Plasma Metabolomics: Application to Canine Obesity

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Abstract

Metabolomics uses high-throughput technology to simultaneously analyze hundreds of low-molecular weight compounds, providing metabolite profiles during normal or diseased conditions. Such a strategy may provide a greater understanding of a disease or metabolic condition of interest and/or identify early biomarkers of the disease. Metabolomics technologies recently have been used to study obesity in humans and rodent models, but little has been published in dogs and cats. This presentation will provide a brief summary of the human and rodent literature and plasma metabolite data from a recent canine weight gain experiment performed in our laboratory.

Glossary of Abbreviations
BCS: Body Condition Score
BMI: Body Mass Index
BW: Body Weight

Introduction

It is estimated that over one-third of dogs in developed countries are overweight or obese.[1] As in humans, pet obesity may be attributed to many factors including genetics, a sedentary lifestyle, neutering, and overconsumption of energy-dense foods. Obese pets have numerous health issues including insulin resistance, diabetes mellitus, hyperlipidemia, musculoskeletal, renal, cardiorespiratory, and neoplastic disorders.[2] Despite the high incidence of obesity, the metabolic events that accompany this disease are still poorly understood at a molecular level.[3]

Metabolomics is a high-throughput technology that provides systematic analysis of low-molecular weight compounds, which can be used to characterize metabolic profiles during normal physiological conditions and pathologies.[4] Metabolomics often uses a multiplatform approach (e.g., nuclear magnetic resonance spectroscopy, mass spectroscopy, liquid chromatography, gas chromatography) with high sensitivity that allows for the determination (untargeted analysis) or quantification (targeted analysis) of biological compounds. This recent emergence of untargeted metabolomics analytical platforms have provided new strategies to: 1) elucidate the mechanisms by which a disease develops and progresses; 2) identify biological molecules that indicate a specific disease stage; and 3) predict effective therapeutic interventions to manage the disease.[5,6]

Using Metabolomics to Study Obesity in Humans and Rodent Models

Untargeted metabolomics strategies have been used in recent years to study obesity and related morbidities in humans and rodent models. Due to ease of access and low cost/equipment needs, metabolite profiles of blood are most commonly studied in human participants. Morris, et al.,[7] provided an overview of some of the initial human metabolomics studies focused on obesity and insulin resistance. The emphasis of that paper was on alterations in amino-acid metabolism, specifically the branched-chain amino acids and related metabolites, and their relationship with insulin resistance and diabetes. In general, increased circulating branched-chain amino acid concentrations are observed in an obese, insulin-resistant condition. Because most studies made cross-sectional associations between metabolites and body-mass index (BMI), it is unknown whether high-circulating amino acids are contributing to insulin resistance or are merely a response to the condition. Insulin resistance is known to cause excess protein breakdown in skeletal muscle.[8] Some amino acids have neuroactive properties,[9,10] which can alter appetite and feeding behaviors that contribute to obesity.

Not surprisingly, metabolites related to lipid and carbohydrate metabolism are also altered with obesity. Kim, et al.,[11] used ultra-performance liquid chromatography and mass spectrometry to study plasma of overweight/obese (n=30) and age-matched normal-weight (n=30) adult men. Their analyses suggested altered metabolism of branched-chain amino acids and aromatic amino acids and alterations in fatty-acid synthesis and oxidation in obese individuals. Similar data have been reported in children. Wahl, et al.,[12] identified three amino acids, nine phospholipids, two acylcarnitines, and 69 metabolite ratios that were different in obese versus lean children. Those researchers studied serum samples of obese (n=80) and normal-weight (n=40) children between 6 and 15 years of age using liquid chromatography and mass spectrometry. Finally, Moore, et al.,[13] studied blood metabolites of 947 participants from three studies performed in the U.S. and China. Those researchers identified

