provided?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(32) Are chew items (e.g., cat-safe grasses) provided?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(33) Does each cat like to play with toys?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(34) Does each cat have toys to chase that mimic quickly moving prey?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(35) Does each cat have toys that can be picked up, carried, and tossed in the air?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

(36) Are toys rotated on a regular basis (at least weekly) to provide novelty?

☐ DK
☐ NA
☐ Yes
☐ No
Other/Comments: —

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

Funding for the present study was supported by the Morris Animal Foundation. The authors gratefully acknowledge the expert contributions of C. A. Buffington and T. Shreyer to this work.

References

[1] American Veterinary Medical Association, "U.S. Pet Ownership & Demographics Sourcebook," 2012, https://www.avma.org/KB/Resources/Statistics/Pages/Marketresearch-statistics-US-pet-ownership.aspx.

[2] Humane Society of the United States, H. Pets by the Numbers, 2012, http://www.humanesociety.org/issues/pet_overpopulation/facts/pet_ownership_statistics.html.

[3] American Society for the Prevention of Cruelty to Animals, Pet Statistics, 2012, https://www.aspca.org/about-us/faq/pet-statistics.

[4] M. D. Salman, J. Hutchison, R. Ruch-Gallie et al., "Behavioral reasons for relinquishment of dogs and cats to 12 shelters," *Journal of Applied Animal Welfare Science*, vol. 3, no. 2, pp. 93–106, 2000.

[5] G. J. Patronek, L. T. Glickman, A. M. Beck, G. P. McCabe, and C. Ecker, "Risk factors for relinquishment of cats to an animal shelter," *Journal of the American Veterinary Medical Association*, vol. 209, no. 3, pp. 582–588, 1996.

[6] R. A. Casey, S. Vandenbussche, J. W. S. Bradshaw, and M. A. Roberts, "Reasons for relinquishment and return of domestic cats (*Felis silvestris catus*) to rescue shelters in the UK," *Anthrozoos*, vol. 22, no. 4, pp. 347–358, 2009.

[7] S. L. H. Ellis, I. Rodan, H. C. Carney et al., "AAFP and ISFM feline environmental needs guidelines," *Journal of Feline Medicine and Surgery*, vol. 15, no. 3, pp. 219–230, 2013.

[8] L. I. Slingerland, V. V. Fazilova, E. A. Plantinga, H. S. Kooistra, and A. C. Beynen, "Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors in the development of feline type 2 diabetes mellitus," *Veterinary Journal*, vol. 179, no. 2, pp. 247–253, 2009.

[9] C. A. T. Buffington, "External and internal influences on disease risk in cats," *Journal of the American Veterinary Medical Association*, vol. 220, no. 7, pp. 994–1002, 2002.

[10] J. Stella, C. Croney, and T. Buffington, "Environmental factors that affect the behavior and welfare of domestic cats (*Felis silvestris catus*) housed in cages," *Applied Animal Behaviour Science*, vol. 160, no. 1, pp. 94–105, 2014.

[11] M. J. Daniels, M. A. Beaumont, P. J. Johnson, D. Balharry, D. W. Macdonald, and E. Barratt, "Ecology and genetics of wild-living cats in the north-east of Scotland and the implications for the conservation of the wildcat," *Journal of Applied Ecology*, vol. 38, no. 1, pp. 146–161, 2001.

[12] T. Tennessen, "Coping with confinement—features of the environment that influence animals' ability to adapt," *Applied Animal Behaviour Science*, vol. 22, no. 2, pp. 139–149, 1989.

[13] C. Cameron-Beaumont, S. E. Lowe, and J. W. S. Bradshaw, "Evidence suggesting preadaptation to domestication throughout the small Felidae," *Biological Journal of the Linnean Society*, vol. 75, no. 3, pp. 361–366, 2002.

[14] D. Turner, "Social organization and behavioral ecology of the free-ranging domestic cat," in *The Domestic Cat: The Biology of Its Behaviour*, D. Turner and P. Bateson, Eds., pp. 63–70, Cambridge Univeristy Press, Cambridge, UK, 2014.

[15] M. Mendl and R. Harcourt, "Individuality in the domestic cat: origins, development and stability," in *The Domestic Cat: The Biology of its Behaviour*, D. C. Turner and P. Bateson, Eds., pp. 47–64, Cambridge Univeristy Press, Cambridge, Mass, USA, 2000.

[16] S. L. H. Ellis, "Environmental enrichment. Practical strategies for improving feline welfare," *Journal of Feline Medicine and Surgery*, vol. 11, no. 11, pp. 901–912, 2009.

[17] E. Heidenberger, "Housing conditions and behavioural problems of indoor cats as assessed by their owners," *Applied Animal Behaviour Science*, vol. 52, no. 3-4, pp. 345–364, 1997.

[18] D. L. Zoran and C. A. Buffington, "Timely topics in nutrition: effects of nutrition choices and lifestyle changes on the well-being of cats, a carnivore that has moved indoors," *Journal of the American Veterinary Medical Association*, vol. 239, no. 5, pp. 596–606, 2011.

[19] E. A. Plantinga, G. Bosch, and W. H. Hendriks, "Estimation of the dietary nutrient profile of free-roaming feral cats: possible implications for nutrition of domestic cats," *The British Journal of Nutrition*, vol. 106, supplement 1, pp. S35–S48, 2011.

[20] A. K. Hewson-Hughes, V. L. Hewson-Hughes, A. T. Miller, S. R. Hall, S. J. Simpson, and D. Raubenheimer, "Geometric analysis of macronutrient selection in the adult domestic cat, *Felis catus*," *Journal of Experimental Biology*, vol. 214, no. 6, pp. 1039–1051, 2011.

[21] A. K. Hewson-Hughes, V. L. Hewson-Hughes, A. Colyer et al., "Consistent proportional macronutrient intake selected by adult domestic cats (*Felis catus*) despite variations in macronutrient and moisture content of foods offered," *Journal of Comparative Physiology B*, vol. 183, no. 4, pp. 525–536, 2013.

[22] J. L. Stella, L. K. Lord, and C. A. T. Buffington, "Sickness behaviors in response to unusual external events in healthy cats and cats with feline interstitial cystitis," *Journal of the American Veterinary Medical Association*, vol. 238, no. 1, pp. 67–73, 2011.

[23] J. Stella, C. Croney, and T. Buffington, "Effects of stressors on the behavior and physiology of domestic cats," *Journal of Applied Animal Behavior Science*, vol. 143, no. 2–4, pp. 157–163, 2013.

[24] F. D. McMillan, "Stress-induced and emotional eating in animals: a review of the experimental evidence and implications for companion animal obesity," *Journal of Veterinary Behavior: Clinical Applications and Research*, vol. 8, no. 5, pp. 376–385, 2013.

[25] L. Colliard, B.-M. Paragon, B. Lemuet, J.-J. Bénet, and G. Blanchard, "Prevalence and risk factors of obesity in an urban population of healthy cats," *Journal of Feline Medicine and Surgery*, vol. 11, no. 2, pp. 135–140, 2009.

[26] E. A. Courcier, D. J. Mellor, E. Pendlebury, C. Evans, and P. S. Yam, "An investigation into the epidemiology of feline obesity in Great Britain: results of a cross-sectional study of 47 companion animal practises," *The Veterinary Record*, vol. 171, no. 22, p. 560, 2012.

[27] E. A. Courcier, R. O'Higgins, D. J. Mellor, and P. S. Yam, "Prevalence and risk factors for feline obesity in a first opinion practice in Glasgow, Scotland," *Journal of Feline Medicine and Surgery*, vol. 12, no. 10, pp. 746–753, 2010.

[28] E. M. Lund, P. J. Armstrong, C. A. Kirk et al., "Prevalence and risk factors for obesity in adult cats from private US veterinary practices," *The International Journal of Applied Research in Veterinary Medicine*, vol. 3, pp. 88–96, 2005.

[29] N. J. Cave, F. J. Allan, S. L. Schokkenbroek, C. A. M. Metekohy, and D. U. Pfeiffer, "A cross-sectional study to compare changes in the prevalence and risk factors for feline obesity between 1993 and 2007 in New Zealand," *Preventive Veterinary Medicine*, vol. 107, no. 1-2, pp. 121–133, 2012.

[30] E. Ward, "Association for Pet Obesity Prevention," 2015, http://petobesityprevention.org.

[31] D. L. Zoran, "The carnivore connection to nutrition in cats," *Journal of the American Veterinary Medical Association*, vol. 221, no. 11, pp. 1559–1567, 2002.

[32] M. Morgan and K. A. Houpt, "Feline behavior problems: the influence of declawing," *Anthrozoos*, vol. 3, no. 1, pp. 50–53, 1989.

[33] C. A. T. Buffington, J. L. Westropp, D. J. Chew, and R. R. Bolus, "Clinical evaluation of multimodal environmental modification (MEMO) in the management of cats with idiopathic cystitis," *Journal of Feline Medicine and Surgery*, vol. 8, no. 4, pp. 261–268, 2006.

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 7393620, 22 pages
http://dx.doi.org/10.1155/2016/7393620

Review Article

Meeting the Vitamin A Requirement: The Efficacy and Importance of β-Carotene in Animal Species

Alice S. Green and Andrea J. Fascetti

Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

Correspondence should be addressed to Andrea J. Fascetti; ajfascetti@ucdavis.edu

Received 20 May 2016; Accepted 29 August 2016

Academic Editor: A. Kate Shoveller

Vitamin A is essential for life in all vertebrate animals. Vitamin A requirement can be met from dietary preformed vitamin A or provitamin A carotenoids, the most important of which is β-carotene. The metabolism of β-carotene, including its intestinal absorption, accumulation in tissues, and conversion to vitamin A, varies widely across animal species and determines the role that β-carotene plays in meeting vitamin A requirement. This review begins with a brief discussion of vitamin A, with an emphasis on species differences in metabolism. A more detailed discussion of β-carotene follows, with a focus on factors impacting bioavailability and its conversion to vitamin A. Finally, the literature on how animals utilize β-carotene is reviewed individually for several species and classes of animals. We conclude that β-carotene conversion to vitamin A is variable and dependent on a number of factors, which are important to consider in the formulation and assessment of diets. Omnivores and herbivores are more efficient at converting β-carotene to vitamin A than carnivores. Absorption and accumulation of β-carotene in tissues vary with species and are poorly understood. More comparative and mechanistic studies are required in this area to improve the understanding of β-carotene metabolism.

1. Introduction

Vitamin A (VA) is an essential nutrient for all vertebrate animal species. There are two dietary sources of VA: preformed retinoids and provitamin A (pro-VA) carotenoids. The proportion of an animal's total VA supply coming from these two sources is dependent upon several major factors: (1) dietary supply; (2) intestinal absorption; and (3) metabolic ability to convert pro-VA carotenoids to retinoids. Although all carotenoids with one or more unsubstituted β-ionone rings can theoretically be VA precursors, β-carotene (BC) appears to be the most important of these. It is certainly the most studied carotenoid, at least in the context of its role as a pro-VA compound. Thus, this review will focus on the metabolism of BC across animal species. However, many of the concepts discussed here likely apply to other pro-VA carotenoids, though there is no doubt of much complexity and variation in the metabolism of those compounds as well.

A comparative approach to understanding the metabolism of BC is appropriate, as the handling of this nutrient varies impressively across animal species. Understanding how different species meet their VA requirement can inform how we feed animals that come into our care, be they production livestock, our companion pets, or exotic species kept in zoos and aquaria or managed wildlife. For this, it is important to understand the efficiency of conversion of BC to VA and how it varies in animal species. In addition, absorption into blood and accumulation in tissues of intact BC is highly variable and species-dependent. This facet of comparative BC metabolism determines to what extent a species may be impacted by the non-pro-VA functions of BC.

2. Vitamin A

2.1. Structure, Units, and Chemistry. Vitamin A is actually a family of compounds that are structurally similar to and have the essential functions and biological activity of retinol. Retinol is a long-chain, unsaturated alcohol containing five double bonds and a β-ionone ring. Retinol and most other naturally occurring retinoids are lipid-soluble compounds.

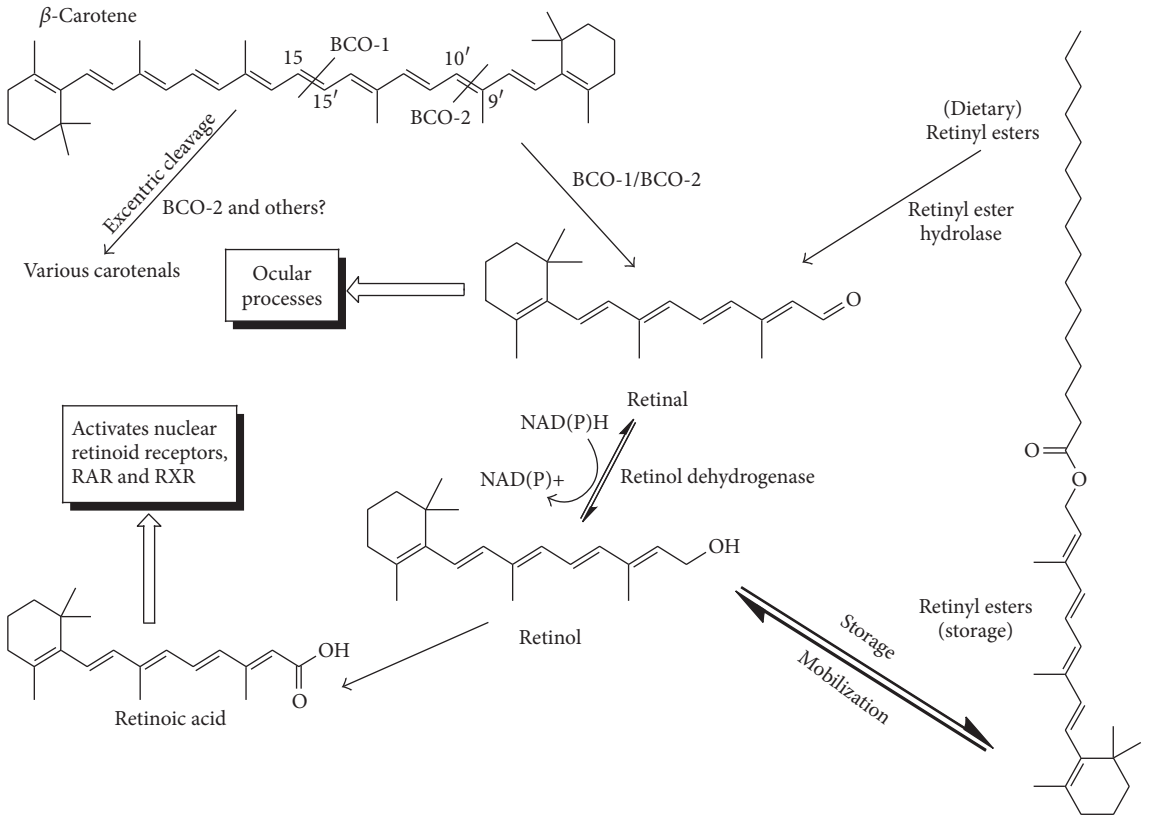


Figure 1: Simplified schematic of the major metabolic pathways of β-carotene and retinoids. BCO-1, β,β-carotene 15,15′-oxygenase; BCO-2, β,β-carotene 9,10′-oxygenase.

The term "vitamin A" is often used to also include pro-VA carotenoids, but in this review, the two are distinguished and "VA" is used to exclusively mean retinoids with the biological activity of retinol. Vitamin A requirements and food values are expressed as *retinol equivalents* (RE), with 1 RE equivalent to 1 μg free retinol. This conversion is important, because much of the preformed dietary VA is in the form of retinyl esters.

2.2. Forms and Functions of Vitamin A. The most physiologically active forms of VA are retinoic acid and retinal. Retinoic acid interacts with the nearly ubiquitously expressed nuclear retinoid receptors (RAR and RXR) to regulate gene expression. Through this mechanism, VA is required for normal cellular differentiation, development, and immune function [1]. Retinal is the prosthetic group of opsins, the light absorbing units of the retina in the eye. It is thus essential for the transduction of light to the neural signals required for vision [2]. Retinol and retinal can be interconverted, and retinol can be irreversibly converted to retinoic acid (Figure 1). Retinoic acid can be utilized for most of the essential functions of VA but can not meet the requirement for vision and some reproductive functions [3].

2.3. Absorption and Metabolism of Vitamin A. Major sources of preformed VA include liver, fish, eggs, and, in human food systems, fortified foods such as milk, cereals, and margarine. Vitamin A is predominantly in the form of retinyl esters in foods. After release from the food matrix by digestion, retinyl esters are hydrolyzed by retinyl ester hydrolases, several of which have been described. Free retinol is taken up by enterocytes, where it is complexed with cellular retinol binding protein (CRBP-II) and then reesterified by lecithin : retinol acyltransferase (LRAT). It is then incorporated into chylomicrons and exported into the lymph along with other components of dietary fat [4]. In the circulation of humans, VA is associated almost exclusively with retinol binding protein (RBP) and is tightly regulated. However, in many other species, particularly carnivores, a significant proportion of circulating VA is in the form of retinyl esters [5]. This aspect of VA metabolism will be discussed in more depth later in this review.

If VA status is adequate, the major storage depot of VA is in the stellate cells of the liver. Hepatic storage generally accounts for 50–85% of total body VA in replete humans and is predominantly in the form of retinyl esters. Vitamin A absorption, metabolism, and storage are complex and affected by multiple proteins and control points that maintain VA homeostasis, and readers are thus referred to more complete reviews of these topics [6–8].

2.4. Vitamin A Deficiency and Requirements. Vitamin A deficiency results in xerophthalmia, metaplasia, epithelial differentiation, anorexia, and compromised function of both

innate and adaptive immunity [4]. VA requirements have been determined using a wide range of indicators of status, including occurrence of night blindness, maintenance of normal pressure in cerebrospinal fluid, and amounts required for optimal growth and reproduction [9]. The lack of standardized indices for determination of a VA requirement makes it difficult to compare requirements across species, particularly those for which there are only one or two studies. Nevertheless, estimated VA requirements for animal species are given in Table 1.

2.5. Vitamin A Toxicity. Acute VA toxicity results in a range of effects within hours: general malaise, anorexia, nausea, peeling skin, hyperirritability, muscular weakness, convulsions, paralysis, and even death [10]. Chronic VA toxicity causes skeletal malformations, spontaneous fractures, and internal hemorrhage [10] and may also be linked to the development of osteoporosis in elderly humans [11]. Both VA deficiency and toxicity can cause teratogenic effects [12, 13]. Ruminants are more tolerant of high dietary VA than omnivores and nonruminant herbivores, because rumen microbes can destroy 40–70% of dietary VA [14]. Of the animal species that have been studied, adult cockatiels are among the least tolerant of high dietary VA [15]. Safe upper limits estimated for different species are summarized in Table 1.

Carnivores appear to be the class of animals most tolerant to high dietary VA. Work in the domestic cat indicates increased VA tolerance compared with other species due to the ability to increase metabolism and excretion of VA and maintain higher concentrations of circulating retinyl esters in blood [16, 17]. Cats increase urinary and fecal excretion of VA when consuming large quantities, as evidenced by the increased excretion of retinol, retinyl esters, and more polar retinol conjugates in cats dosed orally with ^{3}H-retinol [16]. Retinol and retinyl esters have also been measured in the urine of the dog, silver fox, blue fox, and raccoon dog, while no VA was detected in the urine of cows, sheep, horses, rabbits, and rats [18, 19]. No more than trace amounts of VA have been observed in human urine with the exception of cases of pneumonia, kidney disease [20], or infections causing diarrhea or proteinuria [21], when retinol appears to be excreted in urine associated with RBP.

Carnivores also store tremendous amounts of VA in the liver at much higher concentrations than have been found in livers of noncarnivores. This fact is well-known among northern native people and polar explorers, who have found that consumption of polar bear and seal liver causes acute toxicity. Retinyl ester concentration in polar bear and seal livers has been found to range from 2,215 to 10,400 μg/g wet weight and to vary with age and sex [22, 23]. To put this into perspective, hepatic concentrations of 20–300 μg/g wet weight retinyl esters are considered to indicate "adequate" VA status in humans [4]. Similar concentrations, dependent on season, are found in herbivorous free-ranging wild species consuming natural diets [24–26]. Leighton et al. suggested that the large number of stellate cells in polar bear livers compared to other species permits the storage of high concentration of retinyl esters [23]. Species differences in hepatocyte types and proportions may help to explain differences in the ability to store large quantities of VA. Vitamin A tolerance is likely protective for wild carnivores, which may sporadically consume large amounts of VA from the liver of prey species.

2.6. Circulating Forms of Vitamin A in Animal Species. There are significant interspecies differences in circulating forms of retinoids. In humans, some primates, and many other omnivores and herbivores, the majority of circulating VA is in the form of retinol bound to RBP and is tightly controlled, making plasma or serum retinol nonresponsive to VA intake (unless very deficient or toxic) and not a good indicator of status [4]. In contrast, retinyl esters make up a significant proportion of circulating VA in many, but not all, carnivorous species [5]. In domestic cats, a study found 70 ± 17% of total plasma VA by weight was esterified, and of this, 61 ± 6% was retinyl stearate, 36 ± 13% was retinyl palmitate, and 5 ± 3% was retinyl oleate [18]. In a study surveying serum retinoids in 12 captive wild felid species, retinyl esters, predominantly stearate and palmitate, were detected in the serum of all species measured [27]. However, the proportion of VA present as esters ranged widely from 87% in the sand cat and 48% in the fishing cat to as low as 7% in cheetahs and cougars and 4% in servals.

In dogs, it has been shown that circulating retinyl ester concentration is proportional to dietary VA, while circulating nonesterified retinol is unaffected across a range of 210–13,200 RE/kg diet dry matter (DM) [28]. More recently, eight-week-old Labrador Retrievers and Miniature Schnauzers were fed one of four retinyl acetate concentrations to achieve intakes of 5.24, 13.10, 78.60, and 104.80 umol retinol (5000, 12 500, 75 000, and 100 000 IU VA/4184 kJ) (1000 kcal) ME for 1 yr [29]. There was no effect of VA concentration on any hematological or biochemical variables, bone-specific alkaline phosphatase, crossed-linked carboxyterminal telopeptides of type one collagen, and dual X-ray absorptiometry. Total VA concentration did have an effect on total serum retinyl esters, but there was no effect of dose on number, type, or duration of adverse events. The authors concluded that 104.80 umol retinol (100,000 IU VA/4184 KJ) (1000 kcal) ME was a suitable safe upper limit for growing dogs. Similar results were found in ferrets fed a VA supplement of 7500 RE/d as retinyl palmitate for 37 d. There was little difference in plasma retinol between the controls and the supplemented ferrets, while retinyl ester (both palmitate and stearate) concentrations increased more than 6-fold after the supplementation period [30]. Thus, it seems that dogs and ferrets are able to maintain the concentration of retinol, carried by RBP [31], within a range required for homeostasis. The retinol complexed with RBP is available for transport into cells and then interaction with nuclear receptors. The circulating retinyl esters likely represent a type of VA storage for carnivores consuming higher concentrations of VA.

3. β-Carotene and Other Carotenoids

3.1. Carotenoid Structure. Beta-carotene is just one of the more than 600 naturally occurring carotenoids that have

Table 1: Estimated vitamin A requirements and safe upper limits in RE[1]/kg dry matter in animal species. Requirements listed are the minimum requirements or adequate intake values.

Species	Physiological state	Requirement	Upper limit	References
Cat	Growth	1000	80,000	[210]
	Maintenance	1000	100,000	
	Gestation and lactation	2000	100,000	
Catfish	All	450	9999	[10, 202]
Chicken	Growth	450	4500	[10, 195]
	Laying	900	13500	
Cockatiel	Maintenance	600	<3000	[15]
Common carp	All	1200	ND	[10, 202]
Cow, beef	Feedlot	660	19800	[10, 211]
	Pregnant heifers and cows	840	19800	
	Lactating cows and bulls	1170	19800	
Cow, dairy	Growth	1014	19800	[10, 212]
	Lactating cows and bulls	840	19800	
Dog	Growth, gestation, lactation	1212	15,000	[210]
	Maintenance	1212	64,000	
Fox	Growth	732	ND	[213]
Geese	Growth	450	4500	[10, 195]
	Breeding	1200	4500	
Goat	Maintenance	1500	13500	[10, 214]
	Lactation	1750		
Hamster	All	1223	ND	[156]
Horse	Maintenance	549	4800	[215]
	Growth, working	450–690	4800	
	Pregnancy and lactation	825–1110	4800	
Human	Adult male	1200	6000	[10, 160]
Mink	Growing	1779	ND	[213]
Mouse	All	800	ND	[156]
Pig	Growing, 5–10 kg	660	6000	[10, 216]
	Growing, 20–120 kg	390	6000	
	Pregnant swine and boars	1200	12000	
	Lactating	600	12000	
Rabbit	Growing, maintenance	174	4800	[10, 217]
	Gestation	497	4800	
Rainbow trout	All	750	7500	[10, 202]
Salmon	All	750	7500	[10, 202]
Sheep	Replacement ewes, 60 kg	470	13500	[10, 218]
	Pregnancy, 70 kg	992	13500	
	Lactation, 70 kg	714	13500	
	Replacement rams, 80–100 kg	593	13500	
Turkey	Growing and breeding	1500	4500	[10, 195]

[1]1 retinol equivalent (RE) = 1 μg retinol.

been isolated and characterized thus far [32]. All carotenoids are derived from a C_{40} polyisoprenoid skeleton, and their diversity comes from various modifications including substitution, cyclization, addition, elimination, and rearrangement [33]. They are all characterized by a long polyene chain of conjugated double bonds, called the chromophore, and this part of their structure is responsible for absorbing light in the visible region and thus producing the observed color associated with carotenoid compounds. The isomerization that can occur around each double bond contributes in part to the huge number of carotenoids, though most carotenoids in nature are predominantly present in the all-*trans* form [32]. The carotenoids are lipophilic and insoluble in water. Therefore, unless they are associated with proteins or have an

additional polar functional group, carotenoids are generally located in the lipid bilayer of the cell membrane [32].

Carotenoids are divided into two groups, the carotenes and the xanthophylls. The carotenes are nonpolar hydrocarbons and include BC, α-carotene, and lycopene. The xanthophylls have hydroxyl or keto end groups and are thus more polar compounds, including lutein, zeaxanthin, canthaxanthin, and β-cryptoxanthin [34]. Because of their strongly lipophilic nature, carotenes are found in the lipid core of the cell membrane. The carotenes are also rigid molecules, so they are generally aligned parallel to the membrane surfaces. The more polar xanthophylls are more likely to expose their hydroxyl groups to the outer membrane layer, often orienting perpendicular to the membrane and at times being transmembrane in orientation [35]. These properties impact the transport, metabolism, and functions of the different classes of carotenoids. In animals, polar carotenoids seem to be more readily absorbed compared to nonpolar carotenoids [36, 37]. In humans, carotenes are more likely to be transported after absorption with LDL, while xanthophylls are evenly distributed between LDL and HDL [38, 39]. However, important species differences in lipoprotein transport may exist. For example, it has been shown in ferrets supplemented with BC that the majority of circulating BC is associated with HDL [40].

The presence of at least one unsubstituted β-ionine ring confers pro-VA capacity to carotenoids (Figure 1). Beta-carotene has two β-ionine rings, so one molecule of BC can theoretically form two molecules of retinol via retinal. In comparison, β-cryptoxanthin contains only one unsubstituted β-ionine ring (the other contains a hydroxyl group) so it can only yield one molecule of retinol. The structure of lycopene has no β-ionine rings so it cannot be a precursor to VA. Based on structure alone, it is thought that about 50–60 of the known carotenoids have some pro-VA activity [41].

3.2. History of β-Carotene Research. Beta-carotene was first isolated in 1831 by Wachenroder [42], who crystallized the pigment from carrot (*Daucus carota*) root and gave the compound its name. Steenbock [43] provided the first suggestion of an association between yellow carotene pigments and "fat-soluble vitamin" activity in 1919, when he made the observation that rats thrived on a diet based on yellow corn but died within 3 months when fed a diet of white corn, after developing a severe inflammation of the eyes (now known as xerophthalmia). Steenbock's work also fit with the observations of Osborne and Mendel [44], who noted that rats grew normally when the dietary fat source was butterfat but they faced "nutritive disaster" (again characterized by xerophthalmia) and ultimately death if the sole fat source was lard or almond oil. The link between BC and VA was definitively established in 1929 by von Euler et al., who demonstrated that crystallized carotene had VA activity [45]. Moore demonstrated *in vivo* conversion of BC to VA in rats in 1930 [46], and a wealth of new questions regarding the pro-VA function of carotenoids was born.

During the course of the history of the study of BC, the metabolic differences between animal species were recognized early as investigators found disparate results depending on the animal model used. For example, in his studies of BC metabolism in the early 1930s, Ahmad switched from rats to cats in search of a larger animal model, and he found that his results completely changed [47]. Amazingly, the first suggestion that the conversion of BC to VA might occur in the intestine was made during a study of baleen whales published in 1939 [48], though this speculation was not confirmed until 1947 [49]. In the 1960s, radioisotope studies measuring the absorption of BC in the lymph of rats and humans quickly determined that humans are able to absorb intact BC, while rats cannot [50, 51]. Since this time, investigators have been cautious to extrapolate observations from one species to another. In addition, the search for a better animal model for human carotenoid metabolism has led to many detailed studies in animal species, contributing to our comparative understanding of BC metabolism.

3.3. Food Sources of Carotenoids. Fruits such as apricots, peaches, persimmons, citrus, tomatoes, and melon are rich sources of carotenoids [52]. High concentrations are also found in green vegetables like spinach, broccoli, and parsley as well as orange tuber vegetables like carrots and sweet potatoes [52]. In addition, carotenoids accumulate in the tissues and products of animals; butterfat, egg yolk, and salmon (concentrated in the skin, muscle, and ovaries) are excellent sources [52].

3.4. Functions of Carotenoids. Carotenoids are not considered to be essential nutrients for animals. As long as their diets provide adequate VA, animals can live and reproduce while consuming a carotenoid-free diet without exhibiting any specific signs of deficiency. However, some free-ranging birds and fish would likely not be successful breeders without the appropriate dietary carotenoids used as pigments to communicate their viability as mates. In addition to their role as potential precursors to VA, carotenoids also have a variety of other functions that may improve animal health. Carotenoids can serve as antioxidants [53] and improve gap junction communication [54] and immune function [55]. Carotenoid intake has also been associated with reduced risk of heart disease [56], macular degeneration [57], cataracts [58], and some cancers [59, 60] in humans. In addition, BC has been shown to increase the reproductive success of cows and pigs with important implications for animal production systems [61, 62].

Unlike VA, which can be toxic at only 3-4 times the recommended daily allowance in humans, BC does not appear to have any toxicity effects [63]. As will be described in more detail later, the conversion of BC to VA is tightly regulated and dependent upon VA status. Excessive intakes of BC result in decreased efficiency of conversion to VA, thus preventing VA toxicity. This makes dietary supplementation with BC and other pro-VA carotenoids a safe way to improve VA status without risking toxicity as with retinoid supplementation, at least provided the species can utilize pro-VA carotenoids for VA. Very high intakes of BC have been known to result in harmless and transient hypercarotenemia and orange pigmentation of the skin in humans [64].

3.5. Digestion and Absorption of Carotenoids. Carotenoids are lipid-soluble compounds and are absorbed in the digestive tract with dietary fats. However, they must first be released from the food matrix by mastication, gastric actions, and digestive enzymes. Carotenoids in leaves and stems are usually present in the free form, while those in fruits and seeds are often esterified with fatty acids and must be hydrolyzed prior to absorption [65]. Esterified carotenoids may be less digestible than those in the free form [66], though this does not seem to be the case with lutein esters [67]. Free carotenoids in the intestinal lumen are incorporated into mixed micelles and absorbed by the mucosa of the small intestine, apparently by a passive, nonsaturable mechanism. Once in the enterocyte, carotenoids are packaged in chylomicrons and travel via the lymph to the periphery and eventually the liver. Oxidative cleavage of BC to VA occurs mainly at the brush border membrane of the intestine, with some activity in other organs such as the liver, kidney, and lungs [68].

4. Bioavailability of Carotenoids

Bioavailability is defined as the proportion of a consumed nutrient that is available for normal physiological functions and storage. Measuring the bioavailability of pro-VA carotenoids is made more complex by the fact that a portion of the ingested carotenoids is usually converted to VA in the intestine, before it reaches circulation. Thus, to measure true bioavailability of pro-VA carotenoids, it is also necessary to measure bioconversion to VA, which will be discussed later in this review. However, measures of apparent digestibility still provide useful information, particularly when dietary treatments are compared in the same study. In this section, the methods used to measure bioavailability and factors that have been shown to impact carotenoid absorption within a species are discussed. Discussion of interspecies differences in carotenoid absorption will be combined with a later section on interspecies differences in pro-VA activity, as these two processes interact and are difficult to separate experimentally.

4.1. Methods Used to Measure Bioavailability. Several methods have been used to measure carotenoid bioavailability with varying degrees of success. The simplest method involves measuring the serum or plasma response after carotenoid ingestion. This method is useful when comparing bioavailability between doses or dietary treatments; however, it can not quantify true bioavailability because it does not distinguish the dosed carotenoids from endogenous compounds or account for conversion to VA or other metabolic pools of carotenoids, such as those stored in tissues. In order to be distinguished from endogenous carotenoids, large nonphysiological doses must be used, which are likely to be less bioavailable compared with more physiological doses.

A more specific method is to measure the chylomicron response to a carotenoid dose [69, 70]. This method generates an improved estimate of absorption and distinguishes the dose from endogenous carotenoids. Because chylomicron carotenoid concentration is presumably specific to the dosed compounds and separate from endogenous pools, smaller doses can be used than with the serum/plasma response method. However, it is difficult to separate intestinal chylomicrons from liver-derived VLDL. Furthermore, carotenoids can transfer from chylomicrons to LDL or HDL and thus not be accounted for in the chylomicron response method [68].

The oral-fecal balance method can also be used to estimate bioavailability. However, this method gives highly variable results and can not account for carotenoid degradation in the upper (from chemical oxidation) or lower (from microbial degradation) gastrointestinal tract. For example, cattle given a BC preparation with rumen-protected fats had an increased plasma BC response compared with BC given with nonprotected fats [71]. It has also been shown that plant lipoxygenases present in feed plants could quickly destroy BC and lutein *in vitro* in bovine rumen fluid [72]. In addition, bioavailability of BC was higher in germ-free rats compared to those with normal gut microflora [73].

Dosing with isotope-labeled carotenoids appears to be the most promising method for determining carotenoid bioavailability [74]. An isotope-labeled dose allows absolute distinction between endogenous and dosed compounds and thus allows for the use of smaller, more physiological, doses. Some of the most informative early work on carotenoid bioavailability and conversion was conducted using ^{3}H-BC. Concerns about the effects of radiation caused many investigators to turn to stable isotope applications, especially for work with human subjects. However, the impressive detection limits of accelerator mass spectrometry technology have led to a resurgence of the use of ^{14}C-BC with the smallest doses yet [75, 76]. Subjects may be dosed with synthetic preparations or endogenously labeled foods grown in isotope-enriched substrates [77]. The use of isotope-labeled carotenoids determines not only absolute absorption but also postabsorptive metabolism. Thus, the conversion of BC to VA can be qualitatively measured. If a concurrent reference dose of labeled VA is given, BC conversion can also be quantified [74, 77–79].

4.2. Factors Impacting Bioavailability. As discussed earlier, the chemical structure of the carotenoid in question confers some inherent differences in absorption, based on chemical polarity, that impact bioavailability. Other factors affecting bioavailability include food matrix, VA status, interaction with other carotenoids, dietary fat, dietary fiber, and parasitic infection. Most of the work on bioavailability of carotenoids has been conducted in humans or rodents with several studies in ferrets. Little is known about how these factors may be similar or different in other animal species.

4.2.1. Food Matrix. Bioavailability of carotenoids in raw vegetables is estimated to be 5–10% for humans and rats, while it may be as high as 50% when dissolved in oils. Thus, the food matrix has an important impact on bioavailability, and processing such as mincing, liquefying, and mild heating can improve the bioavailability of carotenoids [34, 80]. One study showed that the bioavailability of BC in spinach was improved by liquefying (9.5%) compared with whole leaf spinach (5.1%) [81]. Lutein bioavailability was measured in

the same study and found to be much higher (45–55%) than that of BC and unaffected by processing. Other investigators have also observed that bioavailability of lutein in vegetables is much higher than the bioavailability of BC [37], a finding consistent with the more polar nature of lutein.

Commercially prepared water-soluble BC beadlets are perhaps the most bioavailable form of BC. In ferrets fed naturally occurring BC in carrot juice (in crystalline form in the chloroplasts), bioavailability was only about 30% of that from a water-soluble beadlet form [82]. In preruminant calves, the plasma BC response after supplementation with crystalline BC in oil was only 4% of that measured after supplementation with water-soluble BC beadlets [83].

Cooking vegetables with excessive heat (e.g., boiling) can result in degradation of carotenoids via isomerization and oxidation. While the isomers of BC may have some pro-VA activity, they appear to be less readily absorbed and/or transported compared with all-*trans* β-carotene, the predominant form in raw fruits and vegetables. It has been shown in ferrets, gerbils, and humans that the 9-*cis* and 13-*cis* BC isomers are less bioavailable than the all-*trans* isomer [84, 85].

4.2.2. Vitamin A Status. Current VA status can impact BC absorption, though the data is somewhat mixed. In chicks, an inverse relationship was found between dietary VA concentration and BC absorption from yellow corn [86]. This effect was observed on apparent BC absorption determined with both the oral-fecal balance and chromic oxide methods and also in the BC content of the chicks' serum, liver, and skin. Similar results were found in a study of rats; VA-deficient rats absorbed BC at twice the rate of rats fed an adequate diet [87]. In contrast, Boileau et al. [88] observed decreased *in vitro* BC uptake by isolated brush border membrane vesicles of VA-deficient rats compared with VA-replete rats, leading these authors to postulate that VA deficiency impairs enterocyte function. Lemke et al. [76] demonstrated improved BC absorption (75% versus 55% apparent absorption) in 2 VA-replete women consuming a VA supplement for 3 weeks compared with baseline measurements. As will be discussed later, VA status also influences the efficiency of conversion of BC to VA so it may compensate for changes in bioavailability.

4.2.3. Carotenoid Interactions. In humans, dietary BC appears to decrease the absorption of several other carotenoids when consumed together, including lutein [36, 89], lycopene [89], and canthaxanthin [90]. In these studies, BC absorption was not affected by the other carotenoids. On the other hand, another study [91] found that a concurrent dose of lutein (15 mg) in men decreased the absorption of a BC dose of equal mass, whereas concurrent lycopene did not impact BC absorption. In ferrets, a marked decrease in serum BC was observed following a 10 mg/kg BW dose of both BC and canthaxanthin compared with BC administered alone [92]. Interestingly, the serum canthaxanthin response was 10x lower than the serum BC response, so though canthaxanthin appeared to interfere with the absorption of BC, very little canthaxanthin itself was absorbed. These studies demonstrate that intake of other carotenoids obviously influences the absorption of BC and vice versa, but the effects may vary depending on animal species and interactions with a multitude of other factors, some described here, that impact carotenoid absorption.

4.2.4. Dietary Fat. Dietary fat improves the absorption of BC, because it is required for the formation of the mixed micelles in which BC travels from the intestinal lumen into the enterocyte. In addition, fat aids in the transition of carotenoids from what is often an aqueous substrate (i.e., fruits and vegetables) to solubilization into mixed micelles. Ahmad [47] demonstrated the importance of dietary fat for absorption of BC as early as 1931 in his studies on rats. Human studies have shown that dietary fat is required for optimal absorption of BC from both supplements [64, 93] and vegetable sources [94]. However, only about 3–5 g fat may be required; increasing dietary fat above this level does not appear to improve absorption of BC in humans [95, 96].

However, there appear to be species differences in the amount of fat required for optimal BC absorption. For example, in ferrets supplemented with BC, Lakshman et al. [97] found that dietary fat concentrations of 13% or 23% improved hepatic BC storage compared with 6% fat diet. Hepatic retinol and retinyl ester concentrations were significantly improved in a dose-response manner up to the 23% fat diet, indicating that dietary fat may impact both absorption of BC and its metabolism to VA. In contrast, in horses, Kienzle et al. [98] found no difference in serum BC response to supplementation with 2.5 or 6.6% fat diets. It is possible that some species require more fat than others for optimal carotenoid absorption. From the example of ferrets and horses, one might speculate that fat is more important for optimal BC absorption in carnivores than in herbivores, which evolved consuming very low fat diets and depending on BC as an important source of VA. Regardless, most studies administer some fat with carotenoid supplements to ensure that dietary fat does not limit absorption.

Type of dietary fat may also influence BC absorption. This has been shown both *in vitro* [35, 99] and *in vivo* in rats [100, 101]. Schweigert et al. [100] found that BC accumulation in the livers of rats was higher when administered with olive or arachidonic oil compared with butter fat, lard, tallow, sunflower, soya, or linseed oils. However, sunflower oil resulted in the highest accumulation of BC in the lungs. Type of fat had little impact on plasma or liver VA. However, the animal fat sources resulted in a remarkable increase in spleen VA, up to a 10-fold increase over that observed with the vegetable fats. Thus, type of fat may influence not only BC absorption but also conversion to VA and selective tissue uptake of both BC and VA.

4.2.5. Dietary Fiber. Several studies have shown that pectin, guar, and alginate reduce the absorption of BC supplements [102, 103]. Dietary fiber presumably interacts with bile acids to increase the fecal excretion of fats and other fat-soluble compounds such as carotenoids. In addition, fiber may entrap carotenoids in the intestinal lumen.

4.2.6. Parasitic Infection. Parasitic infection may reduce the bioavailability of BC. A study by Jalal et al. [94] in children demonstrated increased absorption of BC from foods (mostly red sweet potatoes) when the subjects were also given anthelmintic treatment for the intestinal parasite *Ascaris lumbricoides*. Allen [104, 105] showed that chicks inoculated with coccidial infections had decreased plasma, intestinal, and liver carotenoids.

In some birds and fish, carotenoid pigmentation is thought to be an excellent indicator of fitness and is used as such by potential mates. Hamilton and Zuk [106] suggested that coloration might specifically indicate resistance to blood parasites in North American passerines. The mechanism for this could be via parasite effects on carotenoid absorption but is also likely to involve other metabolic processes required to utilize ingested carotenoids as skin and feather pigments. Negative effects of infection on pigment intensity have been reported in yellowhammers infected with *Haemoproteus coatneyi* [107], goldfinches infected with coccidiosis [108], and house finches infected with mycoplasma [109]. Milinski and Bakker [110] demonstrated that three-spined stickleback male fish have decreased intensity of red color after infection with the ciliate *Ichthyophthirius multifiliis* and are thus less likely to be selected as mates by gravid females. Similarly, Houde and Torio [111] showed that when male guppies were infected with the parasite *Gyrodactylus turnbulli*, the intensity of their yellow spots decreased, and they were avoided by females compared with their uninfected brothers.

5. Conversion of β-Carotene to Vitamin A

5.1. Mechanism. The mechanism of cleavage of BC to VA has been the subject of debate since the link between the two compounds was confirmed. Early investigators recognized that there are two chemically feasible routes from BC to retinol: (1) Central cleavage of the BC molecule to yield two molecules of retinal or (2) excentric sequential oxidative attack of the double bonds of BC, eventually leading to the formation of one molecule of retinal. The central cleavage enzyme was characterized in 2001 by Leuenberger et al. [112]. They provided strong evidence that it works as a monooxygenase via a transient carotene epoxide, so the central cleavage enzyme is called β,β-carotene 15,15′-oxygenase (BCO-1; Figure 1). BCO-1 has since been cloned in Drosophila [113], mice [114], chickens [115], and human retinal pigment epithelium [116].

An excentric cleavage pathway was proposed in 1954 by Glover and Redfearn [117] and has been confirmed to occur, if at a lower rate than central cleavage. Early evidence included the appearance of some labeled apocarotenals in intestinal homogenates from humans, primates, rats, and ferrets [118]. The enzyme catalyzing excentric cleavage has since been shown to specifically catalyze the oxidative cleavage of BC at the C-9′,C-10′ double bond so it is called β,β-carotene-9′,10′-oxygenase (BCO-2; Figure 1) [119]. Most animal studies do not distinguish between the cleavage activities of BCO-1 and BCO-2; therefore, we use the more generic term "BCO" to describe enzyme activity measured as the formation of VA.

Conversion of BC occurs most efficiently in the intestine. In rats, Duszka et al. [120] found maximal activity of BCO in the jejunum and noted that activity was much higher in mature functional cells than in stem cells. BC cleavage activity has also been described in liver, lung, kidney, and brain tissues of the rat, though these sites are quantitatively much less important than the intestine [121]. The activity of BC metabolizing enzymes may change with development. Yamaguchi et al. [122] showed that in prehatch chicks, there was no intestinal BCO activity but significant hepatic activity. Posthatch, intestinal cleavage activity was rapidly induced within 24 hr, while activity in the liver decreased.

5.2. Methods Used to Measure β-Carotene Conversion. Several methods have been used for measuring BC conversion or biopotency relative to preformed VA. It is important to point out that each method has its pitfalls and that each gives a slightly different type of answer to the same question. This makes it difficult to compare data from studies using different methods to measure BC conversion. The chylomicron response method and isotope-labeled dosing methods were discussed in the section on methods for measuring absorption of BC. Two other methods have been used to measure conversion of BC to VA and are worth mentioning here, the functional bioefficacy method and enzyme activity assays.

The functional bioefficacy assay was used in some of the early studies in animals [123–125] and humans [125] and contributed much to the understanding of the quantitative value of BC as a source of VA. It is still used frequently with animals. With this method, study subjects first must be depleted of VA stores and verified as such through measurement of liver VA concentration or clinical signs of VA deficiency (dark adaptation was used in the human studies). Groups of subjects are then repleted with different amounts of preformed VA (usually as retinyl acetate or palmitate) or BC (either as a synthetic supplement or as contained in food). VA repletion is determined by measuring liver stores or other functional indices, and regression lines for VA status and both dietary VA and BC are calculated. The ratio of the regression coefficients of the BC line to the VA line gives the potency of BC as a source of VA. The advantage of the functional bioefficacy method is that it is a direct measurement of the utilization of BC, taking into account bioavailability of the BC source. On the other hand, this method does require VA depletion, which can take several years in many animals and may be ethically objectionable. In addition, accurate assessment of depletion and repletion requires measuring hepatic VA stores, which is an invasive procedure. Another disadvantage to this method is that BC conversion to VA has been shown to be more efficient in VA-deplete subjects than in replete subjects [87, 126, 127], so BC conversion efficiency may be underestimated with this method compared to conversion efficiency in normal replete subjects.

Enzyme activity assays have been used with tissue homogenates to determine the rate of conversion of BC to VA in a variety of species [120, 128–131]. Though this method gives quantitative results, the *ex vivo* nature limits the application of the data to calculation of the contribution of

dietary BC to the VA supply. Assay conditions are unlikely to exactly mimic the tissue environment, particularly given the many nutrient interactions that can impact BC metabolism. In addition, reaction conditions have been modified over the years, making it difficult to compare enzyme activity measurements from different studies. Enzyme assays are most useful for determining that an animal is capable of conversion and comparing the enzyme activity of tissues homogenates isolated from different animals species under the same assay conditions.

5.3. Effect of Vitamin A Status on β-Carotene Metabolism. One characteristic of BC metabolism to VA is that its conversion efficiency is affected by VA status. VA-deficient animals are more efficient at converting BC to VA than replete counterparts. Conversely, with increasing consumption of BC, cleavage to VA becomes less efficient, making BC a safe supplement without risking VA toxicity. VA-deficient rats have been shown to have greater intestinal BCO cleavage activity [87, 126] and increased plasma VA in response to a BC dose [126]. In a human study, Ribaya-Mercado et al. [127] demonstrated that Filipino children with poor VA status were more responsive to a dietary BC intervention than those with better baseline VA status.

Some insight into the mechanism of the homeostatic effects of VA status on BC metabolism was provided by James and El Gindi [132]. These authors showed that the activity of the intestinal BCO enzyme in rats decreased *in vivo* in a dose-dependent matter with oral doses of retinyl acetate, BC, and retinoic acid and increased with the administration of a RARα receptor antagonist. They also reported that treatment of VA-deprived chickens with retinoic acid resulted in a significant decrease in intestinal BCO mRNA. Thus, these authors suggest that retinoids and carotenoids regulate intestinal BCO activity at the transcriptional level via RAR interactions.

5.4. Other Dietary Factors Impacting β-Carotene Metabolism. Both the type [132] and dietary concentration of protein [133] have been shown to influence BC metabolism in rats, with moderate to high levels of protein improving VA formation. Higher fat diets appear to promote BC conversion to VA in gerbils [134], ferrets [97], and rats [121]. Total carotene load has an inverse effect on BC utilization such that conversion is most efficient when BC is fed in small amounts, on the order of 1-2x that needed to meet the VA requirement; above this, conversion efficiency declines [135].

6. Species Differences in β-Carotene Metabolism

Animal species handle BC differently with respect to absorption, accumulation in blood and tissues, and metabolism to VA. Although BC metabolism has been studied in many species, the variability in methods used makes it difficult to make quantitative interspecies comparisons. However, some patterns emerge. In the following section, mammals are classified as herbivores, omnivores, and carnivores, and the research on members of each group is reviewed. Birds and fish are also discussed separately, as their metabolism of carotenoids and retinoids is distinctive from land mammals in several respects. Reviewed data are also summarized in Table 2 of conversion efficiency ratios and Table 3, a summary of absorption, tissue accumulation, and conversion to VA in animal species.

6.1. Herbivorous Mammals

6.1.1. Cows. Cows absorb intact BC into circulation, accumulate it in tissues, and utilize BC as an important source of VA. In one study evaluating VA (retinol and retinyl esters) and BC in 59 different mammalian and bird species, animals in the order Artiodactyla (cows and gaurs) were one of the only orders to have BC detectable in plasma [136]. Mora et al. [137] compared plasma and tissue concentrations and intestinal BC cleavage activity in cattle and goats. In cattle, plasma BC concentrations reflected dietary intake. Tissue concentrations of BC in cattle are highest in the corpus luteum [138]. There was significant BC cleavage activity measured in both cattle and goats, though goats had higher activity of the two species [137]. BC conversion to VA in bovine intestinal mucosa isolates has been confirmed in several other studies [128, 137].

Absorption of BC and conversion to VA may vary with cattle breed; it has been noted that Holsteins have less pigmented adipose and milkfat, while Jerseys and Guernseys have yellow adipose and milkfat [9]. Morales et al. [139] found no difference in intestinal and hepatic BCO-1 mRNA expression between cattle with pigmented or less pigmented adipose tissue. There was also no difference in the intestinal BCO enzyme activity between pigmented and less pigmented cattle, though hepatic BCO activity was higher in pigmented cattle. This study did not report the breed or the diets of the cattle; however, it does indicate that adipose pigmentation occurs independently of intestinal BCO activity and is not prevented by increased BCO activity in the liver.

Supplementation with BC has been shown to improve reproductive performance in cattle [61], and BC cleavage to VA in reproductive tissues may play a role in the mechanism of this effect. BCO activity has been measured in bovine ovarian follicles, and a significant positive correlation between follicle maturation and both BC conversion rate and vitamin A concentration in follicular fluid was found [129]. BC cleavage activity has also been measured in the corpus luteum of cattle; activity at mid-ovulation was 2x that measured in intestinal homogenates [140]. Oral supplementation with 2000 mg of BC daily from d 21 before the expected calving date until parturition supported the onset of luteal activity during early lactation in dairy cattle [141]. The dose of BC in this study approximated the amount of intake at grazing during the close-up dry period.

In preruminant calves, oral supplementation with BC results in a plasma BC response [80, 83, 142, 143] as well as increases in tissue BC concentration [80, 143]. Hoppe et al. [144] found a dose-response relationship between dietary BC and plasma, liver, and perirenal BC and hepatic VA concentrations, providing evidence that calves both absorb BC intact and convert it to VA. Kon et al. [145] found that

Table 2: Estimated efficiency of conversion of β-carotene to vitamin A in animals, defined as μg of β-carotene required to form 1 RE (1 μg retinol). The n value given is the total number of animals in the study.

Species	Conversion ratio	Study design	References
Herbivorous mammals			
Cow	8.33	Recommendation extrapolated from study in lambs [122].	[123, 212]
Sheep	5.56–8.33	Recommendation based on review of available studies.	[218]
Sheep	7.36	Depletion/repletion study of BC from corn silage in lambs (n = 56); hepatic VA used to indicate VA status.	[123]
Horse	6–10	Recommendation based on review of available studies.	[215]
Horse	>10	Depletion/repletion study with BC supplement in water-soluble beadlet form; serum retinol and relative dose response test used to indicate VA status (n = 45). Supplementation with 215,00 μg BC/d did not restore VA status, while 21,500 RE as retinyl palmitate did (though not completely).	[151]
Omnivorous mammals			
Rat	2	Recommendation based on review of available studies.	[156]
	6	Depletion/repletion study using dry gelatin beadlet form of BC as supplement; hepatic VA used to indicate VA status.	[157]
Mongolian gerbil	6–13	Depletion/repletion study using water-soluble beadlet form of BC as supplement (n = 80); hepatic and renal VA used to indicate VA status.	[158]
Human	2 in oil 12 in food	Recommendation based on review of available studies.	[166]
Human	2.4	Stable isotope study administering physiological doses of ^{13}C-BC in oil to children for ≤10 wk (n = 35); concurrent administration of ^{13}C-retinyl acetate allowed estimation of BC bioefficacy from serum response.	[75]
Human	9.1	Stable isotope study administering single oral doses of ^{2}H-BC to adults (n = 22); concurrent administration of ^{2}H-retinyl acetate allowed estimation of BC bioefficacy from serum response.	[78]
Human	14.8 in carrots; 20.9 in spinach	Subjects consumed single meal of intrinsically labeled ^{2}H-spinach and ^{2}H-carrots (n = 14); concurrent reference dose of ^{13}C-retinyl acetate allowed estimation of BC bioefficacy from serum response.	[77]
Pig	6.7	Recommendation based on review of available studies.	[216]
Pig	13–27	Depletion/repletion study of BC from corn (n = 171); hepatic VA used to indicate status.	[124]
Pig	40	Gilts were fed low VA diet for only 4 weeks, and there was no indication that they were truly depleted (n = 36); supplemented with 100 mg BC in water-soluble beadlets; hepatic VA used to indicate status.	[169]
Carnivorous mammals			
Ferret	>15	Depletion/repletion study with BC supplement from water-soluble beadlets (n = 145); hepatic VA used to indicate status.	[183]
Birds			
Chicken	2	Recommendation based on review of available studies.	[195]
Chicken	6	Depletion/repletion study using dry gelatin beadlet form of BC as supplement; hepatic VA used to indicate VA status.	[157]
Cockatiel	>2	Depletion/repletion study in which chicks born from breeding pairs fed VA-devoid diet consumed VA or BC (synthetic) supplemented diets for 38 d (n = 27); hepatic VA used to indicate status.	[198]

calves converted orally administered BC to VA in blood and liver, while an intravenous injection of BC did not result in increased VA. The same study found that rats and rabbits could utilize injected BC for VA, so these authors speculated that the lack of extraintestinal metabolism in calves may be at least partly responsible for the accumulation of BC in adipose of cows. However, this is not supported by the previously mentioned work of Morales et al. [139].

Slifka et al. [146] measured serum carotenoids of several more exotic members of subfamily Bovidae Bovinae from

Table 3: Summary of β-carotene metabolism in animal species.

Species	Absorption of intact BC into blood	Accumulation of BC in tissues	Conversion to retinol	References
Herbivorous mammals				
Cows	Yes	Yes, but varies with breed	Yes	[9, 80, 83, 128, 129, 137, 140, 142–146, 223, 224]
Sheep and goats	Yes, but only with high dose	No	Yes	[123, 128, 137, 147, 148]
Other Artiodactyla	No	No (shown in white-tailed deer)	Assumed	[146, 147]
Rabbits	n/a	No	Yes	[130, 147]
Horses	Yes	Yes	Yes	[99, 147, 149–151]
Omnivorous mammals				
Rat	No	No	Yes	[50, 120, 135, 145, 152, 154, 156, 157, 225]
Mongolian gerbil	Yes	Yes	Yes	[134, 158, 159]
Humans	Yes	Yes	Yes	[51, 74, 77–79, 147, 161, 162, 164–166, 168, 226, 227]
Nonhuman primates	Yes	Yes	Yes	[118, 152, 163, 167, 168]
Pigs	Yes, but only with high dose	Yes, but only with high doses	Yes	[124, 125, 147, 155, 169–172]
Carnivorous mammals				
Ferrets	Yes	Yes	Yes (but inefficient)	[82, 92, 118, 178, 182–184]
Canids	Yes, but only with high dose	No	Yes	[147, 173–176, 180]
Felids	Yes, but only with high dose. Wild felids may absorb BC more efficiently than domestic cats	No	Yes (but inefficient)	[18, 27, 47, 130, 177, 185–187]
Birds	No, xanthophylls absorbed preferentially	No	Yes (shown in chickens, cockatiels, canaries, quail, and ducks	[157, 188, 194–200]
Fish	No, xanthophylls absorbed preferentially	No	Yes	[201, 203, 206–209]

zoo collections, including a wisent and banteng, and found relatively high circulating concentrations of BC. However, cattle and their close relatives appear to be unique among the species of the family Bovidae, many of whom have been designated nonaccumulators of carotenoids despite consuming moderate to high dietary levels (see "other Artiodactyla"). BC can be destroyed by rumen bacteria [72] and lipoxygenases present in some forage plants [73], and these factors may contribute to variability of responses to BC consumed by cattle and other herbivores.

6.1.2. Sheep and Goats. Sheep and goats do not appear to readily absorb intact BC but are efficient convertors of BC to VA. Sheep and goats have colorless fat, indicating that they do not accumulate BC in their adipose tissue [147]. Apparent absorption of BC was measured in sheep using the oral-fecal balance method [148]. Sheep were dosed daily with 2.9–8.5 mg BC in alfalfa pellets or a water-soluble synthetic solution, and 90–98% of the dose was recovered in the feces. When the dose was dissolved in fat, fecal recovery was reduced to 50%, but the authors found that this was due to BC destruction in the lower intestinal tract, not increased absorption. In goats supplemented with very high levels of BC, BC did not appear in serum until after 10 days of supplementation [137].

Martin et al. [123] conducted a functional bioassay experiment measuring the VA activity of pro-VA carotenoids (predominantly BC) from corn silage in lambs. By measuring serum and hepatic VA, they determined that, in VA-depleted lambs, 7.4 μg BC = 1 μg retinol (RE). Yang and Tume [128] measured BCO activity in isolated intestinal mucosa of sheep and found a specific activity of 13 pmol BC cleaved/mg protein/hr. Enzyme activity of goats and cattle was measured under the same conditions and found to be 5.5 and 4, respectively. Mora et al. [137] also measured BC cleavage activity in both goats and cattle and found goats to have

higher enzyme activity than cattle. BC is no doubt a critical source of VA in all three herbivorous species, but sheep and goats may convert BC to VA at a higher rate than cattle.

6.1.3. Other Artiodactyla. Artiodactyla comprise a large group of animals, all even-toed ungulates. Artiodactyla include cows, sheep, goats, and pigs, which are all discussed in separate sections. Serum carotenoids have been measured in several other Artiodactyla species, and they bear mentioning because carotenoids are remarkably absent from their serum, despite their herbivorous dietary habits (i.e., high carotenoid, devoid of preformed VA). Slifka et al. [146] found no detectable BC in the serum of pygmy hippos, Bactrian camels, Pere David's deer, okapi, giraffe, sitatunga, klipspringer, eland, kudu, Congo buffalo, and Siberian ibex. Moore [147] also noted that white-tailed deer have colorless adipose tissue. Thus, among the Artiodactyla, cows and their close relatives seem to be distinguished by their accumulation of BC in plasma and tissues. However, the herbivorous dietary habits of most Artiodactyla must require that they successfully convert BC to VA in order to meet their VA requirements.

6.1.4. Rabbits. Rabbits have colorless fat so they are considered nonaccumulators of BC [147]. Several studies have measured BC cleavage activity in the intestinal mucosa of rabbits [130, 131]. In the comparative study of enzyme activity by Lakshmanan et al. [130], the specific activity of BC cleavage in rabbits was similar to that in the chicken, a tortoise species, and a monkey species, but less than that measured in the guinea pig or a freshwater fish. Consistent with their herbivorous diet, BC is likely the sole source of VA for rabbits.

6.1.5. Horses. Horses have yellow fat so they seem to accumulate BC in adipose [147]. Kienzle et al. showed a significant increase in serum BC when horses were supplemented with either a water-soluble beadlet preparation or grass meal containing BC, with no observed difference in response between the two BC preparations [98]. These authors also observed that there was no difference in serum response when supplements were given with diets with 2.5 or 6.6% fat, contrary to results in rats [47], ferrets [97], and humans [64, 94, 95]. Fonnesbeck and Symons [149] showed that plasma BC was correlated to dietary BC intake from several forages with varying concentrations of BC. In addition, horses fed alfalfa, which had the highest BC concentration of the forages tested, had higher plasma retinol compared to those consuming the other diets. Relatively high concentrations of plasma BC have also been measured in free-ranging Przewalski's horses [150].

Greiwe-Crandell et al. [151] supplemented VA-depleted horses with 215 mg of BC in synthetic beadlet form daily for 4 weeks and found that VA status, measured by serum retinol and a relative dose-response test, was not improved as compared to controls and horses supplemented with 22 mg retinyl palmitate. The retinyl palmitate dose was twice that recommended by the NRC. The inefficacy of the BC supplement in this study may indicate that horses require more than 10 μg of BC to form 1 RE, the current NRC estimate for horses. Regardless of their conversion efficiency, the herbivorous dietary pattern of horses indicates that they must utilize carotenoids to meet their VA requirement, as their natural diet contains no preformed VA.

6.2. Omnivorous Mammals

6.2.1. Rodents. Some the first work on BC metabolism was done in rats. Studies dosing rats with ^{14}C-BC demonstrated efficient conversion of BC to retinol in rats but showed that rats do not absorb intact BC into blood [50, 152, 153]. On the other hand, Kon et al. [145] injected rats intravenously with BC preparations and were able to measure BC in both plasma and liver. There was also evidence that the rats converted injected BC to VA, presumably extraintestinally. Thus, though rats do not normally absorb intact BC, they apparently have the ability to accumulate and utilize it for VA should it enter the bloodstream. Rats dosed with very large quantities of BC (175 mg 2x/week for 4 weeks) did accumulate it in liver and lung tissues, but BC was not detectable in plasma [100]. BC cleavage activity has also been demonstrated in intestinal homogenates from rats [120, 154] and guinea pigs [131, 155].

The conversion ratio in rats has been estimated to be 2 μg BC = 1 RE [135, 156], though Marusich and Bauernfeind measured a conversion ratio of 6–10 μg BC (water-soluble beadlet form) = 1 RE based on hepatic VA stores using a functional bioassay model; the higher end of the conversion ratio was for higher levels of BC supplementation [157]. Brubacher and Weiser noted that the conversion ratio may only be as low as 2 μg BC = 1 RE when rats are fed just enough BC to meet their requirement (<0.3 μg/kg BW) [135].

Unlike rats, Mongolian gerbils absorb dietary BC and accumulate it in tissues [134, 158, 159]. Lee et al. [158] estimated the VA equivalency of BC to maintain tissue VA in gerbils at 6–13 μg BC = 1 RE.

6.2.2. Humans. Humans are among the most studied animals with regard to BC metabolism, likely because rats were quickly proven to be unsuitable models because of their inability to absorb BC. Other animal models have been proposed [159], but the use of isotope-labeled BC has enabled detailed noninvasive metabolic studies in humans. These have recently been reviewed [74, 160, 161], so this review will only briefly summarize the data on BC metabolism in humans. BC is readily absorbed in the blood of humans [51, 162] and is found in circulation of humans consuming a wide range of omnivorous diets [163] with correlations between intake and circulating concentrations [164]. Humans also have yellow fat [147], so it is assumed that they accumulate BC in adipose. BC can fulfill the VA requirement for humans, though early estimates of conversion efficiency were based on a depletion-repletion model so it may have overestimated conversion efficiency [165]. According to the Institute of Medicine's current recommendations, the conversion ratio is estimated at 2 μg pure BC in oil = 1 RE and 12 μg BC in food matrix = 1 RE [166]. The study of van Lieshout et al. [79] suggests that estimated conversion efficiency of BC in oil is somewhat inadequate; these authors found 2.4 μg BC in oil = 1 RE in Indonesian children. Some studies have found

even higher conversion ratios; for example, ratios of 9.1 μg BC in oil = 1 RE in adults [78] and 15 and 21 μg BC in carrots and spinach = 1 RE, respectively [77]. Thus, the conversion ratio for BC in humans continues to be the subject of much debate and of great interest because of its role in meeting the VA requirement of people in developing countries, where VA deficiency continues to be a problem.

6.2.3. Nonhuman Primates. Similar to humans, the nonhuman primates that have been studied also seem to absorb BC intact into blood. This has been demonstrated in rhesus macaques [152] and squirrel monkeys [163, 167]. The studies on squirrel monkeys demonstrated selective absorption of carotenoids, with zeaxanthin preferentially absorbed compared with BC. García et al. [168] measured carotenoid concentrations in great ape diets and plasma and noted selective accumulation of lutein in plasma compared with higher dietary concentrations of BC. Krinsky et al. [152] dosed rhesus macaques with ^{14}C-BC and noted a strong response of radioactive retinol in both serum and liver. Intact ^{14}C-BC was also found in serum, liver, colon, heart, kidney, lungs, ovary, pancreas, intestine, spleen, and stomach. BC cleavage activity has also been measured in intestinal homogenates of primates [118, 130].

6.2.4. Pigs. Pigs have white adipose tissue [147] and are assumed to be low absorbers and accumulators of BC. Schweigert et al. [169] was able to demonstrate low concentrations of BC in the plasma, liver, adrenals, and corpora lutea of pigs supplemented with high concentrations of BC (100 mg BC/kg diet for 14 weeks). From hepatic VA stores, the authors of this study estimated a conversion ratio of 40 μg BC = 1 RE, though this low conversion ratio was likely inflated by the high dose of BC used. In pigs dosed with 30 mg ^{14}C-β-carotene, Schweigert et al. reported that labeled BC was detected in both the colon and lung, though not in the plasma, liver, kidney, or intestine [170]. These authors also demonstrated conversion of ^{14}C-BC to labeled retinol, though only 4% of the labeled BC dose was recovered as retinol. BC cleavage activity has also been detected in intestinal homogenates isolated from pigs [155, 171].

Several earlier studies examined the efficiency of utilization of BC for VA in pigs using a functional bioassay model. These found that the VA equivalency of BC from corn ranged in 13–27 μg BC = 1 RE [124, 125, 172], a somewhat more efficient estimate than that from Schweigert et al. and likely more accurate since they used more physiological supplementation rates of 1–10 mg BC/kg diet. Regardless, pigs seem to be relatively inefficient at both absorbing BC and converting it to VA compared with other omnivores studied.

6.3. Carnivorous Mammals

6.3.1. Canids. Dogs have been found to have no to moderate concentrations of BC in circulation when consuming unsupplemented omnivorous diets that likely contain some carotenoids [173–176]. Dogs have colorless fat [147], so it appears that they do not accumulate BC in their adipose. Chew et al. [175] studied the plasma response to oral BC supplementation in beagle dogs. Unsupplemented dogs consuming a commercial canine maintenance diet had no measurable BC in plasma. When dogs were given single oral doses of 50, 100, and 200 mg of BC in water-soluble beadlet preparation, plasma BC response was significant and concentration increased in a dose-dependent manner. Plasma BC also accumulated in plasma, lymphocytes, and neutrophils when dogs were dosed for up to 30 days with BC [175]. However, the plasma response to the BC dose was much lower in dogs compared to similar single dose studies in cats [177], ferrets [178], and calves [142, 143].

Crissey et al. [176] analyzed serum from 6 captive wild canid species (African wild dog, arctic fox, gray wolf, maned wolf, Mexican wolf, and red wolf) and found no detectable carotenoids in any samples. Their analysis included BC, lutein, β-cryptoxanthin, lycopene, and α-carotene. Though the diets of these animals were not analyzed for carotenoids, the dietary composition of the maned wolves in this study included 21% fruit and 21% vegetables so they would have moderate concentrations of carotenoids. Slifka et al. [146] also studied grey wolves and cape hunting dogs consuming zoo diets with moderate to high carotenoid concentrations and found no detectable carotenoids in serum.

In 1934, Turner [179] demonstrated that dogs can utilize BC to meet their VA requirement. Dogs were fed a basal diet of meat and boiled rice and were either not supplemented or supplemented with 150 g fresh carrots with and without an additional 10 mL cod liver oil. The two unsupplemented dogs died after 15 and 49 days. The supplemented dogs from both treatments apparently remained healthy throughout the experiment. After 63–224 days on the supplemented diets, there was no difference in hepatic or renal VA concentration between the dogs receiving the cod liver oil or not. Several years later, Bradfield and Smith [180] confirmed this finding, showing no difference in hepatic VA between dogs supplemented with 200 IU of cod liver oil, BC in oil, or BC from carrots, though it is unclear how the authors defined IU units of BC. This definition would have required some assumption about the conversion efficiency of BC to VA. Thus, quantitative conversion efficiency has not been estimated in dogs, though it is clear that dogs both absorb BC and can utilize it as their sole source of VA. It has also been shown that foxes can utilize BC as a source of VA [181]. Dogs seem to be more efficient at converting BC to VA than either ferrets or felids, consistent with their more omnivorous diet compared to the strict carnivores.

6.3.2. Ferrets. Ferrets have been proposed as an animal model for human BC metabolism and have been studied in some detail. Ferrets absorb dietary BC into the blood and accumulate it, though at very different concentrations, in several tissues, including liver, adipose, lung, spleen, kidney, muscle, and skin [82, 92, 178, 182]. Lederman et al. [183] determined in a functional bioassay (based on hepatic VA) that ferrets do convert BC to VA, but their conversion is inefficient, estimated at 15 μg BC < 1 RE. On the other hand, BCO enzyme activity has been successfully measured in several ferret tissues, including intestine, liver, lung, and adipose tissue [118, 184].

6.3.3. Felids. The absolute requirement for preformed VA and inefficacy of BC was one of the first metabolic idiosyncrasies of domestic cats to be discovered. Ahmad [47] was unable to detect the conversion of BC to VA in liver perfusion or oral dosing experiments with VA-deficient cats. In the experiments of Gershoff et al. [185], most VA-deficient cats did not respond to IV or oral doses of BC, though 1 of the 10 cats tested with an oral dose did show a slight increase in serum VA and BC. Lakshmanan et al. [130] tested intestinal homogenate from cats (perhaps just one, number not stated) and found no measurable BC cleavage activity. Many of these early studies were conducted before the advent of HPLC methods for analysis of retinoids and carotenoids so they suffered from a lack of sensitivity and specificity of analytical methods. Data from our laboratory indicates that cats do have the ability to convert BC to VA, though the conversion efficiency is very low [186]. Regardless, it is clear from the earlier depletion studies that BC cannot replace VA in the diets of cats.

Some early studies were unable to detect absorption of orally administered BC into blood or the liver of domestic cats [47, 185]. However, more recent studies and our own experiments have found absorption of BC supplements into plasma to be quite substantial in cats, though these studies use relatively high doses [177, 186, 187]. Domestic cats consuming commercial diets seem to have very low to no circulating BC [18, 187]. However, Crissey et al. [27] found relatively high concentrations of serum BC in 11 captive wild felid species kept in zoos. Dietary carotenoids were not quantified in this study, but regardless, wild felids seem to readily accumulate BC in blood.

6.4. Birds. Xanthophylls are the most important class of carotenoids for pigmentation in birds, and these are absorbed preferentially over the carotenes and give the bright colors to beak, feathers, legs, and yolk. For example, Capper et al. [188] found that the liver oil of hens on a normal diet (with "greenstuffs") was a deep yellow color, while the liver oil of hens fed a purified diet supplemented with BC was only pale yellow in color. Chickens are generally thought to be poor absorbers and accumulators of carotenes [189]. Chicken yolk is very low in BC, instead having lutein and zeaxanthin as the dominant carotenoids, even when hens are supplemented with dietary BC [190]. A recent study determined that yellow skin color in chickens, the most abundant phenotype, is caused by a regulatory mechanism that inhibits the expression of BCO-2 in the skin. BCO-2 asymmetrically cleaves BC and other carotenoids to colorless apocarotenals and retinal, so its inhibition leaves the colorful carotenoids as skin pigments [191].

While chickens are the most studied of the birds, there is likely great diversity in carotenoid metabolism in other birds. For example, unlike chicken egg yolks, the egg yolks from several wild birds (gulls, coots, and moorhen) were found to contain high concentrations of BC, about 25–30% of total carotenoids [192]. Gulls were also found to have high concentrations of BC in the liver [193]. On the other hand, Slifka et al. [146] measured serum carotenoids in a variety of bird species kept at a zoo and found no detectable serum BC in any of the species studied (included greater flamingo, American flamingo, Mandarin duck, grey gull, scarlet ibis, Inca tern, wood duck, hybrid teal, Hadada ibis, whistling duck, brown pelican, sacred ibis, Humboldt penguin, and Brazilian teal). Canthaxanthin was present in high concentrations in the serum of the flamingos, while lutein + zeaxanthin (coeluting on HPLC) were high in the serum of the remaining species.

Birds do utilize BC as an important source of VA. Several functional bioassays in chickens have demonstrated that BC can replace preformed VA in the diets of chickens [157, 188, 194], and the NRC settled on a conversion ratio of 2 μg BC = 1 RE [195], implying that chickens, along with rats, are among the most efficient animals at converting BC to VA; BC cleavage activity has also been measured *ex vivo* in the intestinal mucosa of chickens [196, 197]. The utilization of BC to meet the VA requirement has also been shown in cockatiels [198], canaries [199], bobwhite quail, and ducks [200]. The data on cockatiels indicates that they are somewhat less efficient than chickens, while canaries appear to be equally efficient as chickens at utilizing BC for VA.

The utilization of BC has not been investigated in carnivorous birds. However one study did report retinol concentrations in birds were higher compared to mammals. Retinyl esters (retinol palmitate and oleate) represented 10–50% of VA in birds of the order Ciconiiformes and Falconiformes [136]. The occurrence of blood VA esters in these two orders of birds and carnivorous mammals may represent an adaptation to a carnivorous diet with a high supply of VA [136].

6.5. Fish. VA exists in two major forms in fish: as retinol, also known as A_1, and as 3,4-didehydroretinol, also known as A_2. A_2 differs from retinol in that it has an additional double bond between carbons 3 and 4 in the β-ionone ring. A_2 is part of the visual pigment porphyropsin and accompanies the retinal protein rhodopsin in the eyes of teleost fish as well as in reptiles, amphibians, and crustaceans [201]. In general, A_2 is the predominant retinoid and visual pigment in freshwater fish, while retinol dominates in marine fish [202], though there appear to be some exceptions to this rule [201]. Retinol can be converted into A_2 but not vice versa [203]. The respective roles and relative potencies of retinol and A_2 for biological functions in fish are not well understood. However, A_2 appears less potent than retinol when it comes to meeting the VA requirement in mammals. The biological activity of A_2 was measured in rats with a growth assay and was found to be 40% of pure retinol [204]. Recently, Kongsbak et al. [205] reported on the effects of a dietary intervention in Bangladeshi children using a small native freshwater fish, *Amblypharyngodon mola*, found to contain predominantly A_2, and concluded that this was a poor source of VA for children.

Fish generally absorb and accumulate dietary xanthophylls more efficiently than carotenes [201]. Several studies have demonstrated efficient conversion of BC and other carotenoids to VA. These studies are generally qualitative in nature and do not attempt to estimate conversion efficiency. Investigators reported difficulty with administering a quantitative dose of carotenoids to fish; much of the dose ended

up in the water [206, 207]. Barua and Goswami administered BC and lutein to depleted *Saccobranchus fossilis*, an Indian catfish. About four hours after the dose, significant quantities of retinoic acid were recovered from the intestines of the fish in almost all cases, with retinol appearing in just a few cases [206]. On the other hand, administration of lutein resulted in almost exclusive recovery of A_2 [206, 207]. Goswami [203] showed in several fish species that β-cryptoxanthin was converted to retinol in fish that naturally accumulate more retinol and to A_2 in fish that naturally contain more A_2. Gross and Budowski [208] reported that guppies and platies form VA (predominantly retinol with some A_2) from BC, isozeaxanthin, canthaxanthin, and astaxanthin. They found that lutein administration resulted in only a very small increase in A_2 in these species. Schiedt et al. [209] confirmed that astaxanthin, canthaxanthin, and zeaxanthin were all VA precursors in VA-deficient rainbow trout but that pro-VA activity was significantly decreased in VA-replete fish. The authors did not administer BC in this study, but they suggested that it must be an intermediate in the pathway from xanthophylls to VA [201, 209].

In summary, the literature demonstrates that several carotenoids are important precursors to both retinol and vitamin A_2 in fish. Which form of VA is produced from pro-VA carotenoids seems to be species-specific. Like in mammals and birds, pro-VA conversion efficiency appears to be regulated by VA status. However, fish may be distinct in having reductive pathways from xanthophylls to BC and lutein to retinoids.

7. Conclusion

The metabolism of BC has proven to be an endless field of study with metabolic variations being equal to the number of animal species studied. Many factors impact the bioavailability of BC and its conversion to VA, and estimation of the value of BC in mixed diets and under various conditions is difficult. Nevertheless, some patterns emerge (Tables 2 and 3). Several omnivores (rat and chicken) appear to be the most efficient converters of BC to VA. Perhaps this is due to the diversity of potential diets in these omnivores, requiring them to be the most flexible and able to readily utilize BC or preformed VA when available. Herbivores also have high conversion efficiency, though they likely do not require maximal BC conversion efficiency since their diets generally have high concentrations of pro-VA carotenoids. It is not surprising that carnivores are less efficient at converting BC to VA, as they evolved consuming diets with abundant preformed VA. It would be useful to conduct quantitative studies of BC conversion in more animal species to better define the spectrum of conversion efficiencies and determine if this general pattern (omnivore > herbivore > carnivore) remains. In particular, only a few species of carnivores have been studied. Domestic cats are often assumed to be a good model species for all carnivores, but the example of dogs and ferrets demonstrates that there is likely a spectrum of BC conversion efficiencies even within carnivores. In addition, there is no data on BC conversion on other faunivores, such as those that eat insects or other invertebrates, foods that have very different concentrations of preformed VA and carotenoids than vertebrate tissue.

The variability between species in the ability to absorb BC intact and accumulate it in tissues is not understood. Early observations that some species had yellow adipose and others had white adipose led investigators to speculate that there was a relationship between conversion efficiency and tissue accumulation. It was thought that white adipose species are perhaps so efficient at intestinal cleavage of BC that very little escapes into circulation. However, the story seems to be more complex. Some white adipose species are relatively poor convertors but do absorb BC into circulation (e.g., cats and ferrets) and among the most efficient convertors are species with both white (e.g., sheep, goats, rats, chickens) and yellow adipose (e.g., cows and humans). Why are there species differences? Little research has been conducted in this area. It is often said that BC absorption occurs via a passive mechanism, but the selective absorption of carotenoids in many species indicates this may not be the case. Some investigators have proposed that there are specific carrier proteins in the gut and other tissues that may regulate the absorption and accumulation of carotenoids [210–222]. Accumulation of carotenoids in the tissues of some species but not others may be related the lack of extraintestinal BCO activity, as has been proposed in cows [145]. In chickens, the inhibition of the expression of BCO-2 in the skin allows for the accumulation of carotenoids in that tissue [191]. Thus, species- and tissue-specific accumulation may be related to the expression and inhibition of BCO enzymes. Perhaps examining BCO activity beyond the gut would improve our understanding of species differences with regard to BC accumulation. Absorption and accumulation of intact BC in animal species determines if BC may have important functions (i.e., antioxidant, immune response, and gap junction communication) beyond its role as a VA precursor.

Competing Interests

There are no competing interests associated with this work.

Authors' Contributions

Alice S. Green was the primary author of the manuscript, and Andrea J. Fascetti revised the manuscript.

Acknowledgments

Alice S. Green was supported by a National Science Foundation Graduate Research Fellowship.

References

[1] P. Chambon, "A decade of molecular biology of retinoic acid receptors," *The FASEB Journal*, vol. 10, no. 9, pp. 940–954, 1996.

[2] J. Saari, "Retinoid in photosensitive systems," in *The Retinoids: Biology, Chemistry, and Medicine*, M. Sporn, A. Roberts, and D. Goodman, Eds., pp. 351–385, Raven Press, New York, NY, USA, 1994.

[3] D. M. Wellik, D. H. Norback, and H. F. DeLuca, "Retinol is specifically required during midgestation for neonatal survival," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 272, part 1, pp. E25–E29, 1997.

[4] A. Ross and E. Harrison, "Vitamin A: nutritional aspects of retinoids and carotenoids," in *Handbook of Vitamins*, J. Zempleni, R. B. Rucker, J. W. Suttie, and D. B. McCormick, Eds., pp. 1–39, CRC Press, Boca Raton, Fla, USA, 4th edition, 2007.

[5] F. J. Schweigert, O. A. Ryder, W. A. Rambeck, and H. Zucker, "The majority of vitamin A is transported as retinyl esters in the blood of most carnivores," *Comparative Biochemistry and Physiology—Part A: Physiology*, vol. 95, no. 4, pp. 573–578, 1990.

[6] E. H. Harrison, "Mechanisms of digestion and absorption of dietary vitamin A," *Annual Review of Nutrition*, vol. 25, pp. 87–103, 2005.

[7] E. H. Harrison and M. M. Hussain, "Mechanisms involved in the intestinal digestion and absorption of dietary vitamin A," *Journal of Nutrition*, vol. 131, no. 5, pp. 1405–1408, 2001.

[8] A. C. Ross and R. Zolfaghari, "Regulation of hepatic retinol metabolism: perspectives from studies on vitamin A status," *Journal of Nutrition*, vol. 134, no. 1, pp. 269S–275S, 2004.

[9] L. R. McDowell, *Vitamins in Animal and Human Nutrition*, Iowas State University Press, Ames, Iowa, USA, 2000.

[10] NRC, *Vitamin Tolerance of Animals*, The National Academies Press, Washington, DC, USA, 1987.

[11] K. L. Penniston and S. A. Tanumihardjo, "The acute and chronic toxic effects of vitamin A," *The American Journal of Clinical Nutrition*, vol. 83, no. 2, pp. 191–201, 2006.

[12] S. Q. Cohlan, "Congenital anomalies in the rat produced by excessive intake of vitamin A during pregnancy," *Pediatrics*, vol. 13, no. 6, pp. 556–567, 1954.

[13] J. G. Wilson, C. B. Roth, and J. Warkany, "An analysis of the syndrome of malformations induced by maternal vitamin a deficiency. Effects of restoration of vitamin a at various times during gestation," *American Journal of Anatomy*, vol. 92, no. 2, pp. 189–217, 1953.

[14] G. E. Mitchell Jr., C. O. Little, and B. W. Hayes, "Pre-intestinal destruction of vitamin A by ruminants fed nitrate," *Journal of Animal Science*, vol. 26, no. 4, pp. 827–829, 1967.

[15] E. A. Koutsos, L. A. Tell, L. W. Woods, and K. C. Klasing, "Adult cockatiels (*Nymphicus hollandicus*) at maintenance are more sensitive to diets containing excess vitamin A than to vitamin A-deficient diets," *The Journal of Nutrition*, vol. 133, no. 6, pp. 1898–1902, 2003.

[16] T. L. Freytag, *Vitamin A Metabolism and Toxicity in the Domestic Cat*, University of California, Davis, Davis, Calif, USA, 2001.

[17] T. L. Freytag, S. M. Liu, Q. R. Rogers, and J. G. Morris, "Teratogenic effects of chronic ingestion of high levels of vitamin A in cats," *Journal of Animal Physiology and Animal Nutrition*, vol. 87, no. 1-2, pp. 42–51, 2003.

[18] J. Raila, U. Mathews, and F. J. Schweigert, "Plasma transport and tissue distribution of β-carotene, vitamin A and retinol-binding protein in domestic cats," *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology*, vol. 130, no. 4, pp. 849–856, 2001.

[19] F. J. Schweigert, E. Thomann, and H. Zucker, "Vitamin A in the urine of carnivores," *International Journal for Vitamin and Nutrition Research*, vol. 61, no. 2, pp. 110–113, 1991.

[20] N. R. Lawrie, T. Moore, and K. R. Rajagopal, "The excretion of vitamin A in urine," *Biochemical Journal*, vol. 35, no. 7, pp. 825–836, 1941.

[21] C. B. Stephensen, J. O. Alvarez, J. Kohatsu, R. Hardmeier, J. I. Kennedy Jr., and R. B. Gammon Jr., "Vitamin A is excreted in the urine during acute infection," *The American Journal of Clinical Nutrition*, vol. 60, no. 3, pp. 388–392, 1994.

[22] K. Rodahl and A. W. Davies, "Vitamin A in seals," *The Biochemical Journal*, vol. 45, no. 4, pp. 408–412, 1949.

[23] F. A. Leighton, M. Cattet, R. Norstrom, and S. Trudeau, "A cellular basis for high levels of vitamin A in livers of polar bears (*Ursus maritimus*): the Ito cell," *Canadian Journal of Zoology*, vol. 66, no. 2, pp. 480–482, 1988.

[24] A. E. Anderson, D. E. Medin, and D. C. Bowden, "Carotene and vitamin A in the liver and blood serum of a rocky mountain mule deer, *Odocoileus hemionus hemionus* population," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 41, no. 4, pp. 745–758, 1972.

[25] W. G. Youalt, D. E. Ullrey, and W. T. Magee, "Vitamin A concentration in livers of white-tailed deer," *The Journal of Wildlife Management*, vol. 40, no. 1, pp. 172–173, 1976.

[26] C. R. Hungerford, "Vitamin A and productivity in Gambel's quail," *The Journal of Wildlife Management*, vol. 28, no. 1, pp. 141–147, 1964.

[27] S. D. Crissey, K. D. Ange, K. L. Jacobsen et al., "Serum concentrations of lipids, vitamin D metabolites, retinol, retinyl esters, tocopherols and selected carotenoids in twelve captive wild felid species at four zoos," *Journal of Nutrition*, vol. 133, no. 1, pp. 160–166, 2003.

[28] F. J. Schweigert and V. Bok, "Vitamin A in blood plasma and urine of dogs is affected by the dietary level of vitamin A," *International Journal for Vitamin and Nutrition Research*, vol. 70, no. 3, pp. 84–91, 2000.

[29] P. J. Morris, C. Salt, J. Raila et al., "Safety evaluation of vitamin A in growing dogs," *British Journal of Nutrition*, vol. 108, no. 10, pp. 1800–1809, 2012.

[30] J. Raila, C. Gomez, and F. J. Schweigert, "The ferret as a model for vitamin A metabolism in carnivores," *Journal of Nutrition*, vol. 132, no. 6, supplement 2, pp. 1787S–1789S, 2002.

[31] J. Raila, I. Buchholz, H. Aupperle, G. Raila, H.-A. Schoon, and F. J. Schweigert, "The distribution of vitamin A and retinol-binding protein in the blood plasma, urine, liver and kidneys of carnivores," *Veterinary Research*, vol. 31, no. 6, pp. 541–551, 2000.

[32] G. Britton, "Structure and properties of carotenoids in relation to function," *The FASEB Journal*, vol. 9, no. 15, pp. 1551–1558, 1995.

[33] G. Britton, S. Liaaen-Jensen, and H. Pfander, "Carotenoids today and challenges for the future," in *Carotenoids*, G. Britton, S. Liaaen-Jensen, and H. Pfander, Eds., pp. 13–26, Birkhäuser, Basel, Switzerland, 1995.

[34] D. M. Deming and J. W. Erdman Jr., "Mammalian carotenoid absorption and metabolism," *Pure and Applied Chemistry*, vol. 71, no. 12, pp. 2213–2223, 1999.

[35] P. Borel, P. Grolier, M. Armand et al., "Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets," *Journal of Lipid Research*, vol. 37, no. 2, pp. 250–261, 1996.

[36] D. Kostic, W. S. White, and J. A. Olson, "Intestinal absorption, serum clearance, and interactions between lutein and β-carotene when administered to human adults in separate or combined oral doses," *American Journal of Clinical Nutrition*, vol. 62, no. 3, pp. 604–610, 1995.

[37] K. H. Van Het Hof, I. A. Brouwer, C. E. West et al., "Bioavailability of lutein from vegetables is 5 times higher than that of β-carotene," *American Journal of Clinical Nutrition*, vol. 70, no. 2, pp. 261–268, 1999.

[38] R. S. Parker, "Absorption, metabolism, and transport of carotenoids," *The FASEB Journal*, vol. 10, no. 5, pp. 542–551, 1996.

[39] B. A. Clevidence and J. G. Bieri, "Association of carotenoids with human plasma lipoproteins," *Methods in Enzymology*, vol. 214, pp. 33–46, 1993.

[40] J. D. Ribaya-Mercado, J. Lopez-Miranda, J. M. Ordovas, M. C. Blanco, J. G. Fox, and R. M. Russell, "Distribution of β-carotene and vitamin A in lipoprotein fractions of ferret serum: effect of β-carotene supplementation," *Annals of the New York Academy of Sciences*, vol. 691, pp. 232–237, 1993.

[41] J. C. Bauernfeind, "Carotenoid vitamin A precursors and analogs in foods and feeds," *Journal of Agricultural and Food Chemistry*, vol. 20, no. 3, pp. 456–473, 1972.

[42] H. Wackenroder, "Ueber das Oleum radicis Dauci aetherum, das Carotin, den Carotenzucker und den officinellen succus Dauci; so wie auch über das Mannit, welches in dem Möhrensafte durch eine besondere Art der Gährung gebildet wird," *Geigers Magazin der Pharmazie*, vol. 33, pp. 144–172, 1831.

[43] H. Steenbock, "White corn vs. yellow corn and a probable relation between the fat-soluble vitamine and yellow plant pigments," *Science*, vol. 50, no. 1293, pp. 352–353, 1919.

[44] T. B. Osborne and L. B. Mendel, "Further observations of the influence of natural fats upon growth," *The Journal of Biological Chemistry*, vol. 20, no. 3, pp. 379–389, 1915.

[45] B. von Euler, H. von Euler, and P. Karrer, "Zur Biochemie der Carotinoide," *Helvetica Chimica Acta*, vol. 12, no. 1, pp. 278–285, 1929.

[46] T. Moore, "Vitamin A and carotene: the absence of the liver oil vitamin A from carotene. VI. The conversion of carotene to vitamin A in vivo," *Biochemical Journal*, vol. 24, no. 3, pp. 692–702, 1930.

[47] B. Ahmad, "The fate of carotene after absorption in the animal organism," *Biochemical Journal*, vol. 25, no. 4, pp. 1195–1204, 1931.

[48] K. H. Wagner, *Vitamin A und β-Carotin des Finn-, Blau- und Spermwals*, Johann Ambrosius Barth, Leipzig, Germany, 1939.

[49] S. K. Kon and S. Y. Thompson, "Site of conversion of carotene to vitamin A," *The British Journal of Nutrition*, vol. 5, no. 1, pp. 114–119, 1951.

[50] H. S. Huang and D. S. Goodman, "Vitamin A and Carotenoids. I. Intestinal absorption and metabolism of ^{14}C-labeled vitamin A alcohol and beta-carotene in the rat," *The Journal of Biological Chemistry*, vol. 240, no. 7, pp. 2839–2844, 1965.

[51] R. Blomstrand and B. Werner, "Studies on the intestinal absorption of radioactive beta-carotene and vitamin A in man. Conversion of beta-carotene into vitamin A," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 19, no. 4, pp. 339–345, 1967.

[52] US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28, May 2016, https://ndb.nal.usda.gov/ndb/.

[53] B. Demmig-Adams and W. W. Adams III, "Antioxidants in photosynthesis and human nutrition," *Science*, vol. 298, no. 5601, pp. 2149–2153, 2002.

[54] J. S. Bertram and H. Bortkiewicz, "Dietary carotenoids inhibit neoplastic transformation and modulate gene expression in mouse and human cells," *The American Journal of Clinical Nutrition*, vol. 62, no. 6, supplement, pp. 1327S–1336S, 1995.

[55] B. P. Chew and J. S. Park, "Carotenoid action on the immune response," *Journal of Nutrition*, vol. 134, no. 1, pp. 257S–261S, 2004.

[56] S. Voutilainen, T. Nurmi, J. Mursu, and T. H. Rissanen, "Carotenoids and cardiovascular health," *The American Journal of Clinical Nutrition*, vol. 83, no. 6, pp. 1265–1271, 2006.

[57] J. T. Landrum, R. A. Bone, and M. D. Kilburn, "The macular pigment: a possible role in protection from age-related macular degeneration," *Advances in Pharmacology*, vol. 38, pp. 537–556, 1997.

[58] M. M. Fernandez and N. A. Afshari, "Nutrition and the prevention of cataracts," *Current Opinion in Ophthalmology*, vol. 19, no. 1, pp. 66–70, 2008.

[59] C. Liu and R. M. Russell, "Nutrition and gastric cancer risk: an update," *Nutrition Reviews*, vol. 66, no. 5, pp. 237–249, 2008.

[60] S. Seren, R. Lieberman, U. D. Bayraktar et al., "Lycopene in cancer prevention and treatment," *American Journal of Therapeutics*, vol. 15, no. 1, pp. 66–81, 2008.

[61] A. H. Rakes, M. P. Owens, J. H. Britt, and L. W. Whitlow, "Effects of adding beta-carotene to rations of lactating cows consuming different forages," *Journal of Dairy Science*, vol. 68, no. 7, pp. 1732–1737, 1985.

[62] M. T. Coffey and J. H. Britt, "Enhancement of sow reproductive performance by beta-carotene or vitamin A," *Journal of Animal Science*, vol. 71, no. 5, pp. 1198–1202, 1993.

[63] R. A. Woutersen, A. P. M. Wolterbeek, M. J. Appel, H. van den Berg, R. A. Goldbohm, and V. J. Feron, "Safety evaluation of synthetic β-carotene," *Critical Reviews in Toxicology*, vol. 29, no. 6, pp. 515–542, 1999.

[64] N. V. Dimitrov, C. Meyer, D. E. Ullrey et al., "Bioavailability of β-carotene in humans," *The American Journal of Clinical Nutrition*, vol. 48, no. 2, pp. 298–304, 1988.

[65] D. E. Breithaupt and A. Bamedi, "Carotenoid esters in vegetables and fruits: a screening with emphasis onβ-cryptoxanthin esters," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 4, pp. 2064–2070, 2001.

[66] C. Dhuique-Mayer, P. Borel, E. Reboul, B. Caporiccio, P. Besancon, and M.-J. Amiot, "β-Cryptoxanthin from *Citrus* juices: assessment of bioaccessibility using an *in vitro* digestion/Caco-2 cell culture model," *British Journal of Nutrition*, vol. 97, no. 5, pp. 883–890, 2007.

[67] P. E. Bowen, S. M. Herbst-Espinosa, E. A. Hussain, and M. Stacewicz-Sapuntzakis, "Esterification does not impair lutein bioavailability in humans," *Journal of Nutrition*, vol. 132, no. 12, pp. 3668–3673, 2002.

[68] K.-J. Yeum and R. M. Russell, "Carotenoid bioavailability and bioconversion," *Annual Review of Nutrition*, vol. 22, pp. 483–504, 2002.

[69] A. J. Edwards, C.-S. You, J. E. Swanson, and R. S. Parker, "A novel extrinsic reference method for assessing the vitamin A value of plant foods," *American Journal of Clinical Nutrition*, vol. 74, no. 3, pp. 348–355, 2001.

[70] T. van Vliet, W. H. P. Schreurs, and H. van den Berg, "Intestinal β-carotene absorption and cleavage in men: response of β-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of β-carotene," *The American Journal of Clinical Nutrition*, vol. 62, no. 1, pp. 110–116, 1995.

[71] J. R. Ashes, R. W. Burley, J. B. Davenport, and G. S. Sidhu, "Effects of dietary supplements of protected lipids on the concentration and transport of beta-carotene and cholesterol in bovine blood and milk: unusual chromatographic behaviour of the high-density lipoprotein with high levels of beta-carotene," *Journal of Dairy Research*, vol. 49, no. 1, pp. 39–49, 1982.

[72] T. W. Larsen, A. Yang, and R. K. Tume, "The in vitro destruction of rumen fluid carotenoids by plant lipoxygenases," *Biochemistry and Molecular Biology International*, vol. 30, no. 2, pp. 197–207, 1993.

[73] P. Grolier, P. Borel, C. Duszka et al., "The bioavailability of α- and β-carotene is affected by gut microflora in the rat," *British Journal of Nutrition*, vol. 80, no. 2, pp. 199–204, 1998.

[74] M. van Lieshout, C. E. West, and R. B. van Breemen, "Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly β-carotene, in humans: a review," *The American Journal of Clinical Nutrition*, vol. 77, no. 1, pp. 12–28, 2003.

[75] C. C. Ho, F. F. de Moura, S.-H. Kim, and A. J. Clifford, "Excentral cleavage of beta-carotene in vivo in a healthy man," *The American Journal of Clinical Nutrition*, vol. 85, no. 3, pp. 770–777, 2007.

[76] S. L. Lemke, S. R. Dueker, J. R. Follett et al., "Absorption and retinol equivalence of β-carotene in humans is influenced by dietary vitamin A intake," *Journal of Lipid Research*, vol. 44, no. 8, pp. 1591–1600, 2003.

[77] G. Tang, J. Qin, G. G. Dolnikowski, R. M. Russell, and M. A. Grusak, "Spinach or carrots can supply significant amounts of vitamin A as assessed by feeding with intrinsically deuterated vegetables," *The American Journal of Clinical Nutrition*, vol. 82, no. 4, pp. 821–828, 2005.

[78] G. Tang, J. Qin, G. G. Dolnikowski, and R. M. Russell, "Short-term (intestinal) and long-term (postintestinal) conversion of beta-carotene to retinol in adults as assessed by a stable-isotope reference method," *The American Journal of Clinical Nutrition*, vol. 78, no. 2, pp. 259–266, 2003.

[79] M. van Lieshout, C. E. West, Muhilal et al., "Bioefficacy of β-carotene dissolved in oil studied in children in Indonesia," *The American Journal of Clinical Nutrition*, vol. 73, no. 5, pp. 949–958, 2001.

[80] C. L. Poor, T. L. Bierer, N. R. Merchen, G. C. Fahey Jr., and J. W. Erdman Jr., "The accumulation of α- and β-carotene in serum and tissues of preruminant calves fed raw and steamed carrot slurries," *Journal of Nutrition*, vol. 123, no. 7, pp. 1296–1304, 1993.

[81] J. J. M. Castenmiller, C. E. West, J. P. H. Linssen, K. H. van het Hof, and A. G. J. Voragen, "The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans," *The Journal of Nutrition*, vol. 129, no. 2, pp. 349–355, 1999.

[82] W. S. White, K. M. Peck, E. A. Ulman, and J. W. Erdman Jr., "The ferret as a model for evaluation of the bioavailabilities of all- trans-β-carotene and its isomers," *Journal of Nutrition*, vol. 123, no. 6, pp. 1129–1139, 1993.

[83] T. L. Bierer, N. R. Merchen, and J. W. Erdman Jr., "Comparative absorption and transport of five common carotenoids in preruminant calves," *Journal of Nutrition*, vol. 125, no. 6, pp. 1569–1577, 1995.

[84] J. M. Gaziano, E. J. Johnson, R. M. Russell et al., "Discrimination in absorption or transport of beta-carotene isomers after oral supplementation with either all-trans- or 9-cis-beta-carotene," *The American Journal of Clinical Nutrition*, vol. 61, no. 6, pp. 1248–1252, 1995.

[85] D. M. Deming, S. R. Teixeira, and J. W. Erdman Jr., "All-trans β-carotene appears to be more bioavailable than 9-cis or 13-cis β-carotene in gerbils given single oral doses of each isomer," *Journal of Nutrition*, vol. 132, no. 9, pp. 2700–2708, 2002.

[86] P. N. Dua, E. J. Day, H. C. Tipton, and J. E. Hill, "Influence of dietary vitamin A on carotenoid utilization, nitrogen retention and energy utilization by the chick," *Journal of Nutrition*, vol. 90, no. 2, pp. 117–122, 1966.

[87] T. van Vliet, M. F. van Vlissingen, F. van Schaik, and H. van den Berg, "β-Carotene absorption and cleavage in rats is affected by the vitamin A concentration of the diet," *The Journal of Nutrition*, vol. 126, no. 2, pp. 499–508, 1996.

[88] A. C. Boileau, C. M. Lee, and J. W. Erdman Jr., "Vitamin A deficiency reduces uptake of β-carotene by brush border membrane vesicles but does not alter intestinal retinyl ester hydrolase activity in the rat," *Journal of Nutritional Biochemistry*, vol. 11, no. 9, pp. 436–442, 2000.

[89] M. E. O'Neill and D. I. Thurnham, "Intestinal absorption of β-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction," *British Journal of Nutrition*, vol. 79, no. 2, pp. 149–159, 1998.

[90] I. Paetau, H. Chen, N. M.-Y. Goh, and W. S. White, "Interactions in the postprandial appearance of β-carotene and canthaxanthin in plasma triacylglycerol-rich lipoproteins in humans," *The American Journal of Clinical Nutrition*, vol. 66, no. 5, pp. 1133–1143, 1997.

[91] H. van den Berg and T. van Vliet, "Effect of simultaneous, single oral doses of β-carotene with lutein or lycopene on the β-carotene and retinyl ester responses in the triacylglycerol-rich lipoprotein fraction of men," *The American Journal of Clinical Nutrition*, vol. 68, no. 1, pp. 82–89, 1998.

[92] W. S. White, K. M. Peck, T. L. Bierer, E. T. Gugger, and J. W. Erdman Jr., "Interactions of oral β-carotene and canthaxanthin in ferrets," *Journal of Nutrition*, vol. 123, no. 8, pp. 1405–1413, 1993.

[93] M. R. Prince and J. K. Frisoli, "Beta-carotene accumulation in serum and skin," *The American Journal of Clinical Nutrition*, vol. 57, no. 2, pp. 175–181, 1993.

[94] F. Jalal, M. C. Nesheim, Z. Agus, D. Sanjur, and J. P. Habicht, "Serum retinol concentrations in children are affected by food sources of β-carotene, fat intake, and anthelmintic drug treatment," *The American Journal of Clinical Nutrition*, vol. 68, no. 3, pp. 623–629, 1998.

[95] P. Jayarajan, V. Reddy, and M. Mohanram, "Effect of dietary fat on absorption of β carotene from green leafy vegetables in children," *Indian Journal of Medical Research*, vol. 71, no. 1, pp. 53–56, 1980.

[96] A. J. C. Roodenburg, R. Leenen, K. H. van Het Hof, J. A. Weststrate, and L. B. M. Tijburg, "Amount of fat in the diet affects bioavailability of lutein esters but not of alpha-carotene, beta-carotene, and vitamin E in humans," *The American Journal of Clinical Nutrition*, vol. 71, no. 5, pp. 1187–1193, 2000.

[97] M. R. Lakshman, Q. H. Liu, R. Sapp, M. Somanchi, and P. R. Sundaresan, "The effects of dietary taurocholate, fat, protein, and carbohydrate on the distribution and fate of dietary β-carotene in ferrets," *Nutrition and Cancer*, vol. 26, no. 1, pp. 49–61, 1996.

[98] E. Kienzle, C. Kaden, P. P. Hoppe, and B. Opitz, "Serum β-carotene and α-tocopherol in horses fed β-carotene via grass-meal or a synthetic beadlet preparation with and without added

dietary fat," *Journal of Animal Physiology and Animal Nutrition*, vol. 87, no. 3-4, pp. 174–180, 2003.

[99] T. Huo, M. G. Ferruzzi, S. J. Schwartz, and M. L. Failla, "Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 22, pp. 8950–8957, 2007.

[100] F. J. Schweigert, A. Baumane, I. Buchholz, and H. A. Schoon, "Plasma and tissue concentrations of β-carotene and vitamin A in rats fed β-carotene in various fats of plant and animal origin," *Journal of Environmental Pathology, Toxicology and Oncology*, vol. 19, no. 1-2, pp. 87–93, 2000.

[101] B. S. Alam, L. R. Brown, and S. Q. Alam, "Influence of dietary fats and vitamin E on plasma and hepatic vitamin A and β-carotene levels in rats fed excess β-carotene," *Nutrition and Cancer*, vol. 14, no. 2, pp. 111–116, 1990.

[102] C. L. Rock and M. E. Swendseid, "Plasma β-carotene response in humans after meals supplemented with dietary pectin," *The American Journal of Clinical Nutrition*, vol. 55, no. 1, pp. 96–99, 1992.

[103] J. Riedl, J. Linseisen, J. Hoffmann, and G. Wolfram, "Some dietary fibers reduce the absorption of carotenoids in women," *Journal of Nutrition*, vol. 129, no. 12, pp. 2170–2176, 1999.

[104] P. C. Allen, "Physiological responses of chicken gut tissue to coccidial infection: comparative effects of *Eimeria acervulina* and *Eimeria mitis* on mucosal mass, carotenoid content, and brush border enzyme activity," *Poultry Science*, vol. 66, no. 8, pp. 1306–1315, 1987.

[105] P. C. Allen, "Effect of coccidiosis on the distribution of dietary lutein in the chick," *Poultry Science*, vol. 71, no. 9, pp. 1457–1463, 1992.

[106] W. D. Hamilton and M. Zuk, "Heritable true fitness and bright birds: a role for parasites?" *Science*, vol. 218, no. 4570, pp. 384–387, 1982.

[107] J. Sundberg, "Parasites, plumage coloration and reproductive success in the yellowhammer, *Emberiza citrinella*," *Oikos*, vol. 74, no. 2, pp. 331–339, 1995.

[108] K. J. McGraw and G. E. Hill, "Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration," *Proceedings of the Royal Society of London B: Biological Sciences*, vol. 267, no. 1452, pp. 1525–1531, 2000.

[109] G. E. Hill, K. L. Farmer, and M. L. Beck, "The effect of mycoplasmosis on carotenoid plumage coloration in male house finches," *Journal of Experimental Biology*, vol. 207, no. 12, pp. 2095–2099, 2004.

[110] M. Milinski and T. C. M. Bakker, "Female sticklebacks use male coloration in mate choice and hence avoid parasitized males," *Nature*, vol. 344, no. 6264, pp. 330–333, 1990.

[111] A. E. Houde and A. J. Torio, "Effect of parasitic infection on male color pattern and female choice in guppies," *Behavioral Ecology*, vol. 3, no. 4, pp. 346–351, 1992.

[112] M. G. Leuenberger, C. Engeloch-Jarret, and W.-D. Woggon, "The reaction mechanism of the enzyme-catalyzed central cleavage of β-carotene to retinal," *Angewandte Chemie—International Edition*, vol. 40, no. 14, pp. 2614–2617, 2001.

[113] J. von Lintig and K. Vogt, "Filling the gap in vitamin A research. Molecular identification of an enzyme cleaving β-carotene to retinal," *The Journal of Biological Chemistry*, vol. 275, no. 16, pp. 11915–11920, 2000.

[114] T. M. Redmond, S. Gentleman, T. Duncan et al., "Identification, expression, and substrate specificity of a mammalian β-carotene 15,15′-dioxygenase," *The Journal of Biological Chemistry*, vol. 276, no. 9, pp. 6560–6565, 2001.

[115] A. Wyss, G. Wirtz, W.-D. Woggon et al., "Cloning and expression of β,β-carotene 15,15′-dioxygenase," *Biochemical and Biophysical Research Communications*, vol. 271, no. 2, pp. 334–336, 2000.

[116] W. Yan, G.-F. Jang, F. Haeseleer et al., "Cloning and characterization of a human $\beta\beta$-carotene-15, 15′-dioxygenase that is highly expressed in the retinal pigment epithelium," *Genomics*, vol. 72, no. 2, pp. 193–202, 2001.

[117] J. Glover and E. R. Redfearn, "The mechanism of the transformation of beta-carotene into vitamin A in vivo," *Biochemical Journal*, vol. 58, pp. 15–16, 1954.

[118] X.-D. Wang, G.-W. Tang, J. G. Fox, N. I. Krinsky, and R. M. Russell, "Enzymatic conversion of β-carotene into β-apocarotenals and retinoids by human, monkey, ferret, and rat tissues," *Archives of Biochemistry and Biophysics*, vol. 285, no. 1, pp. 8–16, 1991.

[119] C. Kiefer, S. Hessel, J. M. Lampert et al., "Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A," *The Journal of Biological Chemistry*, vol. 276, no. 17, pp. 14110–14116, 2001.

[120] C. Duszka, P. Grolier, E.-M. Azim, M.-C. Alexandre-Gouabau, P. Borel, and V. Azais-Braesco, "Rat intestinal β-carotene dioxygenase activity is located primarily in the cytosol of mature jejunal enterocytes," *Journal of Nutrition*, vol. 126, no. 10, pp. 2550–2556, 1996.

[121] A. During, A. Nagao, C. Hoshino, and J. Terao, "Assay of β-carotene 15,15′-dioxygenase activity by reverse-phase high-pressure liquid chromatography," *Analytical Biochemistry*, vol. 241, no. 2, pp. 199–205, 1996.

[122] N. Yamaguchi, T. Yamamoto, K. Suruga, and S. Takase, "Developmental changes in gene expressions of β-carotene cleavage enzyme and retinoic acid synthesizing enzymes in the chick duodenum," *Comparative Biochemistry and Physiology—A Molecular and Integrative Physiology*, vol. 148, no. 3, pp. 690–697, 2007.

[123] F. H. Martin, D. E. Ullrey, H. W. Newland, and E. R. Miller, "Vitamin A activity of carotenes in corn silage fed to lambs," *Journal of Nutrition*, vol. 96, no. 2, pp. 269–274, 1968.

[124] D. E. Ullrey, E. R. Miller, R. D. Struthers, R. E. Peterson, J. A. Hoefer, and H. H. Hall, "Vitamin a activity of fermentation beta-carotene for swine," *The Journal of Nutrition*, vol. 85, pp. 375–385, 1965.

[125] R. H. Wellenreiter, D. E. Ullrey, E. R. Miller, and W. T. Magee, "Vitamin A activity of corn carotenes for swine," *Journal of Nutrition*, vol. 99, no. 2, pp. 129–136, 1969.

[126] S. G. Parvin and B. Sivakumar, "Nutritional status affects intestinal carotene cleavage activity and carotene conversion to vitamin A in rats," *Journal of Nutrition*, vol. 130, no. 3, pp. 573–577, 2000.

[127] J. D. Ribaya-Mercado, F. S. Solon, M. A. Solon et al., "Bioconversion of plant carotenoids to vitamin A in filipino school-aged children varies inversely with vitamin A status," *The American Journal of Clinical Nutrition*, vol. 72, no. 2, pp. 455–465, 2000.

[128] A. Yang and R. K. Tume, "A comparison of β-carotene-splitting activity isolated from intestinal mucosa of pasture-grazed sheep, goats and cattle," *Biochemistry and Molecular Biology International*, vol. 30, no. 2, pp. 209–217, 1993.

[129] F. J. Schweigert, M. Wierich, W. A. Rambeck, and H. Zucker, "Carotene cleavage activity in bovine ovarian follicles," *Theriogenology*, vol. 30, no. 5, pp. 923–930, 1988.

[130] M. R. Lakshmanan, H. Chansang, and J. A. Olson, "Purification and properties of carotene 15,15′-dioxygenase of rabbit intestine," *Journal of Lipid Research*, vol. 13, no. 4, pp. 477–482, 1972.

[131] H. Singh and H. R. Cama, "Enzymatic cleavage of carotenoids," *Biochimica et Biophysica Acta (BBA)—Enzymology*, vol. 370, no. 1, pp. 49–61, 1974.

[132] W. H. James and I. M. El Gindi, "The utilization of carotene. I. As affected by certain proteins in the diet of growing albino rats," *Journal of Nutrition*, vol. 51, no. 1, pp. 97–108, 1953.

[133] S. K. Kamath and L. Arnrich, "Effect of dietary protein on the intestinal biosynthesis of retinol from ^{14}C-beta-carotene in rats," *Journal of Nutrition*, vol. 103, no. 2, pp. 202–206, 1973.

[134] D. M. Deming, A. C. Boileau, C. M. Lee, and J. W. Erdman Jr., "Amount of dietary fat and type of soluble fiber independently modulate postabsorptive conversion of beta-carotene to vitamin A in mongolian gerbils," *Journal of Nutrition*, vol. 130, no. 11, pp. 2789–2796, 2000.

[135] G. B. Brubacher and H. Weiser, "The vitamin A activity of β-carotene," *International Journal for Vitamin and Nutrition Research*, vol. 55, no. 1, pp. 5–15, 1985.

[136] F. J. Schweigert, S. Uehlein-Harrell, G. V. Hegel, and H. Weisner, "Vitamin A (retinol and retinyl esters), α-tocopherol and lipid levels in captive wild mammals and birds," *Journal of Veterinary Medicine A*, vol. 38, no. 1, pp. 35–42, 1991.

[137] O. Mora, J. L. Romano, E. Gonzalez, F. Ruiz, and A. Shimada, "Low cleavage activity of 15,15′ dioxygenase to convert β-carotene to retinal in cattle compared with goats, is associated with the yellow pigmentation of adipose tissue," *International Journal for Vitamin and Nutrition Research*, vol. 70, no. 5, pp. 199–205, 2000.

[138] F. J. Schweigert, "Research Note: chnages in the concentration of β-carotene, α-tocopherol and retinol in the bovinve *Corpus luteum* during the ovarian cycle," *Archives of Animal Nutrition*, vol. 57, no. 4, pp. 307–310, 2003.

[139] A. Morales, A. Gonzalez, A. Varela-Echavarria, A. Shimada, and O. Mora, "Differences in expression and activity of beta,beta′-carotene-15,15′-oxygenase in liver and duodenum of cattle with yellow or white fat," *Journal of Animal Physiology and Animal Nutrition*, vol. 91, no. 7-8, pp. 341–346, 2007.

[140] D. Sklan, "Carotene Cleavage Activity in the corpus luteum of cattle," *International Journal for Vitamin and Nutrition Research*, vol. 53, no. 1, pp. 23–26, 1983.

[141] C. Kawashima, S. Nagashima, K. Sawada, F. J. Schweigert, A. Miyamoto, and K. Kida, "Effect of β-carotene supply during close-up dry period on the onset of first postpartum luteal activity in dairy cows," *Reproduction in Domestic Animals*, vol. 45, no. 6, pp. e282–e287, 2010.

[142] B. P. Chew, T. S. Wong, and J. J. Michal, "Uptake of orally administered beta-carotene by blood plasma, leukocytes, and lipoproteins in calves," *Journal of Animal Science*, vol. 71, no. 3, pp. 730–739, 1993.

[143] C. L. Poor, T. L. Bierer, N. R. Merchen, G. C. Fahey Jr., M. R. Murphy, and J. W. Erdman Jr., "Evaluation of the preruminant calf as a model for the study of human carotenoid metabolism," *The Journal of Nutrition*, vol. 122, no. 2, pp. 262–268, 1992.

[144] P. P. Hoppe, B. P. Chew, A. Safer, I. Stegemann, and H. K. Biesalski, "Dietary β-carotene elevates plasma steady-state and tissue concentrations of β-carotene and enhances vitamin A balance in preruminant calves," *Journal of Nutrition*, vol. 126, no. 1, pp. 202–208, 1996.

[145] S. K. Kon, W. A. McGillivray, and S. Y. Thompson, "Metabolism of carotene and vitamin A given by mouth or vein in oily solution or aqueous dispersion to calves, rabbits and rats," *British Journal of Nutrition*, vol. 9, no. 3, pp. 244–267, 1955.

[146] K. A. Slifka, P. E. Bowen, M. Stacewicz-Sapuntzakis, and S. D. Crissey, "A survey of serum and dietary carotenoids in captive wild animals," *The Journal of Nutrition*, vol. 129, no. 2, pp. 380–390, 1999.

[147] T. Moore, *Vitamin A*, Elsevier, Amsterdam, The Netherlands, 1957.

[148] S. C. Fernandez, P. Budowski, I. Ascarelli, H. Neumark, and A. Bondi, "Low utilization of carotene by sheep," *International Journal for Vitamin and Nutrition Research*, vol. 46, no. 4, pp. 446–453, 1976.

[149] P. V. Fonnesbeck and L. D. Symons, "Utilization of the carotene of hay by horses," *Journal of Animal Science*, vol. 26, no. 5, pp. 1030–1038, 1967.

[150] E. S. Dierenfeld, P. P. Hoppe, M. H. Woodford, N. P. Krilov, V. V. Klimov, and N. I. Yasinetskaya, "Plasma alpha-tocopherol, beta-carotene, and lipid levels in semi-free-ranging Przewalski horses (*Equus przewalskii*)," *Journal of Zoo and Wildlife Medicine*, vol. 28, no. 2, pp. 144–147, 1997.

[151] K. M. Greiwe-Crandell, D. S. Kronfeld, L. S. Gay, D. Sklan, W. Tiegs, and P. A. Harris, "Vitamin A repletion in thoroughbred mares with retinyl palmitate or β-carotene," *Journal of Animal Science*, vol. 75, no. 10, pp. 2684–2690, 1997.

[152] N. I. Krinsky, M. M. Mathews-Roth, S. Welankiwar, P. K. Sehgal, N. C. Lausen, and M. Russett, "The metabolism of [14C]β-carotene and the presence of other carotenoids in rats and monkeys," *The Journal of Nutrition*, vol. 120, no. 1, pp. 81–87, 1990.

[153] J. A. Olson, "The conversion of radioactive beta-carotene into vitamin A by the rat intestine in vivo," *The Journal of Biological Chemistry*, vol. 236, pp. 349–356, 1961.

[154] M. R. Lakshman, I. Mychkovsky, and M. Attlesey, "Enzymatic conversion of all-trans-β-carotene to retinal by a cytosolic enzyme from rabbit and rat intestinal mucosa," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 23, pp. 9124–9128, 1989.

[155] J. Devery and B. V. Milborrow, "beta-Carotene-15,15′-dioxygenase (EC 1.13.11.21) isolation reaction mechanism and an improved assay procedure," *British Journal of Nutrition*, vol. 72, no. 3, pp. 397–414, 1994.

[156] NRC, *Nutrient Requirements of Laboratory Animals*, The National Academies, Washington, DC, USA, 4th edition, 1995.

[157] W. Marusich and J. Bauernfeind, "The biological activity of β-carotene in poultry and rats," *Poultry Science*, vol. 42, no. 4, pp. 949–957, 1963.

[158] C. M. Lee, J. D. Lederman, N. E. Hofmann, and J. W. Erdman Jr., "The Mongolian gerbil (*Meriones unguiculatus*) is an appropriate animal model for evaluation of the conversion of β-carotene to vitamin A," *Journal of Nutrition*, vol. 128, no. 2, pp. 280–286, 1998.

[159] C. M. Lee, A. C. Boileau, T. W. M. Boileau et al., "Review of animal models in carotenoid research," *Journal of Nutrition*, vol. 129, no. 12, pp. 2271–2277, 1999.

[160] H. C. Furr, M. H. Green, M. Haskell et al., "Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans," *Public Health Nutrition*, vol. 8, no. 6, pp. 596–607, 2005.

[161] D. I. Thurnham, "Bioequivalence of β-carotene and retinol," *Journal of the Science of Food and Agriculture*, vol. 87, no. 1, pp. 13–39, 2007.

[162] D. S. Goodman, R. Blomstrand, B. Werner, H. S. Huang, and T. Shiratori, "The intestinal absorption and metabolism of vitamin A and beta-carotene in man," *The Journal of Clinical Investigation*, vol. 45, no. 10, pp. 1615–1623, 1966.

[163] D. M. Snodderly, M. D. Russett, R. I. Land, and N. I. Krinsky, "Plasma carotenoids of monkeys (Macaca fascicularis and Saimiri sciureus) fed a nonpurified diet," *Journal of Nutrition*, vol. 120, no. 12, pp. 1663–1671, 1990.

[164] A. L. van Kappel, J.-P. Steghens, A. Zeleniuch-Jacquotte, V. Chajès, P. Toniolo, and E. Riboli, "Serum carotenoids as biomarkers of fruit and vegetable consumption in the New York Women's Health study," *Public Health Nutrition*, vol. 4, no. 3, pp. 829–835, 2001.

[165] L. Booher, E. Callison, and E. Hewston, "An experimental determination of the minimum vitamin A requirements of normal adults," *Journal of Nutrition*, vol. 17, pp. 317–331, 1939.

[166] Institute of Medicine, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy Press, Washington, DC, USA, 2002.

[167] D. M. Snodderly, B. Shen, R. I. Land, and N. I. Krinsky, "Dietary manipulation of plasma carotenoid concentrations of squirrel monkeys (*Saimiri sciureus*)," *The Journal of Nutrition*, vol. 127, no. 1, pp. 122–129, 1997.

[168] A. L. García, J. Raila, C. Koebnick, K. Eulenberger, and F. J. Schweigert, "Great apes show highly selective plasma carotenoids and have physiologically high plasma retinyl esters compared to humans," *American Journal of Physical Anthropology*, vol. 131, no. 2, pp. 236–242, 2006.

[169] F. J. Schweigert, I. Buchholz, A. Schuhmacher, and J. Gropp, "Effect of dietary β-carotene on the accumulation of β-carotene and vitamin A in plasma and tissues of gilts," *Reproduction Nutrition Development*, vol. 41, no. 1, pp. 47–55, 2001.

[170] F. J. Schweigert, I. Rosival, W. A. Rambeck, and J. Gropp, "Plasma transport and tissue distribution of [14C] beta-carotene and [3H]retinol administered orally to pigs," *International Journal for Vitamin and Nutrition Research*, vol. 65, no. 2, pp. 95–100, 1995.

[171] A. Nagao, A. During, C. Hoshino, J. Terao, and J. A. Olson, "Stoichiometric conversion of all trans-β-carotene to retinal by pig intestinal extract," *Archives of Biochemistry and Biophysics*, vol. 328, no. 1, pp. 57–63, 1996.

[172] D. E. Ullrey, "Biological availability of fat-soluble vitamins: vitamin A and carotene," *Journal of Animal Science*, vol. 35, no. 3, pp. 648–657, 1972.

[173] M. Naziroglu and C. Günay, "The levels of some antioxidant vitamins, glutathione peroxidase and lipoperoxidase during the anaesthesia of dogs," *Cell Biochemistry and Function*, vol. 17, no. 3, pp. 207–212, 1999.

[174] H. Baker, S. M. Schor, B. D. Murphy, B. DeAngelis, S. Feingold, and O. Frank, "Blood vitamin and choline concentrations in healthy domestic cats, dogs, and horses," *American Journal of Veterinary Research*, vol. 47, no. 7, pp. 1468–1471, 1986.

[175] B. P. Chew, J. S. Park, B. C. Weng, T. S. Wong, M. G. Hayek, and G. A. Reinhart, "Dietary β-carotene is taken up by blood plasma and leukocytes in dogs," *Journal of Nutrition*, vol. 130, no. 7, pp. 1788–1791, 2000.

[176] S. Crissey, K. Ange, K. Slifka et al., "Serum concentrations of vitamin D metabolites, vitamins A and E, and carotenoids in six canid and four ursid species at four zoos," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 128, no. 1, pp. 155–165, 2001.

[177] B. P. Chew, J. S. Park, B. C. Weng, T. S. Wong, M. G. Hayek, and G. A. Reinhart, "Dietary beta-carotene absorption by blood plasma and leukocytes in domestic cats," *Journal of Nutrition*, vol. 130, no. 9, pp. 2322–2325, 2000.

[178] E. T. Gugger, T. L. Bierer, T. M. Henze, W. S. White, and J. W. Erdman Jr., "β-Carotene uptake and tissue distribution in ferrets (*Mustela putoriu*s furo)," *Journal of Nutrition*, vol. 122, no. 1, pp. 115–119, 1992.

[179] R. G. Turner, "Effect of prolonged feeding of raw carrots on vitamin A content of liver and kidneys in the dog," *Experimental Biology and Medicine*, vol. 31, no. 7, pp. 866–868, 1934.

[180] D. Bradfield and M. C. Smith, "The ability of the dog to utilize vitamin A from plant and animal sources," *American Journal of Physiology*, vol. 124, pp. 168–173, 1938.

[181] A. I. Coombes, G. L. Ott, and W. Winsnicky, "Vitamin A studies in the fox," *North American Veterinarian*, vol. 21, pp. 601–606, 1934.

[182] J.-R. Zhou, E. T. Gugger, and J. W. Erdman Jr., "The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of beta- and alpha-carotene in the ferret model," *Journal of the American College of Nutrition*, vol. 15, no. 1, pp. 84–91, 1996.

[183] J. D. Lederman, K. M. Overton, N. E. Hofmann, B. J. Moore, J. Thornton, and J. W. Erdman, "Ferrets (Mustela putoius furo) inefficiently convert beta-carotene to vitamin A," *The Journal of Nutrition*, vol. 128, no. 2, pp. 271–279, 1998.

[184] X.-D. Wang, N. I. Krinsky, R. P. Marini et al., "Intestinal uptake and lymphatic absorption of beta-carotene in ferrets: a model for human beta-carotene metabolism," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 263, no. 4, part 1, pp. G480–G486, 1992.

[185] S. N. Gershoff, S. G. Andrus, D. M. Hegsted, and E. A. Lentini, "Vitamin A deficiency in cats," *Laboratory Investigation*, vol. 6, pp. 227–240, 1957.

[186] A. S. Green, G. Tang, J. Lango, K. C. Klasing, and A. J. Fascetti, "Domestic cats convert [$^2H_8$]-β-carotene to [$^2H_4$]-retinol following a single oral dose," *Journal of Animal Physiology and Animal Nutrition*, vol. 96, no. 4, pp. 681–692, 2012.

[187] F. J. Schweigert, J. Raila, B. Wichert, and E. Kienzle, "Cats absorb β-carotene, but it is not converted to vitamin A," *Journal of Nutrition*, vol. 132, no. 6, supplement, pp. 1610S–1612S, 2002.

[188] N. S. Capper, I. M. McKibbin, and J. H. Prentice, "Carotene and vitamin A: the conversion of carotene into vitamin A by fowl," *Biochemical Journal*, vol. 25, no. 1, pp. 265–274, 1931.

[189] A. H. Brush, "Metabolism of carotenoid pigments in birds," *The FASEB Journal*, vol. 4, no. 12, pp. 2969–2977, 1990.

[190] Y. H. Jiang, R. B. McGeachin, and C. A. Bailey, "Alpha-tocopherol, beta-carotene, and retinol enrichment of chicken eggs," *Poultry Science*, vol. 73, no. 7, pp. 1137–1143, 1994.

[191] J. Eriksson, G. Larson, U. Gunnarsson et al., "Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken," *PLoS Genetics*, vol. 4, no. 2, 2008.

[192] P. F. Surai, B. K. Speake, N. A. R. Wood, J. D. Blount, G. R. Bortolotti, and N. H. C. Sparks, "Carotenoid discrimination by the avian embryo: a lesson from wild birds," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 128, no. 4, pp. 743–750, 2001.

[193] P. F. Surai, N. J. Royle, and N. H. C. Sparks, "Fatty acid, carotenoid and vitamin A composition of tissues of free living gulls," *Comparative Biochemistry and Physiology A—Molecular and Integrative Physiology*, vol. 126, no. 3, pp. 387–396, 2000.

[194] C. J. Flegal, P. J. Schaible, and H. H. Hall, "The relative biopotency of fermentation beta-carotene, crystalline beta-carotene and vitamin A for poultry," *Poultry Science*, vol. 50, no. 2, pp. 349–358, 1971.

[195] NRC, *Nutrient Requirements of Poultry: Ninth Revised Edition*, The National Academies Press, Washington, DC, USA, 1994.

[196] H. Bachmann, A. Desbarats, P. Pattison et al., "Feedback regulation of β,β-carotene 15,15′-monooxygenase by retinoic acid in rats and chickens," *Journal of Nutrition*, vol. 132, no. 12, pp. 3616–3622, 2002.

[197] D. Sklan, "Carotene-cleavage activity in chick intestinal mucosa cytosol: association with a high-molecular-weight lipid-protein aggregate fraction and partial characterization of the activity," *British Journal of Nutrition*, vol. 50, no. 2, pp. 417–425, 1983.

[198] E. A. Koutsos and K. C. Klasing, "Vitamin A nutrition of growing cockatiel chicks (*Nymphicus hollandicus*)," *Journal of Animal Physiology and Animal Nutrition*, vol. 89, no. 11-12, pp. 379–387, 2005.

[199] P. Wolf, T. Bartels, H.-P. Sallmann, K. Heisler, and J. Kamphues, "Vitamin A metabolism in recessive white canaries," *Animal Welfare*, vol. 9, no. 2, pp. 153–165, 2000.

[200] K. C. Klasing, *Comparative Avian Nutrition*, CAB International, Cambridge, UK, 1998.

[201] K. Schiedt, "Absorption and metabolism of carotenoids in birds, fish and crustacean," in *Carotenoids*, G. Britton, S. Liaaen-Jensen, and H. Pfander, Eds., pp. 285–358, Birkhauser, Basel, Switzerland, 1998.

[202] NRC, *Nutrient Requirements of Fish*, The National Academies Press, Washington, DC, USA, 1993.

[203] U. C. Goswami, "Metabolism of cryptoxanthin in freshwater fish," *British Journal of Nutrition*, vol. 52, no. 3, pp. 575–581, 1984.

[204] E. Shantz and J. Brinkman, "Biological activity of pure vitamin A_2," *The Journal Biological Chemistry*, vol. 183, pp. 467–471, 1950.

[205] K. Kongsbak, S. H. Thilsted, and M. A. Wahed, "Effect of consumption of the nutrient-dense, freshwater small fish *Amblypharyngodon mola* on biochemical indicators of vitamin A status in Bangladeshi children: a randomised, controlled study of efficacy," *British Journal of Nutrition*, vol. 99, no. 3, pp. 581–597, 2008.

[206] A. B. Barua and U. C. Goswami, "Formation of vitamin A in a freshwater fish. Isolation of retinoic acid," *Biochemical Journal*, vol. 166, no. 1, pp. 133–136, 1977.

[207] A. B. Barua, H. T. Singh, and R. C. Das, "Conversion of lutein into dehydroretinol by the freshwater fish, *Saccobranchus fossilis*," *British Journal of Nutrition*, vol. 30, no. 1, pp. 1–12, 1973.

[208] J. Gross and P. Budowski, "Conversion of carotenoids into vitamins A_1 and A_2 in two species of freshwater fish," *Biochemical Journal*, vol. 101, no. 3, pp. 747–754, 1966.

[209] K. Schiedt, F. J. Leuenberger, M. Vecchi, and E. Glinz, "Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken," *Pure and Applied Chemistry*, vol. 57, no. 5, pp. 685–692, 1985.

[210] NRC, *Nutrient Requirements of Cats and Dogs*, National Academies Press, Washington, DC, USA, 2006.

[211] NRC, *Nutrient Requirements of Beef Cattle*, The National Academies Press, 7th edition, 2000.

[212] NRC, *Nutrient Requirements of Dairy Cattle*, The National Academies Press, Washington, DC, USA, 7th edition, 2001.

[213] NRC, *Nutrient Requirements of Mink and Foxes*, The National Academies Press, Washington, DC, USA, 2nd edition, 1982.

[214] NRC, *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*, The National Academies Press, Washington, DC, USA, 2007.

[215] NRC, *Nutrient Requirements of Horses*, The National Academies Press, Washington, DC, USA, 5th edition, 1989.

[216] NRC, *Nutrient Requirements of Swine*, The National Academies Press, Washington, DC, USA, 10th edition, 1998.

[217] NRC, *Nutrient Requirements of Rabbits*, The National Academies Press, Washington, DC, USA, 2nd edition, 1977.

[218] NRC, *Nutrition Requirements of Sheep*, The National Academies Press, Washington, DC, USA, 5th edition, 1985.

[219] M. R. Lakshman and C. Okoh, "Carotenoid-protein complexes," *Methods in Enzymology*, vol. 214, pp. 74–86, 1993.

[220] J. V. O'Fallon and B. P. Chew, "The subcellular distribution of beta-carotene in bovine corpus luteum," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 177, no. 3, pp. 406–411, 1984.

[221] M. R. Lakshman and M. N. Rao, "Purification and characterization of cellular carotenoid-binding protein from mammalian liver," in *Methods in Enzymology*, pp. 441–456, Academic Press, 1999.

[222] M. N. Rao, P. Ghosh, and M. R. Lakshman, "Purification and partial characterization of a cellular carotenoid-binding protein from ferret liver," *The Journal of Biological Chemistry*, vol. 272, no. 39, pp. 24455–24460, 1997.

[223] B. J. Nonnecke, R. L. Horst, W. R. Waters, P. Dubeski, and J. A. Harp, "Modulation of fat-soluble vitamin concentrations and blood mononuclear leukocyte populations in milk replacer-fed calves by dietary vitamin A and β-carotene," *Journal of Dairy Science*, vol. 82, no. 12, pp. 2632–2641, 1999.

[224] F. J. Schweigert and H. Zucker, "Concentrations of vitamin A, β-carotene and vitamin E in individual bovine follicles of different quality," *Journal of Reproduction and Fertility*, vol. 82, no. 2, pp. 575–579, 1988.

[225] J. A. Olson, "The absorption of beta-carotene and its conversion into vitamin A," *The American Journal of Clinical Nutrition*, vol. 9, no. 4, pp. 1–12, 1961.

[226] S. de Pee, C. E. West, D. Permaesih, S. Martuti, Muhilal, and J. G. A. J. Hautvast, "Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in school children in Indonesia," *The American Journal of Clinical Nutrition*, vol. 68, no. 5, pp. 1058–1067, 1998.

[227] Institute of Medicine, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, National Academy Press, Washington, DC, USA, 2000.

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 6296315, 7 pages
http://dx.doi.org/10.1155/2016/6296315

Review Article

Environmental Aspects of Domestic Cat Care and Management: Implications for Cat Welfare

Judith L. Stella and Candace C. Croney

Center for Animal Welfare Science, Purdue University, West Lafayette, IN, USA

Correspondence should be addressed to Candace C. Croney; ccroney@purdue.edu

Received 30 May 2016; Accepted 6 September 2016

Academic Editor: Maria R. C. De Godoy

Domestic cats (*Felis silvestris catus*) are the most commonly kept companion animals in the US with large populations of owned (86 million), free-roaming (70 million), research (13,000), and shelter (2-3 million) cats. Vast numbers of cats are maintained in homes and other facilities each year and are reliant on humans for all of their care. Understanding cat behavior and providing the highest quality environments possible, including positive human-cat interactions, based on research could help improve the outcomes of biomedical research, shelter adoptions, and veterinary care, as well as overall cat welfare. Often, however, cats' needs are inadequately met in homes and some aspects may also not be well met in research colonies and shelters, despite the fact that similar problems are likely to be encountered in all of these environments. This paper provides a brief overview of common welfare challenges associated with indoor housing of domestic cats. Essential considerations for cage confinement are reviewed, along with implications of poor cat coping, such as weakening of the human-animal bond and relinquishment to shelters. The important role that environmental management plays in cat behavior and welfare outcomes is explored along with the need for additional research in key areas.

1. Introduction: Factors Contributing to the Welfare Problem of Cat Relinquishment

Recent statistics from the American Society for the Prevention of Cruelty to Animals (ASPCA) and the Humane Society of the United States (HSUS) estimate that there are approximately 86 million owned cats and between 50 and 70 million feral and/or free-roaming cats in the United States [1, 2]. Two to three million of these enter shelters each year, of which 70–75% are euthanized.

Through a series of studies by The National Council on Pet Population Study and Policy [3] and others, several reasons for relinquishment and return of cats have been identified. These typically include abandonment/stray (31%), owner circumstances (move, illness, divorce, and financial) (19%), unwanted kittens (14%), and allergies (5%) [4]. Behavioral reasons are thought to be another leading cause of cat relinquishment (approximately 12%) [5, 6], with the most common of these including house soiling, problems between pets, aggression toward people, unfriendliness, fearfulness, and destructive behavior. The owners level of knowledge about species-typical cat behavior also appears to be a factor in relinquishment. Fewer of those who relinquished cats knew that they pounce, scratch, or bite as a form of play and that the number of cats in the home affects cat behavior; relinquishers also exhibited knowledge deficits about cat estrous cycles [7]. Weak owner attachment, not having owned another cat as an adult, and having unrealistic expectations of a particular role for the cat to fill also contributed to surrendering [5]. Additionally, cats at higher risk of relinquishment were younger, mixed breed, and sexually intact. Cats kept confined to basements or garages most of the day, those maintained without access to the outdoors, and those primarily cared for by an adult woman had an increased risk for relinquishment [5].

Interestingly, the welfare of cats in homes is not usually addressed to the extent that it is within a research colony or a shelter, although similar problems are likely to arise in all environments in which cats are confined. For example, Morgan and Houpt [8] found the most common behavior problems of cats in US homes to be scratching furniture (60%),

eating houseplants (42%), conspecific aggression (36%), food stealing (25%), hissing/aggression to people (17%), house soiling (16%), excessive vocalizations (16%), fabric chewing (7%), and "shyness" (4%) [8]. Similarly, Heidenberger's [9] investigation of cats in homes indicated that the most frequent behavior problems cited by cat owners, via a questionnaire, were anxiety (16.7%), scratching furniture (15.2%), feeding problems (10.9%), aggression (10.5%), inappropriate urination (8.2%), and defecation in the house (5.1%) [9]. The most frequently mentioned anxiety-inducing stimulus was a visit by strangers. Neutered females, cats adopted between the ages of 5–12 months, and cats weighing greater than 4 kg were more likely to be anxious. Cats acquired from a shelter, as a stray, or from a friend all tended to show anxiety more often than those born in the owner's home. The number of cats in the home and the amount of available space per cat also were relevant, with multiple cat households and limited space per cat both leading to homes more likely to have a cat described as anxious. Many of these behavioral issues have been observed in response to inappropriate environments under laboratory conditions as well [10].

International studies of cat behavior report similar findings. Amat et al.'s [11] study of 336 cats referred to a behavior clinic in Spain noted that problem behaviors presented for treatment were similar to those cited as problems in the home or as reasons for relinquishment [11]. These included aggression (47%), inappropriate elimination (39%), compulsive behavior (3.5%), excessive vocalization (2.5%), fear and phobias (2.5%), and "other," which was comprised of anorexia, scratching furniture, and overactivity (5.4%). In agreement with other studies, intercat aggression (64% of aggression cases) was found to be a common problem. This may be a species-typical behavioral response for a solitary animal that finds itself unable to disperse and also lacks a well-developed intraspecific communication system, as proposed by Leyhausen and Lorentz [12]. However, many common behavior problems, particularly aggression and inappropriate elimination, are not tolerated well by owners.

Collectively, these studies suggest that owner attention to meeting cats' needs may be the most important determinant of welfare outcomes in homes, given that owner decision-making dictates all of the cat's living conditions. While it is possible that cat behavioral problems may simply be normal behaviors that are unwanted by owners, it is also likely that exhibition of "problem" behaviors could be in response to a poor quality environment or one in which the cat is unable to cope. According to Turner [13] "many behavioral problems result from a lack of consideration of the needs of the cat, poor or changing housing conditions, unrealistic expectations of the owner or inadequate interactions between the owner and the cat" [13]. Regardless of the reason, inadequate housing and handling diminish welfare for the cat.

However, owner self-reports suggest that many do not adequately provide for their cats' needs. For example, Heidenberger [9] found that 24% of companion cats in homes did not have their own food bowls and over half had to share the litter pan with other cats, both of which may lead to resource guarding and defensive behavior in cats. Not surprisingly, cats in groups of two or three exhibited more problem behaviors than did single cats. Having outdoor access was negatively associated with problem behavior, with owners reporting few to no problems with cats that could go outside. Additionally, the quality of owner-cat relationships is often highly variable, despite the finding that those who interact often and regularly with their cats on a daily basis report fewer problem behaviors [9].

Taken together, these findings highlight the need for greater owner attention to cats' behaviors and their overall needs. In particular, understanding the role of the environment in cat behavior and its relationship to the human-animal bond and owner satisfaction is essential to facilitate positive cat-human interactions. Such information may improve retention in homes and better support the welfare of cats in all environments in which they are maintained. The aim of this paper is to outline environmental considerations for confined cats that will lead to improved cat welfare.

2. Macroenvironmental Considerations

Various aspects of the environment may affect the welfare of the cat when confined in homes or cages in shelters, veterinary hospitals, or research facilities. Those of particular importance to cats include the physical components of the macro- and microenvironments and the social environment, which includes the quality of human-animal interactions.

The macroenvironment refers to the cat's housing space (room, building, or barn) and its surroundings and includes factors such as the thermoregulatory environment, lighting, odors, and sounds [14]. Although the thermoregulatory environment exerts a major influence on animal welfare, cats may be unable to express temperature regulating behaviors because of a lack of resources available to them to do so and often the thermoneutral zone of the species is not adequately considered in their housing. For example, the thermoneutral zone for domestic cats is 30–38°C [15] (NRC 2006). Yet most cat housing areas in homes and laboratories are maintained closer to 22 ± 2°C [15]. Thus, thermal discomfort may be a common experience for many cats, despite being an issue that is relatively easy to remedy. Providing opportunities for cats to behaviorally thermoregulate such as provision of warm bedding, resting areas, boxes, or heating elements such as SnuggleSafe® will enable them to more easily cope with the environment.

Another macroenvironmental factor that impacts cat well-being in various housing environments is odor. Because almost all mammals depend more on olfactory cues than do humans, aversive odors can be a source of chronic stress for confined animals. For cats, potentially objectionable odors include the scent of dogs (natural predators of cats), unfamiliar conspecifics, alcohol, cleaning chemicals (including laundry detergent), and citrus scents [16].

Another factor to consider is sound frequency range and intensity. The auditory frequency range of cats exceeds that of humans [17], making assessment of the welfare implications of high frequency noise difficult. Sound intensity in savannah and rain forest habitats ranges from 20 to 40 dB [14], whereas it regularly exceeds 100 dB in shelters and laboratories during routine husbandry [18]. Furthermore, sound intensity of

73 dB has been found to activate the stress response system of rats, leading to a 100–200% increase in plasma corticosterone levels [19]. Based on these findings, it is likely that reducing noise levels and maintaining sound intensity around 60 dB (quiet conversational level) may be beneficial to cats.

3. Microenvironmental Considerations

The micro or cage environment must also be considered relative to animal welfare. Microenvironmental factors include usable floor space, food presentation, elimination facilities, and outlets for the expression of species-typical behaviors. The type, presentation, and availability of these features of the environment can be a source of either stress or enrichment to cats [14, 16].

A particularly important microenvironmental factor to consider is the quantity and quality of space provided to cats. Confined cat spaces or housing environments are often reduced in both quantity and quality of space in comparison to options available to their wild or free-roaming counterparts. Although recommendations for minimum cat cage sizes have been published [20, 21], their basis is questionable as scientific evidence of the welfare implications (particularly adverse consequences) of keeping cats in smaller than recommended cages is not readily available. Thus, it is possible that cats may actually need larger or smaller minimum space allocations than what are currently recommended. Further to this point, the need to provide cats more than the recommended minimum of 0.56 square meters of floor space (6 square feet) is often discussed in animal sheltering communities. However, few studies have been conducted that might help establish precise minimum requirements for short- and long-term cage confinement of cats and thus provide a more informed basis for recommended space allocations for cats.

Recent studies have indicated that the quality of the environment may be more relevant to the cat than the size of the cage during both short and long periods of cage confinement [10, 22–24]. Further, Stella's [24] investigation of the behavior of cats housed in cages providing 1.1 square meters (11.8 square feet) of floor space found no difference in the number of sickness behaviors or in time to adaptation in the first 48 hours than cats housed in cages half that size [23]. Thus, while a minimum cage size clearly exists that affords cats reasonably good welfare, more work is needed to determine optimal cage sizes that also accommodate furnishings which permit both freedom of movement and the ability of cats to engage in species-typical behaviors for which they are highly motivated. In the interim, cats housed in cages for longer periods of time in shelters or research facilities may benefit from being provided daily exercise periods outside of their cages.

Whether kept in homes or other facilities, the type of shelter offered to cats should permit partial isolation from conspecifics and people, as this may be of critical importance to some, enabling them to feel a sense of security that would otherwise not occur [16]. Additionally, variation in the height at which cats can navigate their home environments also appears to be an important component of cat housing. Cats seem to prefer to monitor their surroundings from elevated vantage points and usually welcome provision of climbing frames, hammocks, platforms, raised walkways, shelves, or window seats [25]. Additional furnishing of the environment is often necessary to promote cat health and well-being. Appealing, appropriate objects must be provided to confined cats to permit expression of behaviors that include scratching and marking, which maintain claw health, and to leave both visual and pheromonal territorial marks [16, 25]. In short, the captive environment should be behaviorally relevant, with the quantity and quality of space provided allowing for the development and normal expression of species-typical behavioral patterns.

Another key element is the availability, type, and presentation of food offered to cats. For most cats under human care, food is typically provided in the form of a formulated, uniform, and consistent diet and placed in a single location so that the animal's time and energy related to foraging behavior are greatly reduced. Consequently, boredom that manifests as over- [26] or undereating [10, 23] may result. Attending to how cats are fed therefore becomes an important component of their behavioral and overall welfare management, whether kept singly or in groups and regardless of the type of housing environment in which they find themselves. Offering food in interactive puzzle feeders can provide mental and physical enrichment and is one strategy that may be implemented to minimize boredom and promote exercise.

4. Cat-Human Interactions

As noted previously, the quality and quantity of human-animal interactions experienced by cats are both relevant to their welfare outcomes in various settings. In captivity, acclimation to human presence is an important fitness-determining factor since humans select for tameness and other behaviorally acceptable traits; individuals that do not meet such criteria are often prevented from reproducing. A human-animal relationship can be said to exist if a number of repeated interactions between the animal and human occur, eventually allowing each to make predictions about the other's behavior. Both positive and negative human-animal relationships are important in the context of animal welfare, and this concept is as applicable to cats as to other species. In human-animal relationships, the human generally dictates the number and nature of interactions and hence the relationship, while the animal more often simply reacts to the human's actions.

The predominant reaction of many animals when exposed to humans is fear, and it has been proposed that this occurs because animals often perceive encounters with humans as predatory [27]. Fearful responses can lead to negative caretaker attitudes toward their charges, increasing the likelihood of poor interactions recurring. Given that people's attitudes towards cats are often ambivalent and that several openly express some dislike of cats [28], the caretaker-cat dynamic may be especially vulnerable and in need of consideration. An animal's fear of people can be reduced and desirable behaviors increased, even after receiving poor treatment, especially with attention to offering consistent positive

human-cat interactions. These include utilizing low-stress handling techniques or feeding preferred food items [29].

As with all other aspects of confinement, control and predictability of caretaker behaviors are of great importance to the animal's perceptions of humans [30]. Likewise, in effective cat management, a familiar person appears to be essential. Wild felids are considered to be sensitive to the captive environment, which can result in large numbers of abnormal behaviors and decreased reproductive success. This can be ameliorated by improved keeper-cat relationships. Mellen [31] noted a positive correlation between the quality of keeper interactions and increased reproductive success in small captive felids [32]. Wielebnowski et al. [33] found a negative correlation between fecal cortisol concentrations and the amount of time the primary keeper spent with clouded leopards and a positive correlation between fecal cortisol and the number of keepers. The interpretation of these results was that a higher number of keepers prevented the animals from forming and maintaining predictable relationships with any of the keepers, thus increasing the stress of captivity [33]. As a result, consistent, positive human-animal interactions may facilitate improved cat welfare.

Finally, the social environment is of great importance to cats. The social behavior of domestic cats exhibits great plasticity. It appears to be influenced by ontogeny such that kittens socialized to other cats, humans, dogs, and so forth during the sensitive period of socialization are likely to adapt to life in social groups more readily than are kittens raised by their mothers alone [34]. This social plasticity appears to be distributed across the family Felidae as illustrated by a study of 16 species of small Felidae from five lineages which found that the expression of affiliative behavior toward humans was widely distributed, rather than concentrated in the domestic cat lineage [35].

5. Welfare of Cats Confined in Cages

Each year millions of cats are housed in cages in veterinary hospitals, shelters, and research laboratories. Therefore, understanding aspects of the cage environment that facilitate or prohibit the ability of cats to cope may potentially impact the welfare of large numbers of individuals. Novelty, confinement and the inability to express species-typical behaviors may result in cats experiencing distress [32]. Their related responses may include decreased appetite, withdrawal from social groupings, increases in salivary, blood, and fecal cortisol, increases in urinary cortisol: creatinine ratios, decreases in grooming, and increases in the frequency and intensity of attempts to hide [10, 22, 36]. Medical interventions (e.g., vaccinations, treatment for parasites, and neutering), while potentially beneficial to the cat's physical health, can introduce additional stressors and thus impact the psychological health of the cat.

Because cats evolved in environments where hiding was an adaptive response to threat of predation, it is likely that "pet" cats also display such behavior in threatening environments, like veterinary hospitals and shelters. Because thwarting attempts to hide may contribute disproportionately to overall causes and related measures of stress [37], one form of environmental enrichment often suggested to help cats to cope with confinement has been provision of hiding and perching opportunities. For example, McCune [38] and Rochlitz [25] demonstrated that the ability to hide may be essential to cats when exposed to stressors [25, 38]. Hiding behavior, which is correlated with enhanced ACTH response and increased urinary cortisol concentrations, has been identified as a key indicator of cat stress [36]. These studies suggest that not allowing cats the opportunity to hide may adversely affect their welfare.

In shelters, the idea that allowing cats to hide decreases their chances of adoption often overrides this welfare concern. One study [39] aimed to determine if adding a hide box improved cats' abilities to cope with the environment, allowing the cat to become more comfortable and interactive with unfamiliar people. It was found that cats that were provided boxes approached more often and retreated less than did control cats (those with no box). They were also more often seen sleeping restfully than controls. In contrast, control cats exhibited more vigilance behavior, which is problematic as vigilance has been associated with anxiety-related behavior problems in house cats. Cats in the enriched group were observed in or on their hide boxes 77% of the time, whereas control cats attempted to hide 36% of the time. There was no difference in time to adoption between the groups, disproving the rationale for not providing cats with a hide box. Importantly, cats appeared to be coping, indicated by lower Cat Stress Scores, by day three, whereas control cats exhibited behaviors indicative of a change to chronic stress by the end of the two-week study period. Vinke et al. [40] likewise demonstrated the importance of affording cats the opportunity to hide as means of coping with environmental stressors [40]. Shelter cats provided with a Hide Perch and Go® box were found to acclimate more quickly to a new environment compared to those without a hiding area based on their Cat Stress Scores, suggesting that it provided an effective form of enrichment that facilitated coping [40].

It should be noted that the Cat Stress Score (CSS) is a tool that is often used to assess stress in cats, which describes seven possible stress levels based on cat body postures and behaviors (see [41] for details). Despite its frequent use, it has been proposed that what is really being measured is fear, as evidenced by the three highest scores being labeled as "fearful," "very fearful," and "terrorized." Additionally, it incorrectly assumes that there is a reliable and accurate way to "measure" stress in cats [42]. Therefore, this caveat should be considered when interpreting the results of studies utilizing such scoring.

Nonetheless, given the limited tools available for evaluating stress in cats, CSS was used in another study [43] examining the responses of cats in four different treatments: single housing with usual care, single housing with enrichment, communal housing with usual care, and communal housing with enrichment. Results indicated that CSS were similar in all groups on day one, but thereafter cats in single housing with usual care had higher CSS than all other groups. They also had the lowest adoption rates and the longest length of time waiting for adoption, and they exhibited more fearful behavior than did cats in the other groups. In this study both

housing and handling were manipulated, so either could have produced the effect seen.

In addition, Ottway and Hawkins [44] tested the hypothesis that cats in long-term shelter care housed in groups of unfamiliar conspecifics experience diminished welfare (higher CSS) due to unstable and inappropriate social groupings. A comparison of 12 adult cats unfamiliar with each other, communally housed in a large run, and cats that were either singly housed or pair housed with a familiar conspecific (former housemate) was conducted. The results indicated that the CSS was higher in cats housed communally than in cats housed in single units or with previously familiar conspecifics. Communally housed cats spent more time hiding than single housed cats (26% versus 4%). Play behavior was only observed in 1% of the observation periods and exclusively in singly housed cats or in cats housed with familiar conspecifics. It was therefore concluded that cats housed communally experienced higher levels of stress than cats housed in discrete units and they had more difficulty coping, probably due to the instability of the group, with unstable groups being more stressful than group living itself [44]. Similarly, de Monte and le Pape [45] concluded that for adult cats single housing may not be considered a "totally unfortunate housing situation," especially if the cats have daily positive interactions with humans [45].

The domestic cat has often been used as a model for those interested in identifying and addressing welfare problems in wild felids because these exotic species are easily distressed in captivity. Carlstead et al. [36] imposed a 21-day psychological stressor on singly housed domestic cats that included unpredictable caretaking and mildly aversive handling, a chronic psychological stressor for confined cats. Stressed cats exhibited decreased activity levels and increased attempts to hide compared to controls. They also had increased adrenocortical output (increased urine cortisol concentrations), enhanced adrenal sensitivity to ACTH, and reduced pituitary sensitivity to luteinizing hormone-releasing hormone. The researchers concluded that the environment led to activation of the stress response system in the cats and that hiding was an important behavior for modulating HPA axis activation caused by an unpredictable environment [36].

Similarly, McCobb et al. [46] evaluated stress levels among cats in usual and enriched housing via behavioral assessment (CSS) and monitoring of urine cortisol: creatinine ratios in four different shelters. Results indicated that cats housed in enriched environments had lower stress levels than those housed in traditional shelters. Stress levels among the cats were highest in the morning and decreased throughout the day. A slight negative correlation between the number of days spent in the shelter and the CSS was found with the CSS decreasing with increasing time spent in the shelter. In agreement, the mean morning CSS of the cats in the holding areas was higher than that of the cats in the adoption area. No differences were found between the CSS of owner surrenders and strays. Additionally, 24% of the cats had signs of systemic disease including upper respiratory infections, vomiting, and diarrhea. While no significant relationship was found between the noise level at the shelter and CSS, cats that were housed where they could see, hear, and/or smell dogs had higher urine cortisol: creatinine ratios. Additionally, almost 25% of cats had signs of systemic illness and more than 25% of the urine samples collected had trace amounts of hematuria. The authors concluded that the biggest factor affecting the cats' stress levels in the different types of shelters appeared to be the extent to which they were exposed to dogs. Cats in areas with more exposure to dogs had higher CSS than did cats in other high noise areas. Exposure to dogs appeared to have a cumulative effect on cat health when combined with other environmental stressors in that it increased stress levels more in cats that were obviously ill than in those that had no signs of disease [46]. Stella et al. [23] similarly observed distress in cats in noisy rooms with exposure to disturbances that included recorded sounds of dogs barking [23]. These findings provide strong evidence of the need for both enrichment and consistent management of the cat's environment, particularly with regard to noise to avoid causing cats undue distress and consequently adverse health conditions.

Rochlitz et al. [47] assessed the quarantine experience of cats over six months and observed that the cats required two to five weeks to acclimate to the quarantine situation. The authors concluded that hiding was an important behavior expressed by cats confronted with an aversive situation, such as a novel environment [47]. The withdrawal of friendly human contact was particularly distressful to cats used to receiving a lot of attention and may be important in shelter environments as well and potentially may be worse for owner surrender cats than for strays. Dybdall et al. [48] subsequently investigated this in a study designed to assess the social history of the cats admitted to the shelter. The CSS was used, and the observers were blinded to which group (owner surrender or stray) the cat belonged. Cats were scored for the first three days of housing while in the holding area. No effect of gender or neuter status was found. However, cats surrendered by their owners had higher CSS than did stray cats. Overall, cats that were deemed suitable for adoption had lower CSS than did cats that were deemed unsuitable and subsequently euthanized. Moreover, cats in the owner surrender group became ill significantly sooner than cats in the stray group did [48]. In agreement with the Rochlitz et al. [47] findings, this study indicated that all cats experienced a stress response associated with entry to the shelter, but the owner surrender cats may have experienced an additional psychosocial stressor of forced social separation from their primary caretakers and home environments. Alternatively, owner surrender cats may come from an unfavorable environment that led to behavior problems and relinquishment and may already be more distressed than strays at the time of admission.

Finally, Kessler and Turner [41] assessed cat acclimation to boarding over two weeks and compared the boarding cats' CSS to those of control cats living in a shelter. They evaluated single, pair, and group housing situations. The results indicated that two-thirds of the cats acclimated, one-third found boarding distressful, and 4% never acclimated. Thus, boarding was deemed inappropriate for that group. The daily CSS of the singly housed cats declined significantly from day one to day five, and overall stress levels continued to decrease during the two weeks of boarding [41]. However, in agreement with the findings of earlier studies they never reached

the level of the control cats. This is an important finding since cats in shelters may not have time to acclimate before being rehomed. In fact, most failed adoptions and returns take place within two weeks of adoption. The period of greatest risk for cats appears to fall within the time they are acclimating to the new environment, indicating that current protocols may not be sufficient to allow cats to fully adjust to the new environment and thus impact cat welfare.

6. Conclusions

In summary, it has been suggested that cats do not meet all the criteria for domestication and may best be described as "exploited captives" [49]. Confinement of cats, in homes or other environments, may lead to poor welfare through inadequate environments that do not meet the needs of cats. Ultimately, the environmental needs of the cat are similar whether they are confined to a home or a cage in a shelter, research facility, veterinary hospital, or boarding facility. Aspects of the environment that can be perceived as potential threats or aversive stimuli whether parts of the macro- or microenvironment, human-animal interactions, the social environment, or the predictability and control of the environment all interact to influence a cat's well-being. Poor welfare may be reflected in poor physical health, illness, and disease or behavioral problems such as house soiling and fearful and aggressive behaviors. These factors may lead to a breakdown in the human-animal bond and ultimately to abandonment, relinquishment to a shelter, or euthanasia and thus require further investigation. Research is needed to refine recommendations for the quantity of space needed by confined cats kept both singly and in groups and to better understand the interactions between quantity and quality of space provided to cats. Simple enhancements to improve the quality of cats' living quarters via enrichment such as hiding areas may yield many beneficial effects. Studies on the short- and long-term effects of improving the quality of the housing environment and human-cat interactions on adoption rates, retention outcomes, and even infectious disease incidence are needed to improve cat well-being. In addition, research focused on identifying and understanding the effects of individual differences in coping styles could lead to further improvements in cat welfare. Finally, the etiology and role of owners' attitudes and knowledge about cats are needed to reduce risks to the human-animal bond and to optimize cat well-being.

Competing Interests

The authors declare no competing interests.

References

[1] American Society for the Prevention of Cruelty to Animals, *Pet Statistics*, 2012, https://www.aspca.org/about-us/faq/pet-statistics.

[2] The Humane Society of the United States, *Pets by the Numbers*, 2012, http://www.humanesociety.org/issues/pet_overpopulation/facts/pet_ownership_statistics.html.

[3] NCPPSP, The National Council on Pet Population Study and Policy 2012, http://www.petpopulation.org.

[4] R. A. Casey, S. Vandenbussche, J. W. S. Bradshaw, and M. A. Roberts, "Reasons for relinquishment and return of domestic cats (*Felis Silvestris Catus*) to rescue shelters in the UK," *Anthrozoos*, vol. 22, no. 4, pp. 347–358, 2009.

[5] G. J. Patronek, L. T. Glickman, A. M. Beck, G. P. McCabe, and C. Ecker, "Risk factors for relinquishment of cats to an animal shelter," *Journal of the American Veterinary Medical Association*, vol. 209, no. 3, pp. 582–588, 1996.

[6] M. D. Salman, J. Hutchison, R. Ruch-Gallie et al., "Behavioral reasons for relinquishment of dogs and cats to 12 shelters," *Journal of Applied Animal Welfare Science*, vol. 3, no. 2, pp. 93–106, 2000.

[7] J. C. New, M. D. Salman, M. King, J. M. Scarlett, P. H. Kass, and J. M. Hutchison, "Characteristics of shelter-relinquished animals and their owners compared with animals and their owners in U.S. pet-owning households," *Journal of Applied Animal Welfare Science*, vol. 3, no. 3, pp. 179–201, 2000.

[8] M. Morgan and K. A. Houpt, "Feline behavior problems: the influence of declawing," *Anthrozoos*, vol. 3, no. 1, pp. 50–53, 1989.

[9] E. Heidenberger, "Housing conditions and behavioural problems of indoor cats as assessed by their owners," *Applied Animal Behaviour Science*, vol. 52, no. 3-4, pp. 345–364, 1997.

[10] J. L. Stella, L. K. Lord, and C. A. T. Buffington, "Sickness behaviors in response to unusual external events in healthy cats and cats with feline interstitial cystitis," *Journal of the American Veterinary Medical Association*, vol. 238, no. 1, pp. 67–73, 2011.

[11] M. Amat, J. L. R. de la Torre, J. Fatjó, V. M. Mariotti, S. Van Wijk, and X. Manteca, "Potential risk factors associated with feline behaviour problems," *Applied Animal Behaviour Science*, vol. 121, no. 2, pp. 134–139, 2009.

[12] P. Leyhausen and K. Lorentz, *Motivation of Human and Animal Behavior*, Van Nostrand Reinhold Company, New York, NY, USA, 1973.

[13] D. Turner, "The human-cat relationship," in *The Domestic Cat: The Biology of Its Behaviour*, D. C. Turner and P. Bateson, Eds., pp. 193–206, Cambridge University Press, Cambridge, UK, 2000.

[14] K. N. Morgan and C. T. Tromborg, "Sources of stress in captivity," *Applied Animal Behaviour Science*, vol. 102, no. 3-4, pp. 262–302, 2007.

[15] National Research Council (NRC), *Nutrient Requirements for Dogs and Cats*, National Academy Press, Washington, DC, USA, 2006.

[16] J. L. Stella and T. Buffington, "Environmental strategies to promote health and wellness," in *August's Consultations in Feline Internal Medicine*, S. Little, Ed., vol. 7, chapter 73, pp. 718–736, Elsevier, 1st edition, 2016.

[17] R. S. Heffner and H. E. Heffner, "Hearing range of the domestic cat," *Hearing Research*, vol. 19, no. 1, pp. 85–88, 1985.

[18] C. L. Coppola, R. M. Enns, and T. Grandin, "Noise in the animal shelter environment: building design and the effects of daily noise exposure," *Journal of Applied Animal Welfare Science*, vol. 9, no. 1, pp. 1–7, 2006.

[19] A. L. Baldwin, G. E. Schwartz, and D. H. Hopp, "Are investigators aware of environmental noise in animal facilities and that this noise may affect experimental data?" *Journal of the American Association for Laboratory Animal Science*, vol. 46, no. 1, pp. 45–51, 2007.

[20] NRC, *National Research Council: Guide for the Care and Use of Laboratory Animals*, National Academy Press, Washington, DC, USA, 2013.

[21] Association of Shelter Veterinarians, *Guidelines for Standards of Care in Animal Shelters*, 2014, http://www.sheltervet.org/assets/docs/shelter-standards-oct2011-wforward.pdf.

[22] J. Stella, C. Croney, and T. Buffington, "Effects of stressors on the behavior and physiology of domestic cats," *Applied Animal Behaviour Science*, vol. 143, no. 2–4, pp. 157–163, 2013.

[23] J. Stella, C. Croney, and T. Buffington, "Environmental factors that affect the behavior and welfare of domestic cats (*Felis silvestris catus*) housed in cages," *Applied Animal Behaviour Science*, vol. 160, no. 1, pp. 94–105, 2014.

[24] J. Stella, *Environmental factors that affect the behavior and welfare of domestic cats (Felis silvestris catus) housed in cages [Ph.D. thesis]*, The Ohio State University, 2013.

[25] I. Rochlitz, "Recommendations for the housing and care of domestic cats in laboratories," *Laboratory Animals*, vol. 34, no. 1, pp. 1–9, 2000.

[26] F. McMillan, "Stress-induced and emotional eating in animals: a review of the experimental evidence and implications for companion animal obesity," *Journal of Veterinary Behavior: Clinical Applications and Research*, vol. 8, no. 5, pp. 376–385, 2013.

[27] S. Waiblinger, X. Boivin, V. Pedersen et al., "Assessing the human-animal relationship in farmed species: a critical review," *Applied Animal Behaviour Science*, vol. 101, no. 3-4, pp. 185–242, 2006.

[28] S. R. Kellert, "American's attitudes and knowledge of animals," in *Proceedings of the Transaction of the 45th North American Wildlife and Natural Resources Conference*, vol. 45, pp. 111–124, 1980.

[29] A. M. Claxton, "The potential of the human-animal relationship as an environmental enrichment for the welfare of zoo-housed animals," *Applied Animal Behaviour Science*, vol. 133, no. 1-2, pp. 1–10, 2011.

[30] P. H. Hemsworth, J. L. Barnett, and C. Hansen, "The influence of inconsistent handling by humans on the behaviour, growth and corticosteroids of young pigs," *Applied Animal Behaviour Science*, vol. 17, no. 3-4, pp. 245–252, 1987.

[31] J. D. Mellen, "Factors influencing reproductive success in small captive exotic felids (*Felis* spp.): a multiple regression analysis," *Zoo Biology*, vol. 10, no. 2, pp. 95–110, 1991.

[32] D. M. Broom and K. Johnson, *Stress and Animal Welfare*, Kluwer Academic, Boston, Mass, USA, 2nd edition, 2000.

[33] N. C. Wielebnowski, N. Fletchall, K. Carlstead, J. M. Busso, and J. L. Brown, "Noninvasive assessment of adrenal activity associated with husbandry and behavioral factors in the North American clouded leopard population," *Zoo Biology*, vol. 21, no. 1, pp. 77–98, 2002.

[34] M. Mendl and R. Harcourt, "Individuality in the domestic cat: origins, development and stability," in *The Domestic Cat: The Biology of Its Behavior*, D. C. Turner and P. Bateson, Eds., pp. 47–64, Cambridge University Press, Cambridge, UK, 2000.

[35] C. Cameron-Beaumont, S. E. Lowe, and J. W. S. Bradshaw, "Evidence suggesting preadaptation to domestication throughout the small Felidae," *Biological Journal of the Linnean Society*, vol. 75, no. 3, pp. 361–366, 2002.

[36] K. Carlstead, J. L. Brown, and W. Strawn, "Behavioral and physiological correlates of stress in laboratory cats," *Applied Animal Behaviour Science*, vol. 38, no. 2, pp. 143–158, 1993.

[37] K. L. Overall and D. Dyer, "Enrichment strategies for laboratory animals from the viewpoint of clinical veterinary behavioral medicine: emphasis on cats and dogs," *ILAR Journal*, vol. 46, no. 2, pp. 202–215, 2005.

[38] S. McCune, "The impact of paternity and early socialisation on the development of cats' behaviour to people and novel objects," *Applied Animal Behaviour Science*, vol. 45, no. 1-2, pp. 109–124, 1995.

[39] K. Kry and R. Casey, "The effect of hiding enrichment on stress levels and behaviour of domestic cats (*Felis sylvestris catus*) in a shelter setting and the implications for adoption potential," *Animal Welfare*, vol. 16, no. 3, pp. 375–383, 2007.

[40] C. M. Vinke, L. M. Godijn, and W. J. R. van der Leij, "Will a hiding box provide stress reduction for shelter cats?" *Applied Animal Behaviour Science*, vol. 160, no. 1, pp. 86–93, 2014.

[41] M. R. Kessler and D. C. Turner, "Stress and adaptation of cats (*Felis Silvestris Catus*) housed singly, in pairs and in groups in boarding catteries," *Animal Welfare*, vol. 6, no. 3, pp. 243–254, 1997.

[42] F. D. McMillan, "Stress versus fear in cats," *Journal of the American Veterinary Medical Association*, vol. 240, no. 8, p. 936, 2012.

[43] N. Gourkow and D. Fraser, "The effect of housing and handling practices on the welfare, behaviour and selection of domestic cats (*Felis sylvestris catus*) by adopters in an animal shelter," *Animal Welfare*, vol. 15, no. 4, pp. 371–377, 2006.

[44] D. S. Ottway and D. M. Hawkins, "Cat housing in rescue shelters: a welfare comparison between communal and discrete-unit housing," *Animal Welfare*, vol. 12, no. 2, pp. 173–189, 2003.

[45] M. de Monte and G. le Pape, "Behavioural effects of cage enrichment in single-caged adult cats," *Animal Welfare*, vol. 6, no. 1, pp. 53–66, 1997.

[46] E. C. McCobb, G. J. Patronek, A. Marder, J. D. Dinnage, and M. S. Stone, "Assessment of stress levels among cats in four animal shelters," *Journal of the American Veterinary Medical Association*, vol. 226, no. 4, pp. 548–555, 2005.

[47] I. Rochlitz, A. L. Podberscek, and D. M. Broom, "Welfare of cats in a quarantine cattery," *Veterinary Record*, vol. 143, no. 2, pp. 35–39, 1998.

[48] K. Dybdall, R. Strasser, and T. Katz, "Behavioral differences between owner surrender and stray domestic cats after entering an animal shelter," *Applied Animal Behaviour Science*, vol. 104, no. 1-2, pp. 85–94, 2007.

[49] J. Clutton-Brock, *A Natural History of Domesticated Mammals*, Cambridge University Press, Cambridge, UK, 2nd edition, 1999.

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 1901679, 7 pages
http://dx.doi.org/10.1155/2016/1901679

Research Article

Body Condition Scores and Evaluation of Feeding Habits of Dogs and Cats at a Low Cost Veterinary Clinic and a General Practice

Stephanie A. Sapowicz, Deborah E. Linder, and Lisa M. Freeman

Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA 01536, USA

Correspondence should be addressed to Deborah E. Linder; deborah.linder@tufts.edu

Received 15 April 2016; Revised 8 August 2016; Accepted 15 August 2016

Academic Editor: Agnieszka Rak-Mardyła

This study assessed body condition scores (BCS) and feeding habits for dogs and cats. Eighty-six cats and 229 dogs (and their owners) were enrolled from 2 clinics: a low cost clinic ($n = 149$) and a general practice ($n = 166$). BCS and body weight were recorded. Owners completed a survey which included animal age, sex, and breed; owner demographics; and feeding practices (e.g., diet, rationale for feeding practices). Owners from the low cost clinic had a significantly lower income ($P < 0.001$) and education ($P < 0.001$) compared to those from the general practice. Animals from the low cost clinic were younger ($P < 0.001$) and dogs were less likely to be neutered ($P < 0.001$). Overweight prevalence was 55% overall ($P = 0.083$), with a significantly higher prevalence in the general practice for cats (44% versus 66%; $P = 0.046$), but not for dogs (58% versus 53%; $P = 0.230$). Multivariate analysis showed that only neuter status was significantly associated with BCS ($P = 0.004$). Veterinarians were the most common source of nutritional information, though lack of accurate nutrition knowledge was common among all participants. These findings support the need for enhanced communication about optimal BCS and nutrition regardless of socioeconomic status.

1. Introduction

Pet obesity is a serious and growing concern, with up to 60% of the cat and dog population being overweight or obese [1, 2]. Guidelines for the prevention and treatment of obesity have recently been published [3]. However, effective obesity prevention and treatment require a better understanding of owner attitudes on nutrition and feeding habits so that programs can be tailored to the individual owner and animal.

A study investigating environmental factors associated with obesity in dogs showed that lower owner income and older age, as well as frequency of treats, were risk factors for overweight and obesity [2]. However, the feeding habits and reasons behind the higher risk of this population remain largely unknown. In order to effectively communicate and implement healthy weight management in cats and dogs, more information is needed to understand the feeding habits of pet owners from various income and education levels.

Therefore, the objective of the current study was to evaluate pet obesity prevalence and owner feeding habits from 2 veterinary practices: a low cost clinic and a private general practice. Results may be useful to identify potential barriers to optimal nutrition among different populations of pets and could help guide client communication and education outreach, particularly in underserved communities.

2. Materials and Methods

2.1. Study Participants. The study population included animals and their owners from 2 veterinary clinics: a private general practice veterinary clinic in central Massachusetts and a low cost veterinary clinic run by Cummings School of Veterinary Medicine at Tufts University that provides subsidized care for pets within central Massachusetts underserved communities. The low cost clinic has an income prequalification for clients, including documentation of government assistance for food or housing. All owners with animals that presented to the clinics during the study period (June–August 2013) were asked to participate. Pregnant and lactating animals and those under 1 year of age were excluded. Animals also were excluded if their owner was under 18 years of age or was not the primary caretaker for the animal. For owners

who consented to participate in the study, BCS (on a 1–9 scale) was assigned by a single investigator (SAS) and body weight was recorded [4, 5]. The owners completed a survey about their pet, feeding habits, and rationale for feeding practices. The survey included questions on feeding habits, attitudes towards feeding, important sources of pet nutrition information, and owner and animal demographic information. Owner demographic information collected included the owner's age, sex, income level, and highest level of education completed, while animal demographic information included age, sex, neuter status, and breed. Questions regarding feeding practices included the type of diet fed (home-cooked, commercial dry, or commercial canned), percentage of diet coming from treats or table foods, frequency of meals, and use of supplements. Questions also were included to assess where owners obtained information about pet nutrition, how they determined the amount or type of food to feed to their pet, how many calories their pets required, and factors important in their decision to purchase a commercial pet food (full survey available upon request).

This study was approved by the Tufts University Institutional Review Board and the Cummings School Clinical Studies Review Committee.

2.2. Statistical Analysis. Data were assessed for normality using the Kolmogorov-Smirnov test. Since data were all normally distributed, data will be presented as mean ± SD. Categorical data were compared between the two locations using chi-square analysis, while continuous data were compared using independent t-tests. Multivariate analysis was performed by creating a general linear model with BCS as the outcome variable. Commercial statistical software (SPSS 22.0, IBM Corporation, Armonk, NY) was used for all analyses, and a P value < 0.05 was considered significant.

3. Results

3.1. Owner Demographics. Three hundred and fifteen animals and their owners were enrolled from June to August 2013: $n = 166$ from the low cost clinic and $n = 149$ from the general practice. The low cost clinic owners were younger ($P = 0.010$; Table 1), had significantly lower household income ($P < 0.001$), and completed less education ($P < 0.001$) than owners from the general practice. There was no significant difference in sex of the owners, with a predominance of females at both locations (77% overall).

3.2. Animal Demographics. For both dogs and cats, animals at the low cost clinic were significantly younger (dogs: $P = 0.004$; cats: $P = 0.022$; Tables 2 and 3). Dogs ($P < 0.001$), but not cats ($P = 0.147$), at the low cost clinic were more likely to be intact, with only 6 of 102 (6%) of dogs at the general practice being intact, while 46 of 117 dogs (39%) at the low cost clinic were intact. The distribution of dog breeds was different between the 2 clinics ($P = 0.019$), with the low cost clinic having more American Pit Bull Terriers and Chihuahuas. Dogs ($P = 0.035$), but not cats ($P = 0.267$), at the low cost clinic had a significantly lower body weight than those from the general practice. However, cats ($P = 0.023$), but not dogs ($P = 0.248$), at the low cost clinic had a significantly lower mean BCS. In addition, the cats at the low cost clinic had a significantly lower prevalence of overweight or obesity (i.e., BCS > 5/9; 44% at the low cost clinic compared to 66% at the general practice; $P = 0.046$). The prevalence of overweight and obesity for dogs was not significantly different between clinics (58% versus 53%; $P = 0.230$). Multivariate analysis, which included site, owner demographics, and pet demographics, showed that only neuter status was significantly associated with BCS ($P = 0.004$).

Table 1: Summary of owner demographics from 315 animals enrolled from two veterinary clinics.

Variable	General practice	Low cost clinic	P value
n	149	166	—
Age			0.010
18–30 years	22	29	—
30–45 years	36	57	—
45–60 years	56	61	—
>60 years	32	15	—
Sex			0.157
Male	39	32	—
Female	108	130	—
Annual income			<0.001
<$10,000	2	52	—
$10,000–29,000	4	81	—
$30,000–49,000	11	17	—
$50,000–74,000	17	7	—
$75,000–100,000	20	1	—
>$100,000	80	2	—
Education			<0.001
Some high school	0	13	—
High school graduate	20	105	—
College graduate	76	40	—
Graduate degree	50	5	—

3.3. Feeding Habits. There were some differences in feeding habits between dog and cat owners and between the 2 clinics (Table 4). Most owners at both clinics fed primarily dry food, but the percentage of dry food was lower and the percentage of table food and home-cooked food was higher at the low cost clinic. In addition, more owners at the low cost clinic (40% total for dogs and cats) reported leaving food available at all times for their animals compared to those at the general practice (20% total for dogs and cats). However, most owners at both clinics (52%) fed their animals twice daily. Sixty-two percent of owners at both clinics (194/311) reported feeding treats at least once daily. The most common types of treats were commercial treats ($n = 209$), chews ($n = 127$), fruits/vegetables ($n = 86$), meat/cheese ($n = 74$), peanut butter ($n = 56$), and others ($n = 30$). Commercial treats were more common at the general practice, while peanut butter was more commonly provided as a treat at the low cost

Table 2: Summary of dog demographic information from 229 dogs enrolled from two veterinary clinics (absolute number or mean ± SD). Only breeds reported more than 5 times were included in the table.

Variable	General practice	Low cost clinic	*P* value
n	107	122	—
Age (yrs)	6.8 ± 3.8	5.4 ± 3.5	0.004
Sex			<0.001
Male	52 (47 castrated)	58 (34 castrated)	—
Female	50 (49 spayed)	59 (37 spayed)	—
Breed			0.019
Mixed breed	36	43	—
Am. Pit Bull Terrier	2	13	—
Labrador Retriever	8	5	—
Chihuahua	0	9	—
Shih-tzu	3	5	—
Beagle	5	2	—
Golden Retriever	7	0	—
Pug	2	4	—
German Shepherd	3	2	—
Body weight (kg)	22.0 ± 14.9	17.9 ± 14.5	0.035
Body condition score	6.0 ± 1.2	5.8 ± 1.3	0.248
Percent overweight	62/107 (58%)	65/122 (53%)	0.230

The scale is a 1–9 scale; Am.: American.

Table 3: Summary of cat demographic information from 86 cats enrolled from two veterinary clinics (absolute number or mean ± SD). Only breeds reported more than 5 times were included in the table.

Variable	General practice	Low cost clinic	*P* value
n	42	44	—
Age (yrs)	8.3 ± 3.9	6.2 ± 4.6	0.022
Sex			0.147
Male	16 (15 castrated)	19 (10 castrated)	—
Female	25 (24 spayed)	23 (18 spayed)	—
Breed			
DSH/DLH	34	37	0.337
Body weight (kg)	5.5 ± 1.6	4.9 ± 2.7	0.267
Body condition score	6.5 ± 1.7	5.6 ± 1.8	0.023
Percent overweight	27/41 (66%)	19/43 (44%)	0.046

The scale is a 1–9 scale; DSH/DLH: domestic shorthair/domestic longhair.

clinic. Most types of treats were more commonly provided to dogs compared to cats. Supplements were administered to 31/149 (21%) animals from the general practice and 21/166 (13%) animals from the low cost clinic ($P = 0.052$), with the most common supplements being joint supplements, fatty acids, and multivitamins. Joint supplements were more commonly used at the general practice compared to the low cost clinic and in dogs compared to cats. There also were species differences for types of food fed, feeding frequency, supplement use, and frequency of treats (Table 4).

Of the entire population at both sites, only 4 owners reported knowing how many calories their animal required ($n = 2$ at each site). However, 2 of the 4 responses were not sustainable for life for their animals' sizes. At both clinics, most owners reported looking for their veterinarian for advice on how much to feed [$n = 131$ (42%)], followed by the pet food feeding directions [$n = 93$ (30%)]. Twenty-one percent of owners (65/315) reported feeding an amount based on whether or not the pet looks hungry. This response was significantly more common at the low cost clinic compared to the general practice ($P = 0.007$) and for cats compared to dogs ($P = 0.003$). Veterinarians were the most frequent response as the source for nutrition information at both clinics and for both species.

The 5 most commonly reported responses for important factors in an owner's decision to select a diet for his or her pet were that the food was healthy for the pet [154/315 (49%)], ingredients [146/315 (46%)], pet preference [143/315 (45%)], cost [103/315 (33%)], and pet health needs [96/315 (31%)] (Table 5). A significantly higher proportion of owners at the low cost clinic responded that pet preference was an important factor in selecting the diet compared to the general practice ($P < 0.001$). The manufacturer's reputation was reported to be an important factor by significantly more owners at the general practice (31–39%) compared to the low cost clinic (22–27%; $P = 0.009$). There were no significant differences between clinics in the percentage of owners that chose other factors, such as cost (28–39%), availability (16–21%), convenience (6–11%), or being natural (10-11%) as a factor in choosing which diet to feed. For cat owners, pet health needs ($P = 0.008$), availability ($P = 0.005$), and convenience ($P = 0.037$) were reported more frequently as being important factors in selecting a diet compared to dog owners.

4. Discussion

The prevalence of overweight and obesity in the current study was high, in the range 53–58% for dogs and 44–66% for cats, depending on the site. Lack of nutritional knowledge, such as not knowing calories fed or selecting food based solely on ingredients, was common at both clinics. Multivariate analysis showed that the only variable independently associated with BCS was neuter status, with intact animals less likely to be overweight when compared to their neutered counterparts. This is not surprising since neutering is associated with an increase in appetite and a decrease in calorie requirements [6, 7]. Therefore, if veterinarians do not give specific instructions to reduce animals' calorie intake at the time of neutering or if owners are noncompliant, animals will be at higher risk for the development of overweight and obesity. Since the clinic (low cost versus general) was not significantly associated with BCS on multivariate analysis, the high rate of overweight and obesity appears to be a widespread problem that is not limited to one type of practice. Cats at the low cost clinic did have a lower prevalence of obesity compared to those at the general practice (44% versus 66%). The reason for this difference is unclear but may be related to underlying medical conditions, different

Table 4: Summary of responses from 229 dog and 86 cat owners from two veterinary clinics (general practice, $n = 149$; low cost clinic, $n = 166$). Number of owners providing each response, with percentage in parentheses or median (range).

Variable	General practice		Low cost clinic		P value (clinic)	P value (species)
	Dogs	Cats	Dogs	Cats		
n	107	42	122	44	—	—
Percent food type						
Dry	96 (0–100)	97 (0–100)	90 (0–100)	80 (0–100)	0.017	0.393
Canned	0 (0–100)	1 (0–100)	0 (0–100)	15 (0–100)	0.972	<0.001
Table food	0 (0–20)	0 (0–2)	0 (0–80)	0 (0–5)	<0.001	<0.001
Home-cooked	0 (0–100)	0 (0-0)	0 (0–100)	0 (0-0)	0.011	<0.001
Commercial raw	0 (0-1)	0 (0-0)	0 (0–10)	0 (0-0)	0.075	0.540
Home-prepared raw	0 (0-0)	0 (0-0)	0 (0–10)	0 (0-0)	0.343	0.102
Feeding frequency					0.001	<0.001
Ad libitum	13 (12%)	16 (38%)	36 (30%)	30 (68%)	—	—
One time daily	13 (12%)	6 (14%)	21 (17%)	2 (5%)	—	—
Two times daily	77 (72%)	17 (41%)	61 (50%)	8 (18%)	—	—
Three times daily	3 (3%)	3 (7%)	4 (3%)	3 (7%)	—	—
Treats at least once daily	76 (72%)	10 (24%)	92 (75%)	16 (37%)	0.234	<0.001
Treat types*						
Commercial	81	30	67	31	0.004	0.292
Chews	55	1	71	0	0.349	<0.001
Fruits/vegetables	37	1	47	1	0.497	<0.001
Meat/cheese	29	1	41	3	0.183	<0.001
Peanut butter	19	0	35	2	0.027	<0.001
Other	13	3	12	2	0.487	0.169
Any supplements*	29 (27%)	2 (5%)	19 (16%)	2 (5%)	0.052	0.001
Joint supplements	17 (16%)	2 (5%)	4 (3%)	0 (0%)	<0.001	0.037
Fatty acids	9 (8%)	0 (0%)	7 (6%)	0 (0%)	0.462	0.012
Multivitamins	3 (3%)	0 (0%)	4 (3%)	0 (0%)	0.812	0.101
Probiotics	4 (4%)	0 (0%)	1 (1%)	0 (0%)	0.140	0.167
Herbal supplements	2 (2%)	0 (0%)	2 (2%)	2 (5%)	0.913	0.217
Other	0 (0%)	0 (0%)	2 (2%)	1 (2%)	0.100	0.814

*Owners could select more than 1 answer.

relationships between people and their cats compared to dogs, or younger age of animals at the low cost clinic.

Given the high prevalence of overweight and obesity, even in the low cost clinic population in which fewer animals were neutered, higher rates might occur if more owners elected to neuter their animals. This is an important issue to consider since there is an emphasis in the United States on neutering dogs and cats. The veterinary healthcare team is a critical intervention point, and education on the importance of reducing calorie intake at the time of neutering should be emphasized. The high prevalence of overweight dogs and cats at both practices also underscores the need for veterinarians to perform nutritional screening on all animals at every visit to assess body weight, BCS, muscle condition score, and diet history [8, 9]. If animals do not have an ideal BCS (i.e., 4-5/9), the diet history usually provides important clues for sources of excess calories, and these issues should be discussed with the owner to determine the best approach to achieve safe and effective weight loss.

Results from the current study identified issues that could be specifically addressed with owners of overweight and obese animals. For example, owners at the low cost clinic were more likely to feed *ad libitum* compared to those from the general practice, a factor that may contribute to intake of excessive calories. In addition, 73% of dog owners and 30% of cat owners at both clinics responded that they gave treats, with most offering them at least once daily. Owners at the low cost clinic were also more likely to feed home-cooked foods as part of the diet. Given that the vast majority of home-cooked diets are nutritionally unbalanced [10–12], this could contribute to a nutritionally unbalanced diet in addition to excessive calories. These are issues that would be readily identified from a diet history and addressed with specific recommendations [9].

Table 5: Summary of responses from 229 dog and 86 cat owners from two veterinary clinics (general practice, $n = 149$; low cost clinic, $n = 166$). Number of owners providing each response, with percentage in parentheses.

Variable	General practice		Low income clinic		P value (clinic)	P value (species)
	Dogs	Cats	Dogs	Cats		
n	107	42	122	44	—	—
Know how many calories their pet eats daily	1 (1%)	1 (2%)	1 (1%)	1 (2%)	0.927	0.450
Source for how much to feed*†						
Veterinarian	66 (61%)	17 (41%)	38 (31%)	10 (23%)	<0.001	0.291
Product feeding directions	38 (36%)	14 (33%)	31 (25%)	10 (23%)	0.048	0.724
Pet looks hungry	12 (11%)	9 (21%)	25 (21%)	19 (43%)	0.007	0.003
Veterinarian as source for nutrition information	95 (89%)	34 (81%)	93 (76%)	32 (73%)	0.284	0.644
Factors in selecting a diet*						
Food is healthy for the pet‡	56 (52%)	21 (50%)	59 (41%)	18 (41%)	0.494	0.395
Ingredients	58 (54%)	16 (38%)	55 (45%)	17 (39%)	0.264	0.460
Pet preference	33 (31%)	17 (41%)	63 (52%)	30 (68%)	<0.001	0.058
Cost	30 (28%)	13 (31%)	43 (35%)	17 (39%)	0.169	0.688
Pet health needs	35 (33%)	16 (38%)	25 (21%)	18 (41%)	0.063	0.008
Manufacturer	42 (39%)	13 (31%)	27 (22%)	12 (27%)	0.009	0.490
Availability	18 (17%)	13 (31%)	14 (12%)	13 (30%)	0.299	0.005
Natural	15 (14%)	2 (5%)	13 (11%)	4 (9%)	0.739	0.769
Convenience	6 (6%)	3 (7%)	9 (7%)	9 (21%)	0.128	0.037

*Owners could select more than 1 answer.
†Only the 3 most common answers are shown.
‡"Healthy" was not defined for owners and could have been selected for any reason the owner thought food was healthy for the pet.

Supplements were fed to 17% of animals overall, with use being more common in dogs compared to cats. The most commonly used supplement was joint supplements, with 8% of dogs and 5% of cats using this supplement. This overall prevalence of supplement use is higher than that reported in a large multicenter study of dogs and cats conducted in the United States and Australia in which 9.9% of animals were receiving a supplement [13]. Those data were collected in 2004 so it is unclear whether the populations are different or whether there has been an overall increase in supplement use in pets. In the current study, joint supplements were more commonly used in dogs compared to cats and in the general practice compared to the low cost clinic. This may have been the result of population differences between the 2 clinics (i.e., older, large breed dogs).

Owners from both practices were similar in terms of their reasons for choosing a pet food, with both groups relying most commonly on the ingredient list, which has been shown in previous studies [13–15]. Despite a significantly lower income level for owners from the low cost clinic, there was no significant difference between clinics in the percentages of owners who reported cost or convenience as an important factor in choosing pet food. Another study found that cost was a moderately important factor in selecting pet food and that owners of overweight dogs found cost and special offers of dog food more important than owners of healthy weight dogs [15]. Pet preference in selecting a diet was also found to be an important factor in this previous study, as well as the current study, though no difference was found between owners of healthy weight and overweight dogs. The high importance placed on factors that are not evidence-based suggests that education of various populations of owners should focus on similar factors, that is, collecting diet history information at every visit, teaching owners more objective ways to select pet food than using the ingredient list or pet preference [9], and making specific recommendations for which foods and amounts to feed.

Feeding directions appear to be a particularly important area in need of owner education because many owners used the feeding directions from the pet food label (which are not always a good estimation of an individual animal's needs) or based the amount to feed on whether their animal looked hungry. Veterinarians should provide feeding instructions that help the animal to maintain an ideal BCS; this information is likely to be well accepted because owners from both clinics reported the veterinarian to be 1 of the top 3 most commonly used resources for nutrition (though less commonly at the low cost clinic). Previous studies showed similar results that pet owners perceive veterinarian advice as an important factor in feeding their pet [15]. The result from the current study that only 4 of 315 owners (1.3%) knew how many calories their animals needed [with only 2 of 315 (0.6%) being physiologically possible] suggests that owners need much more education on their pets' calorie needs, how much to feed, and other sources of calories in the diet.

Although there was a relatively large and diverse population included in the study, there are a number of differences in the animals and in the owners between clinics that could

have contributed to bias. Mean body weight was higher and breeds were significantly different, which may be due to the urban versus suburban location of the low cost and general practice, respectively. In addition, owners at the low cost clinic practice had lower household incomes, received fewer years of education, and were younger than owners from the general practice. It would be useful to compare obesity rates in clinics with similar owner and animal demographics in future studies; however, in the current study, owner income, education, and age and dog age, breed, and weight were not significantly associated with BCS on multivariate analysis.

There are a number of additional limitations to the study. Animals enrolled in the study were those being presented to the 2 clinics during the study period and could include both healthy animals and those with medical conditions. Underlying medical issues could influence BCS, body weight, and diet selection so the results from the current study may not be generalizable to a population of healthy animals. However, it likely represents a typical population of animals seen by a veterinarian in general practice. Owners were asked to provide answers regarding pet food while away from their homes, so responses regarding types and amounts of food provided to their animals may not have been accurate.

Despite these limitations, the results suggest that overweight and obesity are common in at least 2 populations of dogs and cats and that this was not significantly related to age, income, and education level of the owner; to the clinic; or to animal factors, such as age or sex, other than neuter status. Most owners at both clinics used the relatively useless ingredient list to decide what to feed, instead of the guidelines set forth in recommendations by the World Small Animal Veterinary Association Global Nutrition Committee, which includes ensuring that the pet food manufacturer has a full-time qualified veterinary nutritionist and assessing the nutritional adequacy statement on the pet food to ensure the food is complete and balanced [9].

In addition, pet owners did not know how many calories their animals needed and relied on feeding directions or their perception of their animals' hunger. This suggests that there are important gaps and missed opportunities for the veterinarian and the veterinary healthcare team to be the primary resource for sound nutritional advice.

5. Conclusion

Overweight and obesity are common in at least 2 socioeconomic populations of dogs and cats, which was not significantly related to owner, clinic, or animal factors, other than neutering. Veterinarians were viewed as important resources for owners from both clinics, which provides an opportunity to assess pets' nutritional status and to provide accurate nutritional information to owners. This is especially important at the time of neutering since overweight and obesity were more common in neutered animals. Additional research on effectiveness of education programs is needed to prevent and treat obesity and to optimize pet health.

Abbreviations

BCS: Body condition score.

Disclosure

This work was presented in abstract form at the 2014 American Academy of Veterinary Nutrition Symposium, Nashville, TN, June 4, 2014.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This project was supported by a student grant from Nestlé Purina PetCare. The project described was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, Award no. KL2TR001063. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

[1] E. A. Courcier, R. O'Higgins, D. J. Mellor, and P. S. Yam, "Prevalence and risk factors for feline obesity in a first opinion practice in Glasgow, Scotland," *Journal of Feline Medicine and Surgery*, vol. 12, no. 10, pp. 746–753, 2010.

[2] E. A. Courcier, R. M. Thomson, D. J. Mellor, and P. S. Yam, "An epidemiological study of environmental factors associated with canine obesity," *Journal of Small Animal Practice*, vol. 51, no. 7, pp. 362–367, 2010.

[3] D. Brooks, J. Churchill, K. Fein et al., "2014 AAHA weight management guidelines for dogs and cats," *Journal of the American Animal Hospital Association*, vol. 50, no. 1, pp. 1–11, 2014.

[4] D. Laflamme, "Development and validation of a body condition score system for dogs," *Canine Practice*, vol. 22, pp. 10–15, 1997.

[5] D. Laflamme, "Development and validation of a body condition score system for cats: a clinical tool," *Feline Practice*, vol. 25, no. 5-6, pp. 13–18, 1997.

[6] M. Hoenig and D. C. Ferguson, "Effects of neutering on hormonal concentrations and energy requirements in male and female cats," *American Journal of Veterinary Research*, vol. 63, no. 5, pp. 634–639, 2002.

[7] S. L. Lefebvre, M. Yang, M. Wang, D. A. Elliott, P. R. Buff, and E. M. Lund, "Effect of age at gonadectomy on the probability of dogs becoming overweight," *Journal of the American Veterinary Medical Association*, vol. 243, no. 2, pp. 236–243, 2013.

[8] K. Baldwin, J. Bartges, T. Buffington et al., "AAHA nutritional assessment guidelines for dogs and cats," *Journal of the American Animal Hospital Association*, vol. 46, no. 4, pp. 285–296, 2010.

[9] L. Freeman, I. Becvarova, N. Cave et al., "WSAVA nutritional assessment guidelines," *Compendium: Continuing Education for Veterinarians*, vol. 33, no. 8, pp. E1–E9, 2011.

[10] J. A. Larsen, E. M. Parks, C. R. Heinze, and A. J. Fascetti, "Evaluation of recipes for home-prepared diets for dogs and cats

with chronic kidney disease," *Journal of the American Veterinary Medical Association*, vol. 240, no. 5, pp. 532–538, 2012.

[11] C. R. Heinze, F. C. Gomez, and L. M. Freeman, "Assessment of commercial diets and recipes for home-prepared diets recommended for dogs with cancer," *Journal of the American Veterinary Medical Association*, vol. 241, no. 11, pp. 1453–1460, 2012.

[12] J. Stockman, A. J. Fascetti, P. H. Kass, and J. A. Larsen, "Evaluation of recipes of home-prepared maintenance diets for dogs," *Journal of the American Veterinary Medical Association*, vol. 242, no. 11, pp. 1500–1505, 2013.

[13] K. E. Michel, K. N. Willoughby, S. K. Abood et al., "Attitudes of pet owners toward pet foods and feeding management of cats and dogs," *Journal of the American Veterinary Medical Association*, vol. 233, no. 11, pp. 1699–1703, 2008.

[14] D. Hutchinson, L. M. Freeman, K. E. Schreiner, and D. G. Terkla, "Survey of opinions about nutritional requirements of senior dogs and analysis of nutrient profiles of commercially available diets for senior dogs," *International Journal of Applied Research in Veterinary Medicine*, vol. 9, no. 1-2, pp. 68–79, 2011.

[15] L. Suarez, C. Peña, E. Carretón, M. C. Juste, I. Bautista-Castaño, and J. A. Montoya-Alonso, "Preferences of owners of overweight dogs when buying commercial pet food," *Journal of Animal Physiology and Animal Nutrition*, vol. 96, no. 4, pp. 655–659, 2012.

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 5686372, 5 pages
http://dx.doi.org/10.1155/2016/5686372

Research Article

Insulin-Like Growth Factor-1 and Selected Insulin-Like Growth Factor Binding Protein Concentrations during an Ultramarathon Sled Dog Race

Matthew W. Brunke,[1] Christopher W. Frye,[2] Corri B. Levine,[2] Cristina Hansen,[3] and Joseph J. Wakshlag[2]

[1]*North Country Veterinary Referral Center, 454 Queensbury Avenue, Queensbury, NY 12804, USA*
[2]*Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Tower Road, Ithaca, NY 14853, USA*
[3]*Department of Biology and Wildlife, The University of Alaska Fairbanks, Fairbanks, AK 99775, USA*

Correspondence should be addressed to Joseph J. Wakshlag; jw37@cornell.edu

Received 20 May 2016; Accepted 4 August 2016

Academic Editor: A. Kate Shoveller

The objective of this study was to investigate the effects of running a 1000-mile (1600 km) endurance sled dog race on serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding proteins 1 and 3 (IGFBP-1 and IGFBP-3). Serum was examined from 12 sled dogs prior to the race, at midrace (approximately 690 km), and again at the finish. IGF-1, IGFBP-1, and IGFBP-3 were assessed using radioimmunoassay or enzyme linked immune-absorbance assays. Mean prerace concentrations were significantly higher than midrace and end-race concentrations at 215.93 ± 80.51 ng/mL, 54.29 ± 25.45 ng/mL, and 55.53 ± 28.25 ng/mL, respectively ($P < 0.001$). Mean IGFBP-1 concentrations were not different across these time periods at 24.1 ± 15.8 ng/mL, 25.7 ± 14.0 ng/mL, and 26.6 ± 17.6 ng/mL, respectively. IGFBP-3 concentrations showed a modest significant decrease across time periods at 3,067 ± 2,792 ng/mL, 2,626 ± 2,310 ng/mL, and 2,331 ± 2,301 ng/mL, respectively ($P < 0.01$). Endurance sled dogs show a precipitous drop in serum IGF-1 concentrations. These differences may be related to fuel utilization and excessive negative energy balance associated with the loss of body condition during racing. The relative stability of IGFBP-1 and IGFBP-3 suggests that IGF-1 anabolic signaling is diminished during ultramarathon racing. Further studies comparing the influence of time and duration of exercise versus negative energy balance on serum IGF-1 status are warranted to better understand exercise versus negative energy balance differences.

1. Introduction

Insulin-like growth factor-1 (IGF-1, somatomedin C) is an anabolic peptide hormone similar in molecular structure to insulin with some insulin-like actions. Growth hormone (GH) stimulates IGF-1 production and release. As IGF-1 concentrations increase in the blood stream, they provide a negative feedback loop upon GH release in the pituitary gland. The primary site of action of IGF-1 is insulin-like growth factor-1 receptor (IGF1R). This cellular receptor is found throughout the body and helps drive anabolic protein synthesis and glucose uptake and utilization similar to insulin receptor actions. Such actions are reflective of IGF-1 acting as a potent stimulator of cell growth, proliferation, and hypertrophy [1].

While IGF-1 is within the bloodstream, 99% is bound to insulin growth factor binding proteins (IGFBPs) [2].

Six IGFBPs have been identified, cloned, and sequenced. These molecules interact with IGF-1 in the serum and all have a higher affinity than the IGF-1 cell receptors and may limit available ligand for biological signaling. Additionally these proteins differ in their structure, and other functions have been proposed including transportation, prolonging IGF half-lives, providing tissue or cell specificity, or even neutralizing and potentiating IGFs [2–5]. IGFBP-3 is the most abundant of the IGFBPs and accounts for 80% of all IGF binding.

IGFBPs 1 and 3 have been examined in human athletes undergoing short bouts of exercise, demonstrating no change

over 30 minutes [6]. In marathon runners IGFBP-1 concentrations were elevated just after the race and returned to normal in 24 hours [7]. Human studies showed no immediate change in IGFBP-3 after marathon running, but mild increases may occur 1 to 3 days after the marathon when plasma volume changes are considered [7].

Conversely, concentration differences in IGF-1 have been noted across different types of exercise. Baseline IGF-1 will be higher in well-trained athletes as compared to sedentary people. While individual serum IGF-1 concentrations appear to remain the same during the course of a marathon, sprinting activities have shown increases [7, 8]. Additionally, endurance athletes running over multiple days have demonstrated decreases in IGF-1 concentrations [9].

Currently there is limited information regarding canine IGF-1 and associated binding proteins [10] and no studies examining the effects of exercise on IGF-1 and IGFBPs in dogs, particularly those undergoing endurance exercise. Given the paucity of information available, we elected to study changes in serum concentrations of IGF-1 and selected IGFBPs within a group of competitive ultramarathon racing sled dogs during the course of the entire 2015 Yukon Quest 1,000-mile International Sled Dog Race.

2. Materials and Methods

2.1. Dogs. Dogs were recruited from 3 teams to participate in the study after providing informed client consent. The study protocol was approved by the Cornell University Institutional Animal Use Committee and the Yukon Quest Board of Directors.

Venipuncture was conducted on 14 dogs from each team approximately 24 hours prior to the start of the race in Whitehorse, Yukon Territory, with plans for further collections at the midway point at Dawson City, Yukon Territory (approximately 480 miles into the race), and then again at the finish in Fairbanks, Alaska (approximately 983 miles). All blood samples were taken within 2 hours of the dogs stopping at the midway point in Dawson City and at the finish in Fairbanks, Alaska. Of the original teams bled, only one team had follow-up blood draws. Fourteen dogs (3 were female and 11 were male) were bled at the midpoint of the race in Dawson; 2 dogs were discontinued from the race between the midway point and the finish leaving only 12 dogs for blood collection in Fairbanks.

2.2. Sample and Data Collection. Ten mL of whole blood was collected using a 22-gauge needle and 12 cc syringe from each dog at the respective time points. The blood was immediately transferred to a coagulation tube and allowed to clot and stored on ice for approximately 30 minutes before centrifugation at 4,000 ×g for 10 minutes. Serum was then stored in 1 mL aliquots and kept frozen on dry ice for transportation to the principal investigator's lab approximately 15 days later. Once received the samples were stored at −80°C until thawed for assays. All dogs had body condition scores evaluated at the time of all blood draws according to the 1–9 scoring system [11] to determine if the dogs changed body condition during the race.

2.3. RIA and ELISA Procedures. The IGF-1 concentrations in each serum sample were assayed using an immunoradiometric commercial kit based on two region-restricted affinities purified polyclonal antibodies (IGF-1: IGFR22 Diasorin, Mediagnost, Reutlingen, Germany) [12] and was used according to the manufacturer's instructions. Intra-assay and interassay coefficients of variation were 5.2% and 21.3%, respectively, using the standard additions method for evaluation of precision and linearity, while a low and high canine IGF-1 standard (E90050Ca, Cloud-Clone, Houston, TX, USA) were a control for accuracy.

Canine IGFBP-1 was measured via enzyme linked immunoabsorbance assay (ELISA; canine insulin-like growth factor; MB022518, MyBioSource, San Diego, CA, USA).

The kit was used according to the manufacturer's instruction using undiluted whole serum which provided values within the standard curve of the assay. High and low limits of detection were 15.6 to 500 ng/mL with inter- and intra-assay coefficients of variation determined by the manufacturer of less than 15% for both.

Canine IGFBP-3 was measured using ELISA (canine insulin-like growth factor; MB022518, MyBioSource, San Diego, CA, USA). The kit was used according the manufacturer's instructions with undiluted whole serum to provide values within the standard curve of the assay. If a value was found to be above the high limit of detection, the sample was diluted 1 : 2 to provide a value within the standard curve generated in the assay (3 samples required dilution). High and low limits of detection were 100 to 6,000 ng/mL with inter- and intra-assay coefficients of variation determined by the manufacturer of less than 10% for both. All samples from a single dog across time points were run on the same plate for both ELISAs. For both IGFBP-1 and IGFBP-3 samples were run in duplicate and if the duplicate values have over a 15% disparity the sample was repeated.

2.4. Statistical Analysis. Results from each assay were assessed for normality using a Shapiro-Wilk test with IGF-1 and IGFBP-1 proving to be normally distributed data, while the IGFBP-3 data proved to be skewed. IGF-1 and IGFBP-1 were statistically evaluated using a mixed model repeated measured analysis of variance, while IGFBP-3 was assessed using a mixed model Friedman's test. All tests underwent Tukey's post hoc analysis to determine if there were any significant differences between preracing samples, midrace, and finishing values for each group. A P value of 0.05 was set as significance for all statistics analyses.

3. Results

3.1. Dogs and Body Condition Scores. All 14 dogs that started the race completed running to the midway point and one dog was dropped from the race at this juncture in Dawson City. Approximately 160 kilometers further another dog was dropped from the race leaving 12 dogs that finished whose serum was evaluated at all three time points and are reported. The median and range body condition score (BCS) at the start of the race was 5.0 (range 4-5). Midrace at Dawson

Table 1: Dog identification, age, gender, and starting and ending body conditions for all dogs examined (n-12).

Dog	Age	Gender	Start	Finish
Sound	4	F	5	3
Cat	2	F	5	3
Bato	2	M	4	3
Basin	4	M	4	3.5
Heath	5	M	4	3
Yukon	5	M	5	3.5
Carbon	5	M	4	3
Braeburn	5	M	5	3.5
Chica	2	F	5	3
Krypton	3	M	4	3
Copper	5	M	5	3
Merc	5	M	5	3

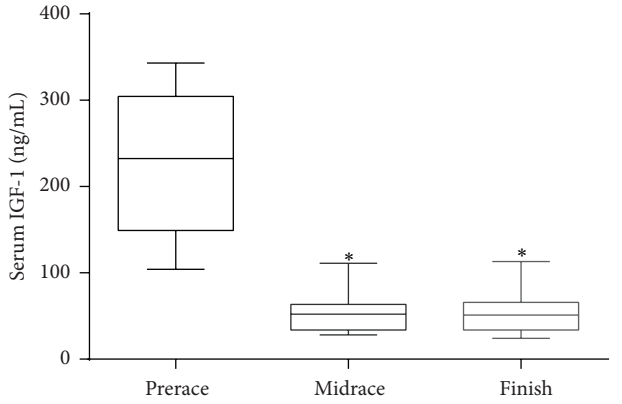


Figure 1: IGF-1 mean and standard deviation at prerace, midrace, and race finish. Boxes represent mean and 75th and 25th percentiles and whiskers represent 1st and 99th percentiles. ∗ indicates a significant difference from the prerace values ($P < 0.01$).

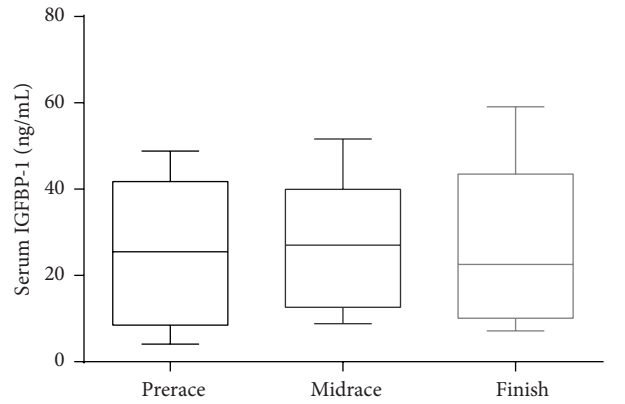


Figure 2: IGFBP-1 at prerace, midrace, and race finish. Boxes represent mean and 75th and 25th percentiles and whiskers represent 1st and 99th percentiles.

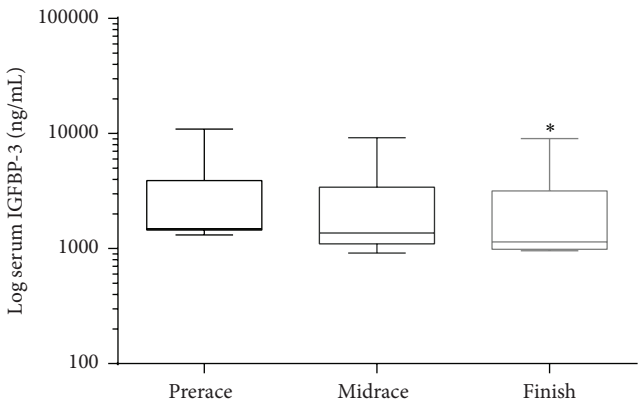


Figure 3: IGFBP-3 at prerace, midrace, and race finish. Boxes represent median and 75th and 25th percentiles and whiskers represent 1st and 99th percentiles. ∗ indicates a significant difference from the prerace values ($P < 0.01$).

the median BCS was 4.0 (4.5–3.5) and the median BCS was 3.0 (3.0–3.5) at the race finish. Dog name, age, gender, and starting and ending body conditions can be found in Table 1.

3.2. IGF-1 Serum Concentrations. IGF-1 concentrations decreased significantly between prerace and midrace in Dawson City. The mean prerace concentration was 215.93 ± 80.5 ng/mL and the mean halfway point concentration was 54.29 ± 25.5 ng/mL ($P < 0.01$). The mean IGF-1 serum concentration at the termination of the race was 55.33 ± 28.3 ng/mL, which was significantly different from the preracing concentrations ($P < 0.01$). There was no significant difference between the midrace and race finish concentrations (Figure 1).

3.3. IGFBP-1 and IGFBP-3 Concentrations. Mean serum IGFBP-1 concentrations at prerace were 23.1 ± 15.8 ng/mL. By midrace the IGFBP-1 concentrations were 25.7 ± 14.0 ng/mL, which was not significantly different from the preracing mean. At completion of the race the serum IGFBP-1 concentrations were 26.6 ± 17.6 ng/mL showing no significant difference from prerace or midrace concentrations. (Figure 2). Serum IGFBP-3 concentrations at prerace were 3,067 ± 2,792 ng/mL and were not significantly different from midrace concentrations, 2,626 ± 2,310 ng/mL, while race finish concentrations were 2,331 ± 2301 ng/mL and were significantly different from preracing values ($P < 0.01$), but not midrace concentrations (Figure 3).

4. Discussion

The IGF-1 concentrations in racing sled dogs responded unexpectedly with a dramatic significant decrease during exercise. Interestingly, no alterations in the IGFBP-1 concentrations were observed. IGFBP-3 in racing sled dogs responded similarly to that of human marathon runners at the halfway point with no significant decrease; however by day 10 of racing IGFBP-3 did decrease significantly compared to prerace values [7]. Most studies of human athletes [13, 14] have shown mild increases in IGF-1 and IGFBP-1 during prolonged exercise such as marathon. It has been suggested that IGFBP-1 has a role in glucose counter regulation by

binding and delivering IGF-1 to tissues maintaining glucose homeostasis in people, particularly muscle tissue [15]. In racing sled dogs the precipitous drop in IGF-1 and relatively low IGFBP-1 suggest other mechanisms are important in delivery of glucose to muscle tissue. IGF-1 concentrations in people will decrease with prolonged exercise over a 7-day period, but not an 80% drop like that experienced in this group of endurance sled dogs [16]. Smith and colleagues found that the decrease in IGF-1 with sustained exercise is similar to that of the decrease of IGF-1 with caloric restriction [16]. In each case the IGF-1 concentrations dropped by approximately 25%, reflecting the extent of negative caloric balance incurred by long duration strenuous exercise in normal individuals. Similarly, IGF-1 and IGFBP-3 decreased approximately 25% and 20%, respectively, in people over a 100-kilometer human marathon race. Participants in this race had access to food and water as needed, suggesting an exercise component to the decreases of serum concentrations [17]. However, a total calorie deficit may have persisted despite access to food. The intensity of exercise is much greater in our racing dogs comparatively as seen by the loss in body condition during the race. This negative energy balance without time for the body to recover is the most likely cause for the dramatic decrease in serum IGF-1 observed in these racing dogs.

The precipitous weight loss observed, which is also observed in human athletes to a lesser degree [18], shows severe negative energy balance in these endurance dogs. Body condition scoring suggests a 7–10% decrease in weight with each one point drop in BCS; therefore the dogs in our study lost a mean 14–20% of their individual body weight during the course of the race [11]. Previous studies by Hinchcliff and colleagues have shown that sled dogs expend approximately 11,250 kilocalories a day and metabolizable energy intake was approximately 10,600 kilocalories per day during a 70-hour race [18]. Another study showed that sled dogs were typically fed 9,500 to 12,000 calories per dog per day and still lost weight (5–10%) throughout the course of the Yukon Quest Ultramarathon sled dog race [19]. Unfortunately, there were logistical problems during the race that precluded collection of body weights and although kilocalories intake was not assessed in this study the feeding pattern of this kennel during racing was examined in a prior study [19].

The precipitous drop in IGF-1 (approximately 80%) is similar to a study where dogs were reduced to approximately 60% of their normal metabolic energy requirement and consequently experienced weight loss [10]. Although IGF-1 changed in our racing dogs, the selected binding proteins were not altered dramatically. On the other hand, a caloric reduction in cats led to downregulation of IGF-1 synthesis by 51% with only a 42.5% restriction in calories, without alteration in the IGFBP-1 or IGFBP-3 from a comparative perspective [20]. Because prolonged exercise in people leads to changes associated with negative energy balance [16] and calorie restriction in companion animals results in decreases of IGF-1 concentrations, it seems intuitive that the IGF-1 and BCS changes seen in our study dogs reflect a prolonged and extreme negative energy balance that is reflected by the largest decrease in IGF-1 ever observed in a canine or human model of exercise.

Little alteration in IGFBPs was observed in this study. IGFBP-1 concentrations did not significantly change during the course of the race, while IGFBP-3 did show a small, but statistically significant decrease by the end of the race. The changes in IGFBP-3 were negligible when compared to the decreases in IGF-1. There have been mixed results with IGFBP-1 concentrations in people pending the duration and type of exercise. Over an 8-week period of concentric training in people there is a decrease in IGFBP-1, while eccentric exercise in humans caused mild increases [21]. In day-long exercise (eccentric or concentric) no significant changes were seen in IGFBP-1 concentrations in people [22]. Marathon runners [7] exhibited modest increases in IGFBP-1. In people, marathon racing and heavy resistance exercise bring about equivocal changes in IGFBP-3 concentrations [7, 22, 23].

Concentrations of IGFBP-3 in these dogs were similar to human athletes and did significantly decrease by the end of racing. However this change, as with IGF-1, may not be a direct reflection of prolonged racing and exercise, but possibly a marker of the energy expenditure leading to overall decreased hepatic synthesis of these binding proteins due to metabolic demands of racing for 9-10 consecutive days.

There are limitations of our study when discussing the dynamics of IGF-1 and IGFBPs during exercise as we did not collect serum until day 4 of racing at midrace; therefore we cannot comment on possible transient IGF-1 or binding protein changes that might occur due to exercise, but the lack of increase over the entire race suggests that exercise plays little to no role in long term homeostasis of IGF-1 in the face of ultramarathon endurance exercise. Lastly, we could not separate the effects of negative energy balance versus exercise on the IGF and IGFBPs we measured but expect that with the loss in body condition that negative energy balance plays a more significant role considering the extent of IGF-1 decreases observed.

5. Conclusion

IGF-1 concentrations decrease similarly to people performing long duration exercise (greater than 24 hours), but to a far greater extent. However, negative energy balance may be playing a significant role (as opposed to exercise directly) over the influence of IGF-1 concentrations in our ultramarathon racers. This same energy balance may also explain the decrease in IGFBP-3 during the course of the race as it is impossible to separate the effects of exercise from that of the energy balance. Examination at earlier time points and different cohorts of exercising canines may shed light into the severity of IGF-1 downregulation due to exercise versus energy intake/expenditure imbalance, providing a better understanding of the influence of exercise on IGF-1 concentrations. The tremendous decrease in IGF-1 sheds light regarding the extreme negative energy balance observed in endurance sled dogs and begs for further understanding of not only the metabolic demands, but also appropriate feeding strategies to fuel these amazing athletes.

Abbreviations

IGF-1: Insulin-like growth factor-1
IGFBP-1: Insulin-like growth factor binding protein 1
IGFBP-3: Insulin-like growth factor binding protein 3
IGF-1R: Insulin-like growth factor-1 receptor.

Competing Interests

None of the authors have any competing interests related to this manuscript.

Acknowledgments

The authors would like to thank the mushers in the 2015 Yukon Quest that allowed them access to their dogs during this study: Brent Sass, Scott Smith, and Rob Cooke. The work was funded by an internal grant from Cornell University Department of Clinical Sciences.

References

[1] M. P. Czech, "Signal transmission by the insulin-like growth factors," *Cell*, vol. 59, no. 2, pp. 235–238, 1989.

[2] D. R. Clemmons, "IGF binding proteins and their functions," *Molecular Reproduction and Development*, vol. 35, no. 4, pp. 368–375, 1993.

[3] D. R. Clemmons, R. C. Camacho-Huber, J. I. Jones, and R. H. McCusker Busby Jr., "Insulin like growth factor binding proteins: mechanism of action at the cellular level," in *Modern Concepts of Insulin-Like Growth Factors*, E. M. Spencer, Ed., pp. 475–486, Elsevier Science, New York, NY, USA, 1991.

[4] K. L. Cohen and S. P. Nissley, "The serum half life of somatomedin activity: evidence for growth hormone dependence," *Acta Endocrinologica*, vol. 83, no. 2, pp. 243–258, 1976.

[5] J. Zapf, M. Waldvogel, and E. R. Froesch, "Binding of nonsuppressible insulinlike activity to human serum. Evidence for a carrier protein," *Archives of Biochemistry and Biophysics*, vol. 168, no. 2, pp. 638–645, 1975.

[6] P. Bang, J. Brandt, M. Degerblad et al., "Exercise-induced changes in insulin-like growth factors and their low molecular weight binding protein in healthy subjects and patients with growth hormone deficiency," *European Journal of Clinical Investigation*, vol. 20, no. 3, pp. 285–292, 1990.

[7] H. Koistinen, R. Koistinen, L. Selenius, O. Ylikorkala, and M. Seppälä, "Effect of marathon run on serum IGF-I and IGF-binding protein 1 and 3 levels," *Journal of Applied Physiology*, vol. 80, no. 3, pp. 760–764, 1996.

[8] Y. Meckel, D. Nemet, S. Bar-Sela et al., "Hormonal and inflammatory responses to different types of sprint interval training," *Journal of Strength and Conditioning Research*, vol. 25, no. 8, pp. 2161–2169, 2011.

[9] C. P. Velloso, "Regulation of muscle mass by growth hormone and IGF-I," *British Journal of Pharmacology*, vol. 154, no. 3, pp. 557–568, 2008.

[10] A. Maxwell, R. Butterwick, M. Yateman, R. M. Batt, A. Cotterill, and C. Camacho-Hübner, "Nutritional modulation of canine insulin-like growth factors and their binding proteins," *Journal of Endocrinology*, vol. 158, no. 1, pp. 77–85, 1998.

[11] D. I. Mawby, J. W. Bartges, A. d'Avignon, D. P. Laflamme, T. D. Moyers, and T. Cottrell, "Comparison of various methods for estimating body fat in dogs," *Journal of the American Animal Hospital Association*, vol. 40, no. 2, pp. 109–114, 2004.

[12] L. Jaillardon, L. Martin, P. Nguyen, and B. Siliart, "Serum insulin-like growth factor type 1 concentrations in healthy dogs and dogs with spontaneous primary hypothyroidism," *Veterinary Journal*, vol. 190, no. 2, pp. e95–e99, 2011.

[13] N. J. Hopkins, P. M. Jakeman, S. C. Hughes, and J. M. P. Holly, "Changes in circulating insulin-like growth factor-binding protein-1 (IGFBP-1) during prolonged exercise: effect of carbohydrate feeding," *Journal of Clinical Endocrinology and Metabolism*, vol. 79, no. 6, pp. 1887–1890, 1994.

[14] A.-M. Suikkari, T. Sane, M. Seppälä, H. Yki-Järvinen, S.-L. Karonen, and V. A. Koivisto, "Prolonged exercise increases serum insulin-like growth factor-binding protein concentrations," *Journal of Clinical Endocrinology and Metabolism*, vol. 68, no. 1, pp. 141–144, 1989.

[15] S. I. Yeoh and R. C. Baxter, "Metabolic regulation of the growth hormone independent insulin-like growth factor binding protein in human plasma," *Acta Endocrinologica*, vol. 119, no. 4, pp. 465–473, 1988.

[16] A. T. Smith, D. R. Clemmons, L. E. Underwood, V. Ben-Ezra, and R. McMurray, "The effect of exercise on plasma somatomedin-C/insulinlike growth factor I concentrations," *Metabolism*, vol. 36, no. 6, pp. 533–537, 1987.

[17] T. Kim, J. S. Chang, H. Kim, K. H. Lee, and I. D. Kong, "Intense walking exercise affects serum IGF-1 and IGFBP3," *Journal of Lifestyle Medicine*, vol. 5, no. 1, pp. 21–25, 2015.

[18] K. W. Hinchcliff, G. A. Reinhart, J. R. Burr, C. J. Schreier, and R. A. Swenson, "Metabolizable energy intake and sustained energy expenditure of Alaskan sled dogs during heavy exertion in the cold," *American Journal of Veterinary Research*, vol. 58, no. 12, pp. 1457–1462, 1997.

[19] M. Yazwinski, J. G. Milizio, and J. J. Wakshlag, "Assessment of serum myokines and markers of inflammation associated with exercise in endurance racing sled dogs," *Journal of Veterinary Internal Medicine*, vol. 27, no. 2, pp. 371–376, 2013.

[20] A. Maxwell, R. Butterwick, R. M. Batt, and C. Camacho-Hübner, "Serum insulin-like growth factor (IGF)-I concentrations are reduced by short-term dietary restriction and restored by refeeding in domestic cats (*Felis catus*)," *The Journal of Nutrition*, vol. 129, no. 10, pp. 1879–1884, 1999.

[21] S. M. Gregory, B. A. Spiering, J. A. Alemany et al., "Exercise-induced insulin-like growth factor I system concentrations after training in women," *Medicine & Science in Sports & Exercise*, vol. 45, no. 3, pp. 420–428, 2013.

[22] S. Hasani-Ranjbar, E. S. Far, R. Heshmat, H. Rajabi, and H. Kosari, "Time course responses of serum GH, insulin, IGF-1, IGFBP1, and IGFBP3 concentrations after heavy resistance exercise in trained and untrained men," *Endocrine*, vol. 41, no. 1, pp. 144–151, 2012.

[23] S. Nassib, W. Moalla, S. Hammoudi-Nassib et al., "The IGF-1/cortisol ratio as a useful marker for monitoring training in young boxers," *Biology of Sport*, vol. 33, pp. 15–22, 2016.

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2016, Article ID 2649093, 7 pages
http://dx.doi.org/10.1155/2016/2649093

Research Article

Cats in Positive Energy Balance Have Lower Rates of Adipose Gain When Fed Diets Containing 188 versus 121 ppm L-Carnitine

M. A. Gooding,[1] D. L. Minikhiem,[1] and A. K. Shoveller[1,2]

[1] *The Iams Company, Mars PetCare, 6571 State Route 503 North, Lewisburg, OH 45338, USA*
[2] *Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, 50 Stone Road East, Guelph, ON, Canada N1G 2W1*

Correspondence should be addressed to A. K. Shoveller; ashovell@uoguelph.ca

Received 19 May 2016; Revised 12 July 2016; Accepted 2 August 2016

Academic Editor: Stephen D. Ginsberg

L-carnitine (LC) is included in select adult feline diets for weight management. This study investigated whether feeding adult cats with diets containing either 188 ppm of LC (LC188) or 121 ppm of LC (LC121) and feeding them 120% of maintenance energy requirement (MER) resulted in differences in total energy expenditure (EE), metabolic fuel selection, BW, body composition, and behavior. Cats ($n = 20$, 4 ± 1.2 yrs) were stratified for BCS and randomly assigned to one of two dietary treatments and fed for 16 weeks. BW was measured weekly, and indirect calorimetry, body composition, physical activity, play motivation, and cognition were measured at baseline and throughout the study. A mixed, repeated measures, ANCOVA model was used. Cats in both treatments gained BW ($P < 0.05$) throughout the study, with no differences between treatments at any time point ($P > 0.05$). There were no differences in body composition between groups at baseline; however, body fat (g) and body fat : lean mass ratio were greater in cats fed LC121 in contrast to cats fed LC188 ($P < 0.05$) on week 16. No other outcomes differed between treatments ($P > 0.05$). Supplying dietary LC at a dose of at least 188 ppm may be beneficial for the health and well-being of cats fed above MER.

1. Introduction

While there is no dietary requirement for L-carnitine (LC) in cats, as it is synthesized endogenously, LC is considered a conditionally essential nutrient, as deficiencies can occur during certain disease states, during aging, and during weight loss/gain, as LC facilitates fatty acid metabolism [1] and energy metabolism [2]. Further, LC is believed to enhance cognition in humans [3] and animal models, such as rats demonstrating cognitive impairments [4]. L-carnitine is a cofactor that facilitates the transport of long chain fatty acids (LCFA) across the inner mitochondrial membrane for subsequent β-oxidation. Furthermore, LC also acts as a cofactor in the transport of acetyl-CoA out of the mitochondria. Increased concentrations of mitochondrial acetyl-CoA can inhibit further β-oxidation. Together these actions regulate the intramitochondrial acetyl-CoA concentrations and release free CoA and acetyl-carnitine that favor the oxidation of pyruvate. Due to the mechanism of action by which LC exerts its effects on fatty acid metabolism, LC may also provide a mechanism for removal of excessive fatty acids that are released during weight loss [2]. This is critically important in cats because lipids released during weight loss are commonly deposited in the liver if they are not oxidized and result in hepatic lipidosis [5]. Weight gain and related metabolic indices in domestic cats are related to diminished physical activity, mainly in the light hours [6]. Similarly, obese cats have lower EE than lean cats [7], and we recently found that overweight and obese cats have reduced activity counts in contrast to lean cats [8]. Recently, dietary LC (100 ppm) fed to cats has been shown to increase EE and lipid oxidation in contrast to cats fed control (30 ppm) during controlled weight loss [9] and for overweight cats fed to weight maintenance [7].

We have previously demonstrated that dietary LC supplementation can positively impact motivation to play in overweight, but not lean, cats [7]. We hypothesized that LC,

partly through actions on energy metabolism and metabolic function, influences central energy sensing that is thought to be part of the underlying control system of predation and play in the cat. In the cat, it is hypothesized that appetitive components of play are related to energy metabolism without an influence on consummatory features once the toy was acquired [10]. Indeed, previous observations in rodents found that LC protected against chronic stress effects and was correlated with a reduction in dopamine to support normal appetitive behavior during food reward trials [11]. In aged dogs, LC has been shown to impact behavior and brain function specifically related to an observed decline in brain function with aging [12] but not after short-term supplementation [13]. Lastly, LC administration has also been demonstrated to increase voluntary physical activity levels when fed to aged rats [14]. It is unclear as to what is the most efficacious level of LC, the population to produce a positive behavioral response, and the appropriate feeding regime to produce the greatest effect.

Previous data suggested that the addition of LC to a maintenance diet had a beneficial effect on supporting healthy metabolism and behavior in overweight, but not lean, cats [7]. Our previous study investigated the effects of LC when cats were fed to maintain body weight, but we do not have any data on the effects of dietary LC when cats are fed above maintenance energy requirement (MER), as often occurs in home environments. In addition, the efficacy of dietary LC has generally been investigated during weight loss [9]. Therefore, the primary objective of the present study was to further investigate the effects of dietary LC on energy and macronutrient metabolism and motivation to play. Results from our previous study were used to power the present study. Our secondary objective was to understand the effects of LC on weight control and body composition. We hypothesized that cats fed a diet containing the higher of two LC concentrations tested would have greater energy expenditure, lower adipose gain, and improved behavioral markers as defined by improved physical activity, play motivation, and cognition when fed above maintenance energy requirements. We chose to investigate two levels of LC, 120 and 200 ppm, as these are two dietary concentrations commonly found in commercial diets. Rather than providing data versus no additional dietary LC, we wanted to understand whether two different supplemental levels of LC provided added benefits.

2. Experimental Methods

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the Iams Company, Procter & Gamble Pet Care.

2.1. Experimental Design. Twenty neutered/spayed cats (N = 20; M = 11 and F = 9) of similar age (4 ± 1.2 years) were randomly assigned to treatment groups balanced for body weight (Group A: BW = 4.48 kg ± 1.15; Group B: BW = 4.50 kg ± 0.99) and maintenance energy intake (Group A: caloric intake = 44.85 kcal ME/kg BW ± 6.96; Group B: caloric intake = 45.85 kcal ME/kg BW ± 0.99). Cats were fed a control diet (CON), with no supplemental LC, for 4 weeks prior to the initiation of the study to allow for washout and baseline measures. Following baseline assessments, groups were stratified based on BW and assigned to receive either the control diet with 121 ppm LC (LC121) or the control diet with 188 ppm LC (LC188) in a parallel study design for a total of 16 weeks.

Table 1: Guaranteed analysis on an "as fed" basis of final products fed to cats. All analyses represent an average of triplicate analyses.

Nutrient	LC121 (121 ppm LC)	LC188 (188 ppm LC)
Fat (%)	16.2	15.8
Ash (%)	6.2	6.0
Crude fiber (%)	1.75	2.07
Moisture (%)	6.11	6.23
Protein (%)	33.5	32.3
L-carnitine (ppm)	121	188
Calculated energy density (kcal/kg)*	3752	3728

* Value calculated using the modified Atwater calculation and not accounting for total dietary fiber.

2.2. Diets. Dry diets were based on Iams ProActive Health Original with Chicken without any L-carnitine tartrate for the baseline period (~30 ppm of endogenous LC), with current supplemental L-carnitine tartrate (LC121), and with added L-carnitine tartrate (LC188) (Carniking™, Lonza Group Ltd.) (Table 1), respectively. Diets were identical formulations and utilized identical batches of ingredients; therefore, minimal differences in macronutrient (protein, fat, ash, and fiber) and micronutrient concentrations are assumed. Final products tested included a total of 121 ppm and 188 ppm LC as analyzed. Each cat was fed to mimic consumer relevant feeding practices while controlling the amount of caloric surplus; thus, cats were offered 120% of their total daily energy requirement (kcal ME/kg BW) and adjusted weekly based on BW. Individual energy requirements were established based on historical records of the individual dietary energy required to maintain body weight and BCS because the management of this colony of cats has been to maintain body condition between BCS of 2.5 and 4.0 and incur little loss or gain of body weight (no more than ±5%). Diets were presented in dry, kibble form and cats were fed once daily individually at 7:00 a.m. and given 60 minutes to eat during food offerings. All remaining food was collected and weighed to account for total (grams) food refusal. On cognition testing days feeding programs were altered for individual cats undergoing testing as food was used as a reward. Cats were fed 25% of their total daily allotment at 7:00 a.m. and the residual 75% of food was offered during T-maze testing. Remaining food was measured following testing and caloric intake calculated. All diets were coded and all researchers were blinded to dietary treatment.

2.3. Body Composition. Body weight was measured weekly prior to the morning feeding. Body composition was measured by dual X-ray absorptiometry (DXA; Hologic Inc.) as previously described on week −1 and week 16 [10]. Cats with

a BW of less than 4.0 kg were not assessed, due to minimum weight allowances of the software, and resulted in exclusion of 7 cats among the two treatment groups.

2.4. Indirect Calorimetry. To assess the effects of LC treatment on energy expenditure (EE) and respiratory quotient (RQ), indirect calorimetry was utilized and conducted as previously described at baseline and at weeks 4, 6, 10, and 14 on cats that had been acclimated to temporary restriction and the calorimetry chambers [7, 15]. Air from each chamber and background air samples were measured for 5 minutes every half hour for a total of 22 h. Background air was used to correct for CO_2 and O_2 of incoming room air. Oxygen (VO_2) consumed and carbon dioxide (VCO_2) produced were measured. Concentrations of O_2 and CO_2 were measured with infrared and O_2 and CO_2 analyzers (Qubit Systems[5], Kingston, Ontario, Canada). The calorimeter is an open circuit, ventilated calorimeter with the room air being drawn through at a rate of 5–10 L/min depending on the body weight of the cat. The rate of airflow was measured with the use of a mass flow meter to enable total volume to be calculated. Calibration of the analyzers and mass flow meters were performed prior to each oxidation study and every 6 hours or when a drift of >5% was observed. Calibration was performed using standard gas mixtures against known calibration standards.

To calculate RQ, EE, fat, and carbohydrate oxidation the following calculations were used:

$$\text{RQ} = \text{liters of } CO_2 \text{ produced/liters of } O_2 \text{ consumed.}$$

$$\text{Resting EE (kcal)} = 3.82 \times \text{liters of } O_2 \text{ consumed} + 1.15 \times \text{liters of } CO_2 \text{ produced [16].}$$

2.5. Behavioral Assessments. Voluntary physical activity was measured using the validated Actical Activity Monitors (Mini Mitter, Bend, OR, USA) over 5 consecutive 24 h periods (Monday to Friday) during weeks −1 (baseline) and 12 when no other collections occurred.

To assess play motivation, an obstruction test was used to measure willingness to work to gain access to a valued toy with the swing door made progressively more difficult to open through the addition of weights (50 g) that were placed into a trough at the bottom of the door (max 600 g). Cats were assessed at baseline and at weeks 1 and 15 of treatment at approximately 5 h after feeding.

A T-maze (stem: 7′ L × 1.5′ W × 1.5′ H; arm: 3.25′ L × 1.5′ W × 1.5′ H) was used to measure cognitive function 6 h after feeding at baseline and at weeks 2, 8, and 16 of the study. A spatial cue (a circular shape and an X shape) was randomly assigned as a positive (rewarded) and negative (nonrewarded) cue for each cat and balanced for diet. Ten trials per day were used to measure number of correct arm entries. Both arms were baited with 1 g of food to ensure that olfactory cues did not influence performance; however, food was only accessible to cats if they entered the correct arm containing that cat's positive (rewarded) cue.

2.6. Statistical Analyses. All data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC) and results expressed as means and standard errors of the means. Sample size was estimated using energy expenditure from our previous study [7] at a difference of 3%, a power of 80%, and α of 0.05. We used these data because there is no data in which cats are in positive energy balance and energy metabolism has been measured and this calculation may have been inappropriate. All data were checked for normality. Results were considered at $P < 0.05$. The calorimetry (area under the curve, AUC), food intake, and median latency to enter the T-maze junction data were analyzed using a repeated analyses of covariance model with baseline outcomes used as a covariate to investigate differences between dietary treatments. The calorimetry analysis was done separately for the fasted period and the entire time the cat was in the oxidation chamber (AUC from 0 to 21 hours after feeding). Maximum door weight (play motivation) and body weight were analyzed using the same model as the calorimetry data with the addition of baseline body weight as a covariate. An ANCOVA model was used to analyze DXA (global fat, global lean, and fat-to-lean ratio) and activity (intensity and duration) data, and baseline body composition was included as a covariate. A day/night indicator variable was added to the activity models. The proportion of correct T-maze arm entries was analyzed using a repeated measures logistic regression model.

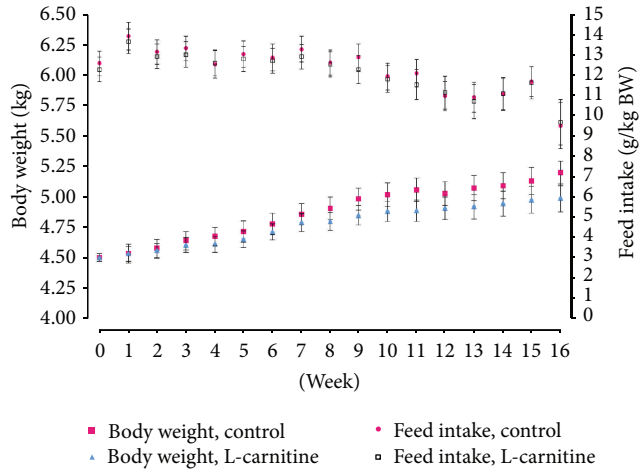


Figure 1: Mean (±SEM) food intake (g/kg/d) and BW (kg) in cats fed LC121 or LC188 receiving 120% of their maintenance energy requirement on a per kg basis during 16-week feeding.

3. Results

3.1. Feed Intake. There was no effect of dietary treatment on total food intake on a g/kg body weight basis over the course of the study ($P = 0.835$). Over the 20-week feeding period, food intake significantly decreased, as a function of BW, in both groups (Figure 1; $P < 0.0001$) despite food allowance being altered based on body weight every week.

3.2. Body Composition and Body Weight. Cats that were fed both diet treatments gained weight throughout the 16-week feeding period ($P < 0.0001$), but treatment groups did not

Table 2: Mean (±SEM) absolute or global fat (g), lean (g), and fat/lean ratio in adult cats fed LC121 or LC188 supplemented diets modified ad libitum receiving 120% of their estimated daily energy requirement.

		LC121 ($N = 6$)	LC188 ($N = 7$)	Pairwise P value
Global fat (g)	Baseline	794.1 ± 255.4	1044.6 ± 236.4	0.49
	Week 16	1460.4 ± 112.1	1106.9 ± 110.6	0.047
Global lean (g)	Baseline	4112.5 ± 218.5	4029.9 ± 202.3	0.79
	Week 16	4242.9 ± 74.7	4285.4 ± 65.8	0.67
Global fat/lean ratio	Baseline	0.189 ± 0.056	0.257 ± 0.052	0.40
	Week 16	0.353 ± 0.028	0.259 ± 0.027	0.038

Table 3: Fasting and fed adjusted AUC for RQ and EE in adult cats fed LC121 or LC188 supplemented diets above their maintenance energy requirement at baseline and during the 16-week LC feeding.

	Week	LC121 ± SE ($N = 10$)	LC188 ± SE ($N = 10$)	Pairwise P value
Fasting RQ*	Baseline	0.756 ± 0.006	0.740 ± 0.006	0.05
	4	0.785 ± 0.013	0.776 ± 0.012	0.61
	6	0.811 ± 0.014	0.784 ± 0.013	0.16
	10	0.839 ± 0.013	0.821 ± 0.012	0.33
	14	0.783 ± 0.015	0.763 ± 0.010	0.26
Fasting EE (kcal/(kg∗d))*	Baseline	45.27 ± 3.78	47.81 ± 3.78	0.64
	4	42.91 ± 4.09	43.16 ± 2.02	0.96
	6	41.15 ± 4.07	40.53 ± 2.42	0.89
	10	37.29 ± 3.86	37.06 ± 2.53	0.96
	14	40.52 ± 4.48	39.05 ± 1.87	0.78
Postprandial RQ*	Baseline	0.81 ± 0.007	0.80 ± 0.007	0.20
	4	0.82 ± 0.008	0.81 ± 0.007	0.22
	6	0.86 ± 0.008	0.85 ± 0.009	0.33
	10	0.86 ± 0.008	0.85 ± 0.009	0.33
	14	0.81 ± 0.008	0.80 ± 0.01	0.38
Postprandial EE (kcal/(kg∗d))*	Baseline	42.97 ± 3.25	44.69 ± 3.25	0.72
	4	41.84 ± 1.81	43.10 ± 1.69	0.58
	6	35.24 ± 1.72	34.73 ± 1.14	0.81
	10	33.52 ± 1.77	33.21 ± 1.32	0.89
	14	41.37 ± 2.58	42.46 ± 1.13	0.70

∗ denotes a significant ($P < 0.05$) time effect.

differ from each other at any point throughout the study ($P > 0.05$; Figure 1) and there was no time ∗ treatment effect ($P > 0.05$). There were no differences in global or absolute adipose or lean body mass between treatment groups at baseline ($P > 0.05$; Table 2). Global body fat ($P = 0.047$) and global fat : lean ratio ($P = 0.038$) significantly increased through the duration of the study for both treatment groups ($P < 0.05$), but lean body mass did not change through the duration of the study for either treatment group ($P > 0.05$). Furthermore, there was a time ∗ treatment effect ($P < 0.05$) for adipose and global fat : lean ratio ($P > 0.05$). Gains in adipose and global fat : lean ratio were significantly less for cats consuming LC188 versus cats consuming LC121 ($P < 0.05$).

3.3. Fasted Energy Metabolism. Fasted RQ was significantly lower in cats fed LC188 at baseline ($P = 0.05$) and used as a covariate to generate adjusted LS means for RQ in the subsequent analyses for weeks 4, 6, 10, and 14. Respiratory quotient did not differ between groups in the fasted state ($P = 0.232$; Table 3). Energy expenditure (kcal/(kg∗d)) in the fasted state did not differ between groups at baseline and at week 4, 6, 10, or 14 (Table 3; $P > 0.05$). A significant effect by week was detected ($P < 0.05$), but there was no treatment ∗ week interaction.

3.4. Fed Energy Metabolism. Postmeal mean AUC for RQ did not differ between treatment groups at baseline or at any point during the study (Table 3; $P > 0.05$). When data were analyzed over the postprandial (0–5.25 h), fed (5.25–10.50 h), return to fasted (10.50–15.75 h), and fasted (15.75–21 h) states, there were no effects of diet on RQ ($P > 0.05$, *data not shown*). There was no effect of diet on postmeal mean AUC

Table 4: Adjusted means (±SEM) for physical activity in adult cats fed LC121 and LC188 supplemented diets above maintenance energy requirements.

Parameter	Period*	LC121 ($N = 9$)	LC188 ($N = 10$)	Pairwise comparison P value
Physical activity intensity (activity counts per hour)	Total	1244 ± 88	1328 ± 103	0.52
	Light	1594 ± 83	1599 ± 76	0.96
	Dark	985 ± 134	1158 ± 155	0.39
Physical activity duration (proportion of activity time within a day)	Total	0.097 ± 0.007	0.100 ± 0.004	0.61
	Light	0.117 ± 0.007	0.117 ± 0.006	0.98
	Dark	0.081 ± 0.008	0.088 ± 0.006	0.46

*Light and dark were defined in accordance with the sunrise and sunset time.

Table 5: T-maze testing and proportion of correct arm entries (baseline adjusted mean (SE)) of cats fed LC121 or LC188 diets.

	LC121 ($N = 10$)	LC188 ($N = 10$)	P value
Week 2	0.64 (0.05)	0.73 (0.05)	0.265
Week 8	0.73 (0.07)	0.76 (0.05)	0.737
Week 16	0.76 (0.05)	0.80 (0.04)	0.533
Overall	0.72 (0.03)	0.76 (0.02)	0.219

for EE (kcal/(kg∗d); Table 3; $P > 0.05$). Energy expenditure analyzed over the postprandial (0–5.25 h), fed (5.25–10.50 h), return to fasted (10.50–15.75 h), and fasted (15.75–21 h) states did not differ between groups ($P > 0.05$, *data not shown*) at baseline or at any point throughout the study. A significant effect by week was detected ($P < 0.05$), but there was no treatment ∗ week interaction.

3.5. Behavioral Assessments. Voluntary physical activity did not differ between groups for total movement ($P = 0.527$) and time spent moving ($P = 0.610$) or when the data were split between daytime and night activity ($P > 0.05$, Table 4). Performance during T-maze testing did not differ between dietary treatments ($P = 0.219$, Table 5). Mean latency to make a selection for the T-maze was not different between cats fed LC188 and LC121 ($P = 0.711$). There was no effect of diet on play motivation or mean weight and the cats were willing to push open to gain access to a toy (LC121 = 292.45 g ± 62.82 and LC188 = 339.58 ± 74.27; $P = 0.626$).

4. Discussion

Cats in positive energy balance fed diets containing 188 ppm LC have lower body fat deposition than cats fed diets containing 121 ppm LC in a 16-week feeding study. Despite these significant changes in body composition, there were no differences in LC treatments for measures of energy metabolism, or physical activity; therefore, the mechanism of action for the reduced adipose gain with the 188 ppm LC fed cats remains unclear. Furthermore, there was no effect of diet on any behavioral outcomes including play motivation and cognitive performance, although the study was not powered against outcomes of physical activity or behavior, which are more variable than more physiological outcomes. Overall, feeding LC, particularly higher doses, to cats in positive energy balance may be beneficial to help mitigate weight and more importantly adipose gain.

The effect of decreased adipose gain in cats fed LC188 is not surprising. The presence of LC in 3T3-L1 adipocyte culture resulted in increased hormone-sensitive lipase, carnitine palmitoyltransferase I-a, and acyl-coenzyme A oxidase, suggesting that LC may act to increase lipid oxidation [17]. Furthermore, the expressions of peroxisome proliferator-activated receptor-γ and adipose-specific fatty acid binding protein were downregulated by LC in 3T3-L1 adipocytes, suggesting a decrease in adipogenesis [17], which supports the findings in the current study. Similarly, a cocktail of red grape extract, soy isoflavone, and LC was found to inhibit body weight and adipose gain in C57BL/6J mice consuming a high fat diet [18], although the use of a cocktail including other compounds is difficult to compare to the current study. Another cocktail of *Garcinia cambogia*, soy peptide, and LC reduced visceral fat accumulation in Sprague Dawley rats fed high fat diets [19] but again is not solely the action of LC. Similarly, a cocktail of an Egyptian herbal formula and LC resulted in reduced body weight gained and a better metabolic profile compared to rats that did not receive the supplement [20]. A longer treatment period and/or more animals may be necessary to elucidate the positive effects on biomarkers and warrants further investigation. This same experimental paradigm approach combined with ad libitum feeding may also demonstrate significant effects on total body weight gain and warrants further investigation. Indeed, providing a nutritional technology that could mitigate weight gain, especially in households with multiple cats, would significantly help owners manage individual cat body weight.

Although we observed a difference between diets on adipose tissue deposition, there were no effects of diet on EE or metabolic fuel selection as underlying mechanisms for the observed compositional changes. Previous data collected in cats fed supplemental LC have been completed during maintenance or restricted (for weight loss) feeding paradigms in which LC supplementation contributes to an increase in EE and fatty acid oxidation [7, 9]. Feeding above energy requirements in the current study did not produce similar results as there were no differences in EE and RQ between cats fed the diet with 121 ppm LC and cats fed the diet with

188 ppm LC. As cats fed both treatments gained weight, fasting EE (kcal/kg BW/d) decreased, but overall there was no difference between dietary treatments. There was a significant response over time in fasting RQ, but there was no difference between diets. Fasting RQ and postprandial EE and RQ appeared to follow a less clear pattern over time. The lack of metabolic response within a feeding paradigm above energy requirements may be due to (1) the overriding effect of total caloric intake on LC effectiveness and (2) the theory that the population of animals that would mostly benefit from LC treatment are those with LC deficiency, namely, cats undergoing weight loss, which are at risk for the development of hepatic lipidosis [5, 9, 21].

Overall, the dietary concentration of LC within the diet had no effect on voluntary physical activity, motivation to play, and performance during a T-maze test. Several studies have reported that treatment with LC in populations with LC insufficiency, primarily due to disease or fatigue syndrome, can lead to reductions in subjective feelings of low energy [22–24]. Further, supplementation with acetyl-L-carnitine and LC has been shown to increase voluntary physical activity levels when fed to aged rats [14]. However, the effect of LC on ambulatory behavior is not always apparent [24], which is consistent with our findings, as we did not observe an effect of LC on physical activity in healthy, adult cats. Previously, LC supplementation at 100 mg/kg contributed to an increased motivation to play in overweight cats (BCS > 3.5) [7], but a similar response was not observed within the current study. The lack of effect of LC on play motivation may have been due to the absence of effect on energy metabolism (particularly EE) which has historically been linked to increases in play motivation in cats [7]. Similar to previous studies in humans and animals, the effects of LC may be more apparent in populations at risk for LC insufficiency or those exhibiting declines or impairments in cognitive function, activity, and play. Indeed, the cats used in the present study were healthy and young and received a lot of cat-cat and human socialization and may not be the suitable cohort to observe effects on play motivation or physical activity.

In conclusion, these results suggest that diets containing 188 ppm LC and fed above maintenance energy requirements will result in less fat gain than diets with 121 ppm LC and may be beneficial for the health and well-being of cats that may be overfed. However, LC may be required at different levels depending on total caloric intake and level of adiposity, and a dose-dependent comparison in multiple experimental paradigms is necessary to elucidate the mechanism of action of LC on adipose gain and how the individual physiological state (e.g., level of adiposity) of cats affects the expected outcome.

Abbreviations

BCS: Body condition score
BW: Body weight
EE: Energy expenditure
RQ: Respiratory quotient
ME: Metabolizable energy.

Competing Interests

All authors have financial interest in the Procter & Gamble Company as shareholders and M. A. Gooding and D. L. Minikhiem are employees of the Iams Company, Mars PetCare, North America.

Acknowledgments

This work was supported by Procter & Gamble Pet Care, Mason, Ohio, USA.

References

[1] P. J. Armstrong and G. Blanchard, "Hepatic lipidosis in cats," *Veterinary Clinics of North America: Small Animal Practice*, vol. 39, no. 3, pp. 599–616, 2009.

[2] E. Liepinsh, I. Kalvinsh, and M. Dambrova, "The regulation of energy metabolism pathways through L-carnitine homeostasis," in *Role of the Adipocyte in Development of Type 2 Diabetes*, C. Croniger, Ed., chapter 6, pp. 107–128, InTech, Rijeka, Croatia, 2011.

[3] S. A. Montgomery, L. J. Thal, and R. Amrein, "Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease," *International Clinical Psychopharmacology*, vol. 18, no. 2, pp. 61–71, 2003.

[4] S. Ando, S. Kobayashi, H. Waki et al., "Animal model of dementia induced by entorhinal synaptic damage and partial restoration of cognitive deficits by BDNF and carnitine," *Journal of Neuroscience Research*, vol. 70, no. 3, pp. 519–527, 2002.

[5] S. A. Center, J. Harte, D. Watrous et al., "The clinical and metabolic effects of rapid weight loss in obese pet cats and the influence of supplemental oral L-carnitine," *Journal of Veterinary Internal Medicine*, vol. 14, no. 6, pp. 598–608, 2000.

[6] K. R. Belsito, B. M. Vester, T. Keel, T. K. Graves, and K. S. Swanson, "Impact of ovariohysterectomy and food intake on body composition, physical activity, and adipose gene expression in cats," *Journal of Animal Science*, vol. 87, no. 2, pp. 594–602, 2009.

[7] A. K. Shoveller, D. L. Minikhiem, K. Carnagey et al., "Low level of supplemental dietary L-carnitine increases energy expenditure in overweight, but not lean, cats fed a moderate energy density diet to maintain body weight," *International Journal of Applied Research in Veterinary Medicine*, vol. 12, no. 1, pp. 33–43, 2014.

[8] M. R. C. de Godoy and A. K. Shoveller, "Overweight adult cats have significantly lower voluntary physical activity than adult lean cats and physical activity is profoundly related to the presence of humans," *Journal of Feline Medicine and Surgery*, In press.

[9] S. A. Center, K. L. Warner, J. F. Randolph, G. D. Sunvold, and J. R. Vickers, "Influence of dietary supplementation with L-carnitine on metabolic rate, fatty acid oxidation, body condition, and weight loss in overweight cats," *American Journal of Veterinary Research*, vol. 73, no. 7, pp. 1002–1015, 2012.

[10] M. A. Gooding, G. M. Davenport, J. L. Atkinson, I. J. H. Duncan, and A. K. Shoveller, "Dietary avocado derived mannoheptulose results in increased energy expenditure after a 28 day feeding trial in cats," *International Journal of Applied Research in Veterinary Medicine*, vol. 12, no. 2, pp. 130–140, 2014.

[11] F. Masi, B. Leggio, G. Nanni et al., "Effects of long-term acetyl-L-carnitine administration in rats—II: protection against the disrupting effect of stress on the acquisition of appetitive behavior," *Neuropsychopharmacology*, vol. 28, no. 4, pp. 683–693, 2003.

[12] N. W. Milgram, J. A. Araujo, T. M. Hagen, B. V. Treadwell, and B. N. Ames, "Acetyl-L-carnitine and α-lipoic acid supplementation of aged beagle dogs improves learning in two landmark discrimination tests," *The FASEB Journal*, vol. 21, no. 13, pp. 3756–3762, 2007.

[13] L.-A. Christie, W. O. Opii, E. Head et al., "Short-term supplementation with acetyl-L-carnitine and lipoic acid alters plasma protein carbonyl levels but does not improve cognition in aged beagles," *Experimental Gerontology*, vol. 44, no. 12, pp. 752–759, 2009.

[14] T. M. Hagen, R. T. Ingersoll, C. M. Wehr et al., "Acetyl-L-carnitine fed to old rats partially restores mitochondrial function and ambulatory activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 16, pp. 9562–9566, 1998.

[15] M. A. Gooding, I. J. H. Duncan, J. L. Atkinson, and A. K. Shoveller, "Development and validation of a behavioral acclimation protocol for cats to respiration chambers used for indirect calorimetry studies," *Journal of Applied Animal Welfare Science*, vol. 15, no. 2, pp. 144–162, 2012.

[16] J. B. de Weir, "New methods for calculating metabolic rate with special reference to protein metabolism," *The Journal of Physiology*, vol. 109, no. 1-2, pp. 1–9, 1949.

[17] M.-S. Lee, H.-J. Lee, H.-S. Lee, and Y. Kim, "L-carnitine stimulates lipolysis via induction of the lipolytic gene expression and suppression of the adipogenic gene expression in 3T3-L1 adipocytes," *Journal of Medicinal Food*, vol. 9, no. 4, pp. 468–473, 2006.

[18] J. S. Kang, W. K. Lee, C. W. Lee et al., "Improvement of high-fat diet-induced obesity by a mixture of red grape extract, soy isoflavone and L-carnitine: implications in cardiovascular and non-alcoholic fatty liver diseases," *Food and Chemical Toxicology*, vol. 49, no. 9, pp. 2453–2458, 2011.

[19] Y. J. Kim, K.-Y. Kim, M. S. Kim, J. H. Lee, K. P. Lee, and T. Park, "A mixture of the aqueous extract of *Garcinia cambogia*, soy peptide and L-carnitine reduces the accumulation of visceral fat mass in rats rendered obese by a high fat diet," *Genes & Nutrition*, vol. 2, no. 4, pp. 353–358, 2008.

[20] K. A. Amin and M. A. Nagy, "Effect of carnitine and herbal mixture extract on obesity induced by high fat diet in rats," *Diabetology & Metabolic Syndrome*, vol. 1, pp. 17–31, 2009.

[21] G. R. Blanchard, B. M. Paragon, F. Milliat, and C. Lutton, "Dietary L-carnitine supplementation in obese cats alters carnitine metabolism and decreases ketosis during fasting and induced hepatic lipidosis," *Journal of Nutrition*, vol. 132, no. 2, pp. 204–210, 2002.

[22] F. Graziano, R. Bisonni, V. Catalano et al., "Potential role of levocarnitine supplementation for the treatment of chemotherapy-induced fatigue in non-anaemic cancer patients," *British Journal of Cancer*, vol. 86, no. 12, pp. 1854–1857, 2002.

[23] R. Anty, S. Marjoux, S. Bekri et al., "Plasma carnitine is associated with fatigue in chronic hepatitis C but not in the irritable bowel syndrome," *Alimentary Pharmacology and Therapeutics*, vol. 33, no. 8, pp. 961–968, 2011.

[24] G. Gramignano, M. R. Lusso, C. Madeddu et al., "Efficacy of L-carnitine administration on fatigue, nutritional status, oxidative stress, and related quality of life in 12 advanced cancer patients undergoing anticancer therapy," *Nutrition*, vol. 22, no. 2, pp. 136–145, 2006.