Small Dog Metabolism and Other Unique Characteristics

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Abstract

Small dogs have many inherent attributes that are unique. In addition to the obvious difference in their size, small dogs have higher mass-specific basal metabolic rates; fixed alleles, including those around insulin-like growth factor and its receptor; faster growth rates; decreased bone mineral density; and increased longevity. Additionally, we have found differences in the metabolism of small dogs as compared to non-small dogs. Small dogs have a decreased antioxidant system, differences in amino acid metabolism, and may have a unique system involved in kidney biology. As work continues to characterize the unique nature of small dogs at the whole-animal and molecular level, it will further our understanding of the extreme diversity seen in *Canis lupus familiaris*.

Glossary of Abbreviations
BMR: Basal Metabolic Rate
DEXA: Dual-Energy X-Ray Absorptiometry
IGF-1: Insulin-Like Growth Factor 1
SNP: Single-Nucelotide Polymorphism

Introduction

The species *Canis lupus* contains very diverse members. Subspecies include wolves, dingos and dogs (*Canis lupus familiaris*). Even within the subspecies of dogs, there is an incredible diversity among its members. Domestication events between 15,000 and 100,000 years ago and artificial selection hundreds of years ago has led to the creation of modern dog breeds. The creation of these breeds has introduced fixed phenotypes. These phenotypes include behavioral traits (e.g., herding, hunting) as well as morphological traits (e.g., body size, coat color). Of these, dogs display the greatest diversity in body size of any land mammal.[1] Weight can vary between 1 kg and 100 kg, though some dogs have been reported to weigh as much as 150 kg. Height can vary as much as 50 times among some dogs. Taking these large differences into account, it still begs the question, "Isn't a dog, a dog?" This paper will give a brief review of unique characteristics of small dogs, as compared to larger dogs, as well as highlight some research we have recently conducted.

General Metabolic Considerations

Basal metabolic rate (BMR) represents the required energy to sustain basal metabolism. This is typically measured in a post-absorptive animal that is at rest in a thermal neutral environment. As dog breed sizes increase, the BMR increases.[2] However, on a per body weight basis, small dogs have a higher BMR (mass-specific BMR).[2,3] Papillons were shown to have a roughly 50% higher mass-specific BMR than Great Danes.[3] Some of this can be explained by an increase in surface area per weight in small dogs. Intuitively, this makes sense since weight is driven by volume, in which units are cubed, and by surface area, in which units are squared. The increase in surface area per weight would require a higher mass-specific BMR to maintain body temperature.

Another factor playing a role in BMR is proportion of lean body mass. Lean-body mass as measured by skeletal muscle accounts for up to 61% of BMR in dogs.[4] Small dogs have been shown to have a significantly higher percentage of lean tissue.[5] We recently conducted a study on small dog metabolism (explained in more detail later), which shows similar findings. In our study, small dogs showed a trend for a higher percentage of lean mass ($P = 0.06$), as determined by dual-energy X-ray absorptiometry (Table 1). However, some reports have indicated that small dogs have a lower percentage of lean-body mass.[2] These reports also show inconsistent results depending on the study reviewed. Since lean-body mass accounts for such a large part of BMR, more work will need to be done to elucidate how lean body mass relates to the size of the animal and its metabolic rate.

Growth Rate, Maturity and Longevity

Generally, relative growth rates are faster in small dogs as compared to larger ones. This results in a significant

Table 1. Selected clinical parameters in small dogs as compared to non-small dogs (Others). Mean, standard error of the mean (SEM) and adjusted *P* values are shown. DEXA = dual-energy X-ray absorptiometry; TAS = total antioxidant status.