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nearly 40 metabolites that were significantly associated with BMI, including 19 lipids and 12 amino acids. In addition to increased branched-chain amino acids, butyrylcarnitine and 2-hydroxybutyrate were positively associated with BMI. Both of those metabolites are markers of excessive fatty-acid oxidation.[14,15] Glycerol, the backbone of triglycerides and marker of fatty-acid breakdown, and lathosterol, a cholesterol precursor, were increased with increasing BMI in that study. In contrast, several membrane lipids (e.g., phosphocholines, sphingomyelin) were decreased with increasing BMI. In regard to carbohydrates, mannose and lactate were increased and 1,5-anhydroglucitol (biomarker of short-term glucose control) was decreased with increasing BMI.[16]

Schäfer, et al.,[17] reported similar findings in a genetically obese mouse strain (Berlin Fat Mouse Inbred), with the obese having decreased circulating phosphatidylcholines and carnitine metabolites and increased amino acids compared to lean controls (n=5-9). Rodent experiments have also allowed for tissue collection and analysis. Kim, et al.,[18] used ultra-performance liquid chromatography gas chromatography and mass spectrometry to analyze liver and serum samples of high-fat diet-induced obese versus lean C57BL/6 mice. Liver and serum samples from the obese mice had shifts in amino acids, carnitine metabolites and lipid profiles. In that study, several lysophosphatidylcholines were altered in obese animals, but the response was dependent on the fatty acid chain. Although lysophosphatidylcholines have been positively associated with oxidative stress, inflammation and atherogenesis,[19-21] more research is necessary to understand their role in these biological processes.

Similarly, Cummins, et al.,[22] used liquid chromatography, gas chromatography and mass spectrometry to analyze the metabolite profiles of white adipose tissue in C57BL/6 mice fed a high-fat diet compared to lean controls (n=7). Those researchers identified approximately 50 metabolites that were different in obese versus lean mice, including aromatic and branched-chain amino acids, taurine, glycerophospholipids, carnitine metabolites, and several glycolytic and TCA cycle intermediates. Many of the changes observed suggest a diminished mitochondrial respiratory chain, altered metabolism and autophagy that may be contributing to the whitening of adipose tissue with obesity. More of these experiments are required to increase our understanding of tissue metabolism and identify circulating markers that may accurately describe what is taking place in tissues.

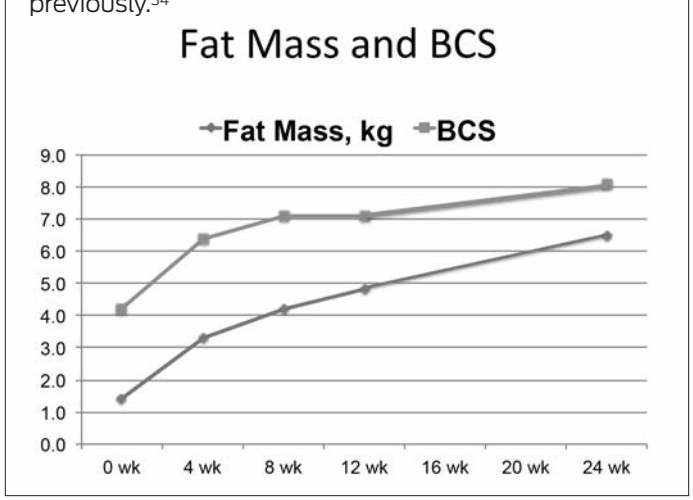
Because gut microbiota play a key role in the breakdown and metabolism of ingested substances and have been associated with obesity, their impact on host metabolism and metabolomic profiles must also be considered. Urine,[23] blood[24] and gastrointestinal digesta[25] samples have been analyzed using high-throughput untargeted assays to assess the impact of diet (e.g., protein or fiber content) or age on metabolomic profiles in humans and rodents. Although microbiota and microbial-derived metabolites are impacted by dietary treatments and have aided in the discrimination among treatment groups, the microbiota have typically not been the primary focus of most experiments. However, the use of metabolomics to increase understanding of gut microbiota metabolism and their impact on host health recently has been suggested.[26,27]

Using Metabolomics to Study Nutrition and Obesity in Dogs and Cats

High-throughput untargeted metabolomics strategies recently have been used to study various disease states in dogs, including bladder cancer,[28] epilepsy[29] and inflammatory bowel disease.[30] Two metabolomics experiments focused on nutritional interventions in dogs and cats have also been published in the past two years.[31,32] To our knowledge, however, untargeted metabolomics strategies have not been used until

Figure 1. Mean food intake of dogs undergoing rapid weight gain as presented previously.[34]

Figure 2. Body fat mass and body condition score (BCS) of dogs undergoing rapid weight gain as presented previously.[34]



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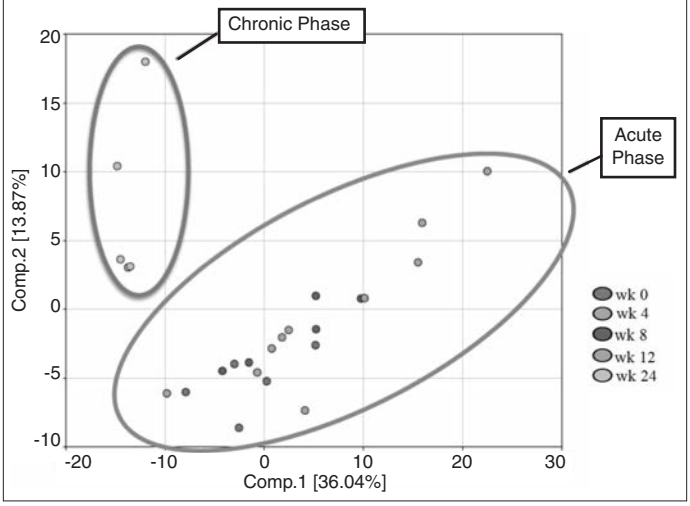
now to study canine or feline obesity.[33] A recent experiment in our laboratory was performed to complement and extend the results of the transcriptional profiling of dogs undergoing rapid weight gain previously described by Grant, et al.[34]

Figure 3. Principal component analysis representing the metabolite profiles of dogs undergoing rapid weight gain as presented previously.[33]



In the study by Grant,[34] five intact female Beagles (4 yr of age) were studied. Dogs had an initial mean body weight (BW) of 8.36 kg and body condition score (BCS) of 4.2 on a 9-point scale (1= extremely thin, 9= morbidly obese).[35] A high-calorie dry kibble commercial diet (*Purina Pro Plan SPORT* Performance 30/20 Formula) was fed *ad libitum* over 24 weeks. Food intake data are presented in Figure 1. At the end of the experimental period, the dogs had a BW of 14.64 kg and a BCS of 8.1. Body-fat mass was increased by about 50% at 4 weeks after baseline, and by 88% at 12 weeks. A mean BCS of 7 was already observed at 8 weeks (Figure 2). Obese dogs had greater ($p<0.05$) blood leptin, triglyceride, insulin, and adiponectin concentrations.[34,36] At baseline and after 4, 8, 12, and 24 weeks of *ad libitum* feeding, blood plasma was collected after an overnight fast and stored at -80°C until analysis. Biochemical profiling was performed using LC/MS and GC/MS analysis as previously described.[37] Metabolites were identified by comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison to Metabolon's reference library entries of purified standards. Currently, more than 1,000 commercially available purified

Table 1. Plasma metabolite alterations in amino acid and carbohydrate metabolism of dogs transitioning from a lean to an obese phenotype[1,2,3]

Metabolic Pathway	Biochemical Name	Fold Change					Correlation		
		4|0	8|0	12|0	24|0	24|12	Trig	BW	Leptin
Amino Acid Metabolism									
Glycine, serine and threonine metabolism	glycine	1.82	1.58	1.26	6.63	5.27	0.6172	0.7200	0.7384
	sarcosine (N-Methylglycine)	1.94	1.51	1.39	11.77	8.48	0.6457	0.7397	0.8009
	serine	1.72	1.88	1.55	5.34	3.45	0.6078	0.7596	0.7670
	betaine	0.95	1.07	1.36	0.87	0.64			
Alanine and aspartate metabolism	asparagine	1.3	1.61	1.24	4.51	3.64	0.6112	0.7721	0.7955
Glutamate metabolism	glutamate	1.35	1.36	1.09	3.5	3.2	0.6847	0.6846	0.6557
	glutamine	0.94	0.96	1.13	0.8	0.71			
Phenylalanine & tyrosine metabolism	phenol sulfate	0.54	0.6	0.75	0.44	0.59		-0.4640	-0.4255
Tryptophan metabolism	3-indoxyl sulfate	0.6	0.64	0.66	0.39	0.59		-0.6792	-0.5872
Valine, leucine and isoleucine metabolism	indolepropionate	0.85	0.64	1.05	0.56	0.53			
	3-methyl-2-oxobutyrate	1.29	1.39	1.77	0.75	0.43			
	3-methyl-2-oxovalerate	1.15	1.31	1.75	0.71	0.41			
	beta-hydroxyisovalerate	1.02	0.99	1.24	0.38	0.31			-0.5628
	isoleucine	1.06	1.32	1.54	0.99	0.65			
	leucine	1.04	1.24	1.47	1.07	0.73			
	valine	1.03	1.25	1.51	0.91	0.6			
Cysteine, methionine, SAM, taurine metabolism	cysteine	1.24	1.22	1.32	4.06	3.08	0.6630	0.7730	0.7935
	S-methylcysteine	1.22	1.47	1.37	4.65	3.39	0.7077	0.6725	0.6195
	cystine	1.7	1.22	0.97	18.69	19.25	0.7081	0.6730	0.7323
	homocysteine	0.87	1.42	1.06	3.25	3.07	0.5864	0.6784	0.6784
Urea cycle; arginine-, proline-, metabolism	dimethylarginine (SDMA + ADMA)	0.9	1	1.2	0.59	0.5	-0.5771		-0.6060
	ornithine	1.06	1.05	1.22	4.41	3.62	0.6750	0.7326	0.7363
	citrulline	0.84	1.06	2.24	1.65	0.73			
Creatine metabolism	creatinine	0.92	0.9	0.98	0.66	0.67		-0.5423	-0.6307
Butanoate metabolism	2-aminobutyrate	1.13	1.27	1.51	0.95	0.63			
Glutathione metabolism	glutathione, reduced (GSH)	1	1	1	1.9	1.9		0.6660	0.6915
	5-oxoproline	0.99	1.04	1.33	0.45	0.34	-0.5905	-0.4562	-0.6269
Dipeptide derivative	carnosine	0.99	1.12	1.26	0.77	0.61			-0.4955
	anserine	0.86	1.17	1.27	0.79	0.62			
gamma-glutamyl	gamma-glutamylvaline	1.15	1.18	1.5	0.99	0.66			
	gamma-glutamylleucine	1.02	1.06	1.51	0.99	0.66			
	gamma-glutamylisoleucine	0.93	1.05	1.31	0.69	0.53			
	gamma-glutamylalanine	1.04	1.21	1.56	2.78	1.79	0.5919	0.6798	0.6829
Polypeptide	bradykinin, des-arg(9)	1.92	1.13	2.16	0.17	0.08			
Carbohydrate Metabolism									
Aminosugars metabolism	erythronate*	0.91	0.95	0.93	0.67	0.72		-0.5716	-0.6808
Fructose, mannose, galactose, starch, and sucrose metabolism	fructose	0.7	0.7	0.95	0.09	0.1	-0.5203	-0.5698	-0.5507
	mannose	1.18	1.11	1.53	1.74	1.14	0.5085	0.6179	0.4553

[1] For each metabolite, mean value is the group mean of re-scaled data to have median equal to 1.

[2] Mean values in shaded cells were significant with p- and q-values <0.05. p values were calculated from one way ANOVA; q-values were used to estimate the false discovery rate (FDR) in multiple comparisons. Fold change values >1.0 were increased; fold change values <1.0 were decreased.

[3] Correlation coefficients in shaded cells were significant with p- and q-values <0.05.

standard compounds have been acquired and registered into Metabolon LIMS for distribution to both the LC and GC platforms for determination of their analytical characteristics. The combination of chromatographic properties and mass spectra provided an indication of a match to the specific compound or an isobaric entity.

A total of 284 named metabolites were identified in that study, with 175 metabolites being altered over time. Two distinct clusters were formed by principal component analysis (Figure 3). The first cluster was comprised of plasma metabolite profiles from 0, 4, 8, and 12 weeks, whereas the second cluster was comprised only of plasma metabolite profiles from 24 weeks. A clear and complete separation of canine plasma metabolite profiles from week 24 versus those from weeks 0 to 12 indicated that the onset of obesity was characterized by two distinct phases. Phase one reflected an acute response to body weight (BW) gain, whereas phase two revealed a chronic or late response to weight gain and/or accretion of fat, showing a complete shift in metabolite signature. This outcome is in agreement with results previously reported from our laboratory, suggesting that differences in adipose tissue gene expression and circulating blood hormones of dogs during the onset of obesity was also a two-phase event, comprised of an acute and a chronic phase.[34] The metabolomics data suggest that metabolic response in relation to BW gain is not only related to the amount of fat deposition (acute phase) but also to the length of time from the onset of obesity (chronic phase).[33] An abbreviated list of metabolites associated with lipid, carbohydrate, and amino-acid metabolism, xenobiotics, and cofactors and vitamins are listed in Tables 1 and 2. All but one metabolite reported by Moore, et al.,[13] was altered in dogs gaining weight in this study.

Conclusion

High-throughput metabolomics assays may be used in combination with traditional laboratory measurements and physiologic data to increase our understanding of canine and feline metabolism of healthy animals and those afflicted with disease, including obesity. Little has been published in dogs and cats to date, but concepts and obesity-related data have been provided by those studying humans and rodent models. While biomarkers of obesity per se are not needed — the body condition score or body weight of a pet will identify that — metabolomics strategies may highlight metabolite profiles that provide mechanism(s) by which obesity leads to metabolic dysfunction and/or early biomarkers of related disease states.

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Table 2. Plasma metabolite alterations related to the metabolism of lipid, cofactors and vitamins, and xenobiotics of dogs transitioning from a lean to an obese phenotype[1,2,3]

Metabolic Pathway	Biochemical Name	Fold Change					Correlation		
		4|0	8|0	12|0	24|0	24|12	Trig	BW	Leptin
Lipid Metabolism									
Medium and long chain fatty acid	caprate (10:0)	1	1.23	1.67	0.08	0.05			
	undecanoate (11:0)	0.93	1.17	1.25	0.19	0.16	-0.5315	-0.5226	-0.6395
	laurate (12:0)	0.9	1.05	1.1	0.15	0.14	-0.5989	-0.6395	-0.7378
	stearidonate (18:4n3)	3.41	4.49	6.49	2.76	0.42			
	dihomo-linoleate (20:2n6)	1.27	1.51	2.13	0.98	0.46			
	arachidonate (20:4n6)	1.3	1.71	2.17	1.22	0.56			
Glycerolipid metabolism	phosphoethanolamine	1.63	1.34	1	4.76	4.76		0.6209	0.5684
	glycerol	1.32	1.34	1.75	1.59	0.9		0.5600	
Ketone bodies	3-hydroxybutyrate (BHBA)	1.1	1.29	1.46	0.59	0.41			-0.5047
Lysolipid	2-oleoylglycerophosphoethanolamine	1.4	0.92	1.43	2.09	1.45	0.6008	0.4989	0.5272
	1-linoleoylglycerophosphoethanolamine	0.85	0.85	0.91	0.64	0.7			
	2-linoleoylglycerophosphoethanolamine	0.9	0.9	0.89	1.96	2.19	0.7221	0.5796	0.7272
	1-arachidonoylglycerophosphoethanolamine	0.88	1.01	1.3	0.65	0.5			
	2-arachidonoylglycerophosphoethanolamine	0.57	0.87	0.8	3.14	3.91	0.8150	0.5526	0.5289
	2-arachidonoylglycerophosphocholine	0.98	1.01	1.88	4.31	2.3	0.6403	0.6940	0.6463
	1-docosapentaenoylglycerophosphocholine	1.69	1.23	2.07	0.86	0.42			
	2-docosapentaenoylglycerophosphocholine	1.23	1.21	1.32	3.02	2.29	0.5413	0.6252	0.5773
	1-docosahexaenoylglycerