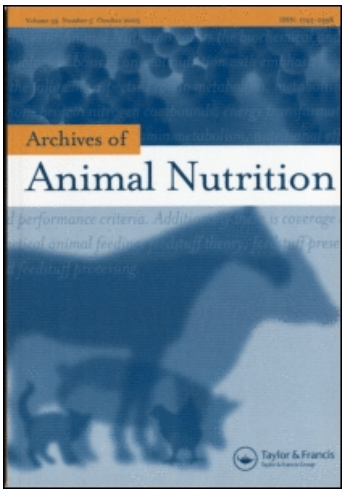

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Adipose tissue gene expression profiles of healthy young adult and geriatric dogs

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Obesity is a major problem in today's dog population, with aged animals having an increased susceptibility to obesity-related comorbidities. A molecular approach to studying adipose tissue may enhance our understanding of its role in energy homeostasis and the disease process. Thus, the objective of this study was to use canine microarrays to compare gene expression profiles of adipose tissue from geriatric and young adult dogs. Adipose tissue samples were collected from six geriatric (12 year-old) and six young adult (one-year-old) female beagles after being fed one of two diets (animal protein-based vs. plant protein-based) for 12 months. RNA samples were hybridised to canine microarrays. Statistical analyses indicated that age had the greatest impact on gene expression, with 65 differentially expressed gene transcripts in geriatric dogs. Diet had a minor impact on gene expression, altering the expression of only 19 gene transcripts. In general, adipose tissue of geriatric dogs had increased expression of genes associated with cell cycle and growth, cell development and structure, cellular trafficking and protein processing, immune function, metabolism, and transcription and translation, as compared with that of young adults. Overall, our mRNA data suggest either an increased population of macrophages or increased inflammatory nature of adipocytes in adipose tissue of aged dogs.

Keywords: dogs; adipose tissue; aging; gene expression, canine microarrays

1. Introduction

Approximately 35% of the canine population in the US and other developed nations is considered overweight or obese (Lund et al. 2006). Similar to humans, canine obesity is a risk factor for numerous disease states, including metabolic abnormalities, endocrinopathies, orthopedic disorders, cardiorespiratory disease, urogenital diseases, and cancer (German 2006). Plasma metabolic and hormonal profiles of obese dogs have been reported (Yamka et al. 2006), but metabolic and transcriptomic changes within tissues of lean vs. obese dogs have not been extensively studied. Because aged animals are believed to have an increased susceptibility to the toxic effects of lipid overload (lipotoxicity) associated with overnutrition (Slawik and Vidal-Puig 2006), research in aged dogs is needed.

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Adipose tissue is an active endocrine organ that plays a pivotal role in energy homeostasis, and thus, obesity incidence. Adipose tissue is highly regulated by hormones and responds to numerous physiological conditions such as aging, nutritional status, and exercise. Thus, research aimed at identifying adipose tissue gene expression changes with age or diet may enhance our understanding of basal adipose metabolism and provide a foundation for obesity research.

Recent advances in genomic biology have provided canine sequence data and tools to study molecular events occurring in specific cells or tissues. Gene expression analyses may aid in our understanding of adipocyte biology and its role in obesity and comorbidities or age-related metabolic changes. The expression of genes in canine adipose tissue associated with lipid and glucose metabolism, including peroxisome proliferator-activated receptor-γ and its target genes, inflammatory cytokines, and other adipokines have recently been studied *in vitro* and *in vivo* (Eisele et al. 2005; Gayet et al. 2007; Leray et al. 2008). Despite this recent progress, most canine experiments have focused on a small number of target genes in lean vs. obese populations of young adults.

DNA microarrays allow the measurement of thousands of gene transcripts simultaneously, providing a global view of gene expression. Over the past decade, microarrays have been used to characterise the molecular events occurring in numerous complex systems. Gene expression changes during adipocyte differentiation in cell culture (Guo and Liao 2000; Burton et al. 2002) and differences between human preadipocytes and adipocytes (Urs et al. 2004) have been evaluated using microarray technology. To our knowledge, however, molecular analysis of geriatric vs. young adult adipose tissue using microarray technology has not yet been performed in dogs. Previously, we reported numerous metabolic, physiologic, and transcriptomic differences in young adult vs. geriatric beagles fed different diets (Swanson et al. 2004, 2009; Kuzmuk et al. 2005). This study extends our previous findings with the primary objective of determining changes in canine adipose tissue transcriptional activity as a function of diet and age.