Biological Area	Clinical Measure	Mean Small	SEM Small	Mean Others	SEM Others	Adj. P Value
Body Composition	DEXA Tissue, Lean (%)	75.43	1.05	70.88	1.41	0.059
	DEXA Bone Density (gm/cm2)	0.65	0.01	0.78	0.01	3.07E-10
Antioxidant	Serum TAS (mmol/L)	1.49	0.03	1.60	0.02	0.031
	Serum Total Bilirubin (mg/dL)	0.10	0.01	0.13	0.01	0.016
Metabolism	Protein Digestibility (%)	88.00	0.38	85.99	0.57	0.007

positive correlation between number of weeks to reach 50% of adult weight and current body mass.[2,6] Controlling growth rates is important for bone growth in large-breed puppies. Overnutrition can lead to an increased bone growth that results in a less-dense skeletal structure and malformation.[7,8] While small dogs do not appear to have this issue, they do have a lower level of bone mineral density.[5] In our study, we found similar results with small dogs having significantly lower bone mineral density as compared to non-small dogs (0.65 and 0.78 g/cm,[2] respectively) (Table 1). Aside from an increased incidence in some types of fractures in toy breeds, usually from falling after being held, little is known regarding bone fracture frequency in small dogs as compared to non-small dogs.

Maturity also occurs faster in small dogs. Small breeds reach maturity (as measured by 99% of their adult body weight) in as little as 42 weeks, while giant breeds can take as long as 65 weeks.[9] However, it should be noted that there are exceptions as some medium breeds reach maturity in a shorter period as compared to some small breeds.

Mammals, typically, show a positive correlation between longevity and body mass; however, dogs show a negative correlation. In a survey representing nearly 16,000 deaths of purebred dogs in the U.K., the median age at death was 11 years and 2 months.[10] Toy breeds (3), small breeds (9), and medium breeds (2) represented the 14 breeds with the highest median age at death. Giant breeds (6), large breeds (2), and medium breeds (2) were included in the 11 breeds with the lowest median age at death. Similarly, a meta-analysis noted that small dogs lived longer than larger dogs.[2] Toy Poodles had the longest average life span (13.2 years), and the shortest was the Bloodhound (5.5 years). Whether the intention of creating breeds by artificial selection was to select differences in longevity and/or age of maturity, these differences did end up being an inherent part of dog breeds.

Molecular Aspects

The identification of quantitative trait loci associated with skeletal variation in Portuguese Water Dogs is one of the earliest examples of determining the genetic basis of canine body size.[11] Building on this, a haplotype with single-nucleotide polymorphisms (SNPs) was identified in small-breed dogs, mostly not shared with giant-breed dogs, and spanned the insulin-like growth factor 1 (IGF1) gene.[12] This work also showed that circulating IGF1 levels were lower in animals with this haplotype. IGF1 is a protein involved in mediating growth and development and regulates skeletal size in other mammals.[13,14]

Subsequently, a polymorphism in the receptor for IGF1 also was found to be associated with breed body size.[15] Since then, multiple studies have identified loci associated with various morphometric differences in dogs including body, dental and cranial size, body dimension, and dental, cranial and bone shape.[1,16]

In order to further understand unique characteristics of small dogs at the molecular level, we have studied the metabolism of small dogs as compared to non-small dogs (83 dogs: 34 small, 49 non-small). Small dogs included Fox Terriers, Miniature Schnauzers and Beagles. Non-small dogs included Labrador Retrievers, English Setters, Siberian Huskies, and Rottweilers. All dogs were fed the same diet and housed at the same location (except Rottweilers). Plasma metabolomics, clinical measures, and fecal microbiome analysis was performed. Additionally, small dog module detection was performed taking into account relationships (clustering and correlations) between metabolites and clinical measures. Of these analyses, antioxidant status, amino-acid metabolism, and measures associated with kidney function were the most pronounced differences identified.

Antioxidant status was significantly lower in small dogs based on multiple parameters: total antioxidant status, urate, bilirubin,

Table 2. Selected metabolites significantly different in small dogs as compared to non-small dogs (Others). Ratios of median transformed values and Benjamini-Hochberg adjusted *P* values are shown.

Biological Area	Metabolite Name	Ratio (Small/Others)	Adj. *P* Value
Antioxidant	urate	0.72	1.58E-04
	bilirubin (E,E)	0.50	0.031
	gamma-glutamylphenylalanine	0.71	3.66E-06
	gamma-glutamylisoleucine	0.78	4.15E-04
	gamma-glutamylleucine	0.76	4.15E-04
	gamma-glutamylvaline	0.77	4.64E-04
	gamma-glutamyltyrosine	0.75	0.002
	gamma-glutamylmethionine	0.77	0.008
	5-oxoproline	0.78	3.28E-07
	ophthalmate	0.47	0.023
	cysteine-glutathione disulfide	0.81	0.038
Amino Acid	phenylalanine	0.84	7.97E-05
	glutamine	0.89	0.002
	tyrosine	0.86	0.010
	arginine	1.15	0.015
	lysine	0.75	0.049
	hydroxyproline	0.72	7.97E-05
	prolylhydroxyproline	0.19	0.005
	p.cresol.sulfate	1.48	1.05E-04
	phenol.sulfate	1.74	6.12E-04

and several glutathione metabolites (Tables 1 and 2). Total antioxidant status represents circulating total antioxidant potential. Urate is a powerful antioxidant that is converted to allantoin by urate oxidase; this is similar in most mammals except in humans where the urate oxidase gene is silenced.[17] Bilirubin associated with heme catabolism is complementary to water-soluble antioxidants as it is lipophilic.[18] The lower levels of bilirubin seen in small dogs were measured in both metabolomics and in a conventional clinical assay.

Glutathione is present in high intra-cellular concentrations and is one of the most important scavengers of reactive oxygen species. Glutathione is involved in a variety of biological processes including transcription, proliferation, apoptosis via its involvement in peroxide detoxification, leukotriene biosynthesis, maintaining thiol disulfide balance, and amino acid transport.[19] Multiple glutathione metabolites were lower in small dogs. Since the ratio of reduced glutathione to oxidized glutathione was not measured, it is difficult to conclude its true relevance. However, based on the number of different metabolites and clinical measures represented here, small dogs appear to have a decreased antioxidant system. This may be a reflection of a small dog's increased mass-specific BMR since an increase in metabolic rate could tax the neutralization of free radicals produced during metabolism.

Multiple circulating amino acids were different between small dogs and non-small dogs with the majority being lower in small dogs (Table 2). Interestingly, protein digestibility was greater in small dogs (Table 1). Of the amino acids at different levels, the essential amino acids phenylalanine, tyrosine, and lysine were lower in small dogs. Arginine was higher in small dogs. Arginine and lysine share the same cationic amino acid transport system. One of these amino acids can competitively inhibit the other, which could explain the negative correlation among them.[20]

Tyrosine and phenylalanine share many of the same metabolic pathways. Both of these were lower in small dogs. Due to microbial fermentation, tyrosine can be metabolized to p-Cresol and indoxyl.[21] These compounds can then be sulfated by the host liver into p-Cresol sulfate and phenol sulfate. Both of these compounds were higher in small dogs indicating the metabolism of the precursor amino acids, tyrosine and phenylalanine, by microbes (Table 2). Additionally, the genus *Bacteroides* was present in higher levels of feces in small dogs as measured by 16S microbiome analysis. *Bacteroides fragilis* produces phenols from tyrosine,[21] providing further evidence of the amino-acid metabolism by microbes in small dogs. Since this would affect protein digestibility measures, microbial contributions may need to be taken into account when making conclusions based on protein digestibility.

In order to better understand the interrelationships of the metabolites and their relationship to clinical measures, a module with the highest ability to distinguish small dogs from non-small dogs was identified based on hierarchical clustering of metabolites and their correlation to clinical measures. Of the 64 metabolites belonging to this module, over half have evidence of being associated with kidney function, development, or disease. Nearly every clinical measure that correlated to these metabolites also have evidence of associating with kidney biology. Since the animals used in this study did not have clinical signs of kidney disease, this module likely represents biological differences in kidney function. This represents an area for future work as it opens up new markers of kidney function as well as how they interrelate.

Conclusion

Progress in the areas of growth, longevity, metabolic rate as well as the genetic basis of small dogs has made immense strides in the past 10 to 15 years. We are now at a stage where we have begun to understand metabolic differences inherent to small dogs. This is important not only for a greater understanding of these biological systems and its application toward human health and disease but also for improving the health and wellness of small dogs.

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