2. Materials and methods

2.1. Animals and diets

Six geriatric (average age = 11.1 years old at baseline; Kennelwood Inc., Champaign, IL, USA) and six weanling (8 weeks old at baseline; Marshall Farms USA, Inc., North Rose, NY, USA) female beagles were used in this experiment. Three dogs of each age were assigned to one of two dietary treatments. Diets tested in this experiment were previously reported to manipulate whole body metabolism, including circulating concentrations of cholesterol, alanine transaminase, alkaline phosphatase, corticosteroid-induced alkaline phosphatase (Swanson et al. 2004; Kuzmuk et al. 2005). Dry kibble diets were manufactured by Wenger Manufacturing Company (Sabetha, KS) as described by Swanson et al. (2004) and fed for 12 months. One diet was an animal-protein based diet (APB) and was composed primarily of highly digestible ingredients and animal-derived protein and fat sources (brewer's rice, poultry by-product meal, poultry fat) and was formulated to contain 28% protein, 23% fat, and 5% dietary fibre. The other diet was a plant-protein based diet (PPB) and was composed primarily of moderately digestible plant-derived ingredients (corn, soybean meal, wheat middlings, and meat and bone meal) and was formulated to contain 26% protein, 11% fat, and 15% dietary fiber (Swanson et al.

2004). Although the two diets were very different in terms of ingredient and chemical composition, both were formulated to meet or exceed all nutrient requirements for canine growth according to the Association of American Feed Control Officials (AAFCO 2008).

Young dogs were fed *ad libitum* to allow for adequate growth, while geriatric dogs were fed to maintain baseline bodyweight (BW) throughout the experiment. Young adult dogs maintained a healthy body condition score (5/9 to 6/9) throughout the experiment. Most geriatric dogs had a slightly greater body condition score than young adults, but were more variable (3/9 to 7/9). In addition to age, it is possible that these differences in body condition also affected adipose tissue gene expression.

To produce the desired metabolic effects, the PPB diet was formulated to contain a lower energetic density (APB = 22.51 kJ/g; PPB = 19.87 kJ/g) and have a lower nutrient digestibility than the APB diet. Thus, dogs fed the PPB diet needed to consume a greater ($p < 0.05$) quantity of food (237 g/d; 4699 kJ/d) than dogs fed the APB diet (166 g/d; 3736 kJ/d) to grow (young dogs) or maintain BW (old dogs). Even though metabolic indices were altered, mean BW among dietary treatments was not different at any time over the course of the study for young or geriatric dogs (Swanson et al. 2004).

At the time of tissue collection, the mean age of geriatric and young adult dogs were 12.1 years and 14 months of age, respectively. Dogs were housed individually in kennels (1.1 × 0.9 m) in temperature-controlled (22.2°C) rooms with a 12-h light:12-h dark cycle at the Edward R. Madigan Laboratory on the University of Illinois campus. The Institutional Animal Care and Use Committee approved all animal care procedures prior to initiation of the study.

2.2. *Tissue sample collection and handling*

After 12 months on experiment, animals were fasted for 12 h and then given a lethal dose (130 mg/kg BW) of sodium pentobarbital (Euthasol®, Virbac Corp., Fort Worth, TX, USA) intravenously into the left forearm. Death was confirmed by lack of respiration and a corneal reflex, and absence of a heartbeat detected with a stethoscope placed under the left elbow. Abdominal subcutaneous adipose tissue samples were collected immediately after death was confirmed, flash frozen using liquid nitrogen, and stored at −80°C until further analysis.

2.3. *RNA extraction*

Total cellular RNA was isolated from adipose samples using the Trizol reagent as suggested by manufacturer (Invitrogen, Carlsbad, CA, USA). RNA concentration was determined using a ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA integrity was confirmed using a 1.2% denaturing agarose gel. Since it was critical to measure inter-animal variation, tissue samples were not pooled in this experiment. Thus, each animal was analysed as an individual experimental unit.

2.4. *Microarray analyses*

All RNA samples were prepared and hybridised to the Affymetrix GeneChip® Canine Genome 2.0 Arrays (Affymetrix, Santa Clara, CA, USA). All reactions were

performed using Affymetrix GeneChip® Expression 3′-Amplification Reagents (One-Cycle Target Labeling and Control Reagents package) according to the manufacturer's instructions and as described by Swanson et al. (2009).

Following hybridisation, chips were washed and stained with streptavidin-conjugated phycoerythrin dye (Invitrogen) enhanced with biotinylated goat anti-streptavidin antibody (Vector Laboratories, Burlingame, CA, USA) utilising an Affymetrix GeneChip® Fluidics Station 450 and GeneChip® Operating Software. Finally, images were scanned using an Affymetrix GeneChip® Scanner 3000.

Affymetrix's Canine Genome 2.0 array contains 42,860 probe sets, which interrogate over 18,000 canine EST/mRNA-based transcripts plus over 20,000 non-redundant canine gene predictions. The chip design was based on content from *Canis familiaris* UniGene Build #11 (April, 2005), GenBank® mRNAs up to 15 April 2005, and the BROADD1 gene predictions. In addition to the recommended Affymetrix quality control measures, we performed both graphical and quantitative QC assessments of the arrays using the affy (Gautier et al. 2004), made4 (Culhane et al. 2005), and affyPLM (Bolstad, 2004) packages from the Bioconductor Project (Gentleman et al. 2004). All arrays passed the QC assessments.

Each probe set consists of 11 perfect match (PM) and 11 mismatch (MM) probes. The raw PM and MM probe-level data were pre-processed into one number per probe set using the GCRMA algorithm in Bioconductor's affy (Gautier et al. 2004) and GCRMA (Irizarry et al. 2003) packages. GCRMA does a background correction based on GC-content, performs quantile normalisation and then summarises the PM values into one number using median polish. We used Affymetrix's call detection algorithm (Affymetrix manual, 2002) to assess which probe sets were reliably detected above background on each array. Probe sets were discarded from further analysis if it was not called present on at least one array or marginal on two arrays. Of the 42,860 probe sets, 26,268 passed this filter and were assessed for differential expression due to age and diet (described below). MetaCore (GeneGo, Inc., St. Joseph, MI, USA) was used to build gene networks and interpret microarray data. Functional attribution was made according to the database SOURCE (http://source.stanford.edu) (Diehn et al. 2003).

2.5. *Statistical analyses*

Differential expression of the microarray data was assessed using the limma package (Smyth 2004). A linear model for the four age × diet groups was fit for each probe set; then differences between groups were extracted from the model as contrasts. An empirical Bayes 'shrinkage' method was employed on the standard errors to improve power for small sample sizes (Smyth 2004). A multiple test correction of p-values was done using a false discovery rate (FDR) method (Benjamini and Hochberg 1995). Gene transcripts having >1.5-fold change and FDR <0.10 were considered significantly altered.

3. Results and discussion

Because they provide a global view of tissue gene expression, DNA microarrays have been a popular tool for the study of complex biological systems. In this experiment, our objective was to use microarray technology to identify genes and/or biological pathways differentially expressed in adipose tissue of aged vs. young adult canines

fed different diets. The diets fed in this experiment were practical, yet distinct enough to result in numerous physiological differences (Swanson et al. 2004; Kuzmuk et al. 2005). The overall adipose tissue gene expression patterns, however, were only mildly impacted by diet. Because the majority of gene expression differences were due to age, they are the primary focus of the discussion.

Using our statistical parameters, 84 transcripts (65 due to age; 19 transcripts due to diet) were differentially expressed. Of these transcripts, we identified 43 and 15 non-redundant, annotated genes that were affected by age (Table 1) and diet (Table 2), respectively. Many age $\times$ diet interactions were noted. According to gene ontology classification (http://source.stanford.edu; Diehn et al. 2003), age-related gene expression changes grouped into several broad functional categories, including cell cycle and growth, cell development and structure, cellular trafficking and protein processing, immune function, metabolism, and transcription and translation.

In agreement with a previous study performed in mice (Wu et al. 2007a), mRNA abundance of numerous genes associated with immune function or inflammatory response were increased with age in the current study. Several chains of MHC class II, CD74, chemokine (C-C motif) receptor 5 (CCR5), and lymphocyte cytosolic protein 2 were all increased by at least 4-fold in aged dogs. CD74 is a type II transmembrane protein that functions as a MHC class II chaperone and is also an important signaling molecule in several pathways. CD74 has been shown to be a high-affinity binding protein for the proinflammatory cytokine, macrophage migration inhibitory factor, which is an upstream activator of monocytes/macrophages (Leng et al. 2003). Lymphocyte cytosolic protein 2 is expressed in platelets, neutrophils, mast cells, macrophages, and natural killer cells and is a signal transducer downstream of T-cell receptors (Koretzky et al. 2006).

CCR5 is expressed on memory T-cells, monocytes, macrophages, and other immune cells and is a receptor for chemokines involved in the immunological response at sites of inflammation. CCR5 is the main receptor for macrophage inhibitory proteins 1-α (MIP-1α) and MIP-1β and RANTES (regulated on activation normal T-cell expressed and secreted), and is thought to provide a strong signal for the recruitment of macrophages and T-cells (Blaak et al. 2000). Adipose tissue RANTES and CCR5 mRNA levels have been demonstrated to be increased in obese mice and humans (Wu et al. 2007b), but to our knowledge, not in aged individuals.

Increased folate receptor expression, as was observed in aged dogs consuming PPB, has been suggested to occur during macrophage activation (Antohe 2006). SLC37A2, upregulated in aged dogs consuming APB, is a macrophage-enriched transcript restricted to spleen, thymus, and white adipose tissue and dramatically increases ($\sim$45-fold) during macrophage differentiation (Kim et al. 2007). Kim et al. (2007) postulated that the function of SLC37A2, a putative sugar transporter, is central to the metabolism of the macrophage population specifically present in white adipose tissue.

Expression of septin 5, septin 9, GPNMB, and DDX11, all involved with cell cycle and growth, were up-regulated in aged dogs. The gene having by far the greatest fold change in geriatric vs. young adult dogs, was glycoprotein nmb (GPNMB; previously known as osteoactivin). Although its functional classification falls under cell cycle and growth, GPNMB appears to have functions pertaining to inflammatory response as well. Ahn et al. (2002) first reported the expression of GPNMB in numerous dendritic cell subsets, but its function was unknown at that time. Recent research demonstrated a strong up-regulation of GPNMB during

Table 1. Genes differentially expressed in adipose tissue of geriatric versus young adult dogs.

Functional classification	Gene name	Gene symbol	Fold change (APB*)	Fold change (PPB#)
Cell cycle and growth				
Cell cycle	Septin 5	SEPT5	2.9	
Cell cycle	Septin 9	SEPT9		2.2
Cell cycle	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11	DDX11		1.6
Cell proliferation	Glycoprotein nmb	GPNMB	88.4	61.2
DNA replication	Replication factor C4	RFC4		−10.4
Cell development and structure				
Cell-cell adhesion	Lectin, galactoside-binding, soluble, 3 binding protein	LGALS3BP	3.7	
Cell-cell adhesion	Plakophilin 2	PKP2	4.2	
Cell-cell adhesion	Roundabout 1 isoform A	ROBO1	2.3	
Cytolysis	Monocyte to macrophage differentiation associated	MMD		−3.1
Development	Blood vessel epicardial substance	BVES	1.7	
Development	Fibulin 1	FBLN1	3.3	4.4
Protein modification	Lysyl oxidase	LOX	3.4	
Cellular trafficking and protein processing				
Transport	Copper transporter homolog	CUTC		−2.2
Transport	Folate receptor 2	FOLR2		6.4
Transport	Lysosomal associated multispanning membrane protein 5	LAPTM5	11.2	11.3
Transport	Solute carrier family 37 (glycerol-3-phosphate transporter), member 2	SLC37A2	3.6	
Transport	Solute carrier family 40 (iron-regulated transporter), member 1	SLC40A1		6.2
Immune function				
Immune function	Chemokine (C-C motif) receptor 5	CCR5	5.3	
Immune function	Immunoglobulin mu chain, C region			14.4
Immune function	MHC class II, DR alpha chain	HLA-DRA	4.7	6.5
Immune function	MHC class II, DQ alpha chain	HLA-DQA	5.7	6.6
Immune function	MHC class II, DQ beta chain	HLA-DQB	7.1	5.2
Immune function	MHC class II, invariant chain	CD74		5.8
Immune function	Lymphocyte cytosolic protein 2	LCP2	4.0	
Metabolism				
AA† metabolism	Branched chain aminotransferase 1	BCAT1	2.5	
CHO‡ metabolism	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH		−2.4
CHO metabolism	Phosphorylase kinase alpha 2	PHKA2		3.2
CHO metabolism	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1	B4GALT1		1.8
Lipid metabolism	Acyl-CoA thioesterase	ACOT7	2.6	
Lipid metabolism	Acyloxyacyl hydrolase	AOAH	1.6	

(*continued*)

Table 1. (*Continued*).

Functional classification	Gene name	Gene symbol	Fold change (APB*)	Fold change (PPB#)
Metabolism	Chitinase 3-like-1	CHI3L1	15.7	
Metabolism	Cytochrome P450 2B11	CYP2B11		14.0
Myoinositol metabolism	Myoinositol oxygenase	MIOX		12.9
Transcription-translation				
RNA catabolism	Decapping enzyme homolog B	DCP1B		−1.5
RNA processing	Cytoplasmic polyadenylation element binding protein 1		4.4	
Transcription	Ecdysoneless homolog	ECD		−2.9
Transcription	Liver-specific bHLH zip transcription factor			1.7
Transcription	Non-histone chromosomal protein high mobility group-17	HMG-17	4.7	4.5
Miscellaneous and unknown				
Unknown	Coagulation factor C homolog, cochlin	COCH	5.8	4.3
Unknown	EF hand calcium binding domain 1	EFCAB1	3.3	
Unknown	Family with sequence similarity 62, member A	FAM62A	−4.4	
Unknown	G protein-coupled receptor 137B	GPR137B	2.4	
Unknown	Macrophage expressed gene 1	MPEG1	4.1	2.7

Notes: *APB, animal protein-based diet; #PPB, plant protein-based diet; †AA, amino acid; ‡CHO, carbohydrate.

macrophage differentiation and stimulation by interferon-γ and lipopolysaccharide (Ripoll et al. 2007). These researchers also performed functional analysis and suggested that GPNMB acts as a negative regulator of macrophage inflammatory responses, potentially impacting inflammatory disease processes (Ripoll et al. 2007). To our knowledge, this is the first time GPNMB has been reported as being highly upregulated in adipose tissue of aged animals, a finding that justifies further research pertaining to its role in adipose tissue.

Several genes having a role in cell development and/or structure were also upregulated in aged dogs. Galectin 3 binding protein (LGALS3BP) was increased in geriatric vs. young adult dogs consuming APB. Galectins are a family of carbohydrate-binding proteins that function as modulators of cell adhesion and play key roles in many pathologic conditions, including inflammation associated with diabetes, hypertension, and rheumatoid arthritis (Wada and Makino 2001; Ohshima et al. 2003). Galectin 3 and its binding protein are not only involved with the inflammation associated with rheumatoid arthritis, but are thought to also play an important role in the activation of synovial fibroblasts (Ohshima et al. 2003).

Similar results have been reported in adipose tissue, as galectin 3 has been shown to stimulate preadipocyte proliferation and be up-regulated in growing adipose tissue (Kiwaki et al. 2007). Galectin 3 is a chemoattractant for monocytes and macrophages and it is thought to be involved in modulating integrin activity during the inflammatory process (Sano et al. 2000). Modifying integrin activity may not only promote cell motility during extravasation by weakening adhesion to the

Table 2. Genes differentially expressed in adipose tissue of geriatric dogs fed an animal protein-based (APB) versus a plant protein-based (PPB) diet.

Functional classification	Gene name	Gene symbol	Fold change
Cell development and structure			
Cell-cell adhesion	Roundabout 1 isoform A	ROBO1	2.3
Development	Blood vessel epicardial substance	BVES	1.8
Protein modification	Lysyl oxidase	LOX	3.4
Protein processing			
Protein catabolism	Ubiquitin specific peptidase 15	USP15	1.6
Protein catabolism	Osteopetrosis associated transmembrane protein 1	OSTM1	1.8
Immune function			
Immune function	Immunoglobulin mu chain, C region		−14.5
Metabolism			
AA* metabolism	Branched chain aminotransferase 1	BCAT1	2.5
Lipid metabolism	Acyl-CoA thioesterase	ACOT7	2.6
Lipid metabolism	Acyloxyacyl hydrolase	AOAH	1.6
Metabolism	Chitinase 3-like-1	CHI3L1	13.9
Myoinositol metabolism	Myoinositol oxygenase	MIOX	−13.3
Transcription-translation			
Transcription	Eukaryotic translation initiation factor 4E binding protein 1	EIF4EBP1	−1.6
Miscellaneous and unknown			
Unknown	EF hand calcium binding domain 1	EFCAB1	3.4
Unknown	G protein-coupled receptor 137B	GPR137B	2.4

Note: *AA, amino acid.

endothelial layer, but extending the time macrophages are present at the site of inflammation by enhancing their adhesion to the extracellular matrix (Hughes 2001). Chitinase 3-like-1, another protein associated with conditions of increased matrix turnover and tissue remodeling, was upregulated in aged dogs fed APB. Ling and Recklies (2004) suggested a physiological role for this protein in limiting the catabolic effects of proinflammatory cytokines. In our experiment, increased chitinase 3-like-1 mRNA may have been in response to a greater inflammatory state in old dogs.

Lysyl oxidase is expressed in preadipocytes and dramatically decreases during adipose conversion, serving as an important early marker of adipocyte differentiation (Dimaculangan et al. 1994). In this study, lysyl oxidase expression was greater in geriatric vs. young adult dogs, suggesting lower adipocyte conversion in the aged population. Others have also reported a reduced capacity of preadipocytes to differentiate with aging (Kirkland et al. 1994). This change in adipogenic potential has been implicated in many processes related to aging, including stress response, inflammation, insulin sensitivity, and lipid metabolism, and may have contributed to the age-related gene expression differences noted with this study.

In addition to mRNA changes pertaining to inflammation or cell adhesion or development, several genes associated with cellular transport or metabolism were differentially expressed in aged dogs. Lysosomal-associated multispanning

membrane 5 (LAPTM5), a transmembrane protein residing in lysosomes and expressed primarily in hematopoietic cells (Adra et al. 1996), was up-regulated in aged dogs fed both diets. Lysosomes appear to play a role in lipid metabolism and energy balance. Mice with lysosomal storage disease have increased inflammatory proteins and lean mass versus controls, suggesting a shift from adipose triglycerides to lysosomal storage (Woloszynek et al. 2007). Although the function of LAPTM5 is poorly defined, some suggest that it is involved with lysosomal movement in immune cells and possibly in transport of molecules destined to be released from the lysosome once at the plasma membrane (Pak et al. 2006). The significance of increased LAPTM5 expression in aged dogs in the current study is not clear at this time.

Expression of ACOT7 and acyloxyacyl hydrolase (AOAH) were upregulated in aged dogs fed APB. Acyl-CoA thioesterases (Acots) modulate cellular levels of activated fatty acids (acyl-CoAs), free fatty acids, and CoA. Although Acots, in general, may cleave a broad range of activated CoA-ester substrates, ACOT7 has a high specificity for arachidonoyl-CoA, an important precursor molecule for proinflammatory eicosanoids (Forwood et al. 2007). Because ACOT7 was highly expressed in macrophages and upregulated by lipopolysaccharide exposure, it has been suggested to play a role in eicosanoid synthesis and inflammation (Forwood et al. 2007). AOAH is a lysosomal enzyme of neutrophils and macrophages that deacylates lipids from bacterial cell walls, but its function in adipose is not known.

As previously stated, there were few dietary effects on adipose tissue mRNA abundance in the current study. Fifteen non-redundant, annotated gene transcripts were differentially expressed between dogs fed APB and PPB. One gene family with sequence similarity 62 member A (FAM62A), of which a function is not known, was upregulated (4.3-fold) in young adult dogs fed APB vs. PPB. The other 14 genes differentially expressed due to diet occurred in aged dogs fed APB vs. PPB (Table 2), many of which (11/14) were also listed in Table 1.

Given our small sample size and the small number of genes that were identified as being differentially expressed, these data must be interpreted with caution. Nonetheless, genes associated with cell development and/or structure, protein processing, and amino acid and lipid metabolism were increased in aged dogs fed APB as compared to aged dogs fed PPB. Lysyl oxidase, a gene differentially expressed in aged vs. young adult dogs, was ~3-fold greater in aged dogs fed APB vs. PPB, suggesting that aged dogs fed APB had a lower adipocyte conversion than those fed PPB. Increased expression of roundabout 1 isoform 1 (ROBO1) and blood vessel epicardial substance (BVES) support this notion and suggest that cellular resources were prioritised to support existing cells rather than the differentiation of new cells.

ACOT7 and AOAH were upregulated in aged dogs fed APB vs. PPB. Both genes are functionally classified under lipid metabolism, but are also associated with an inflammatory response. Similarly, chitinase 3-like-1, which has been suggested to minimise the catabolic effects of proinflammatory cytokines (Ling and Recklies 2004), was also greatly increased (~14-fold) in aged dogs fed APB vs. PPB. In contrast, immunoglobulin mu chain, C region was largely down-regulated (~15-fold) in aged dogs fed APB vs. PPB. Increased expression of genes associated with amino acid and protein catabolism in aged dogs fed APB vs. PPB suggests increased protein turnover. Diet may affect adipose tissue gene expression in aged dogs, but because the diets tested in this study were largely different from one another (e.g. nutrient source, fat and fibre concentrations) the results may not be attributed to any

specific nutrient and were difficult to interpret. Research focused on testing specific dietary changes (e.g. antioxidant supplementation, fat concentration, fatty acid type) may prove beneficial.

In summary, our mRNA abundance data suggest either an increased population of macrophages or increased inflammatory nature of adipocytes in the adipose tissue of aged dogs. Because geriatric dogs had slightly higher, yet more variable, body condition scores, it is possible that gene expression differences were due to differences in age and body condition. Nevertheless, in addition to adipocytes, adipose tissue is comprised of several cell types including macrophages, fibroblasts, and endothelial cells. Non-fat cells in adipose tissue are known to contribute to gene expression and hormone release. Although adipose tissue macrophages usually have an anti-inflammatory phenotype, they have the capability to produce excessive amounts of pro-inflammatory mediators (Zeyda et al. 2007). In fact, recent studies have demonstrated that a majority of inflammatory cytokines secreted by adipose tissue is synthesised by non-adipocyte cells such as macrophages (Fain et al. 2006). Wu et al. (2007a) reported similar macrophage numbers in adipose tissue of young and old mice, and hypothesised that the increased inflammation in old mice was due to functional changes within the tissue. The scope of the current experiment did not include histological analyses or mRNA abundance of specific cell types within adipose tissue. However, our mRNA data demonstrate the need for further research in the aged dog population to determine which cell types are responsible for producing these proinflammatory mediators, what impact they have on obesity-related comorbidities, and if dietary intervention can be used to minimise their release. Identifying genotypic-phenotypic associations and changes due to age or specific dietary intervention will promote our understanding of adipocyte biology and energy homeostasis.

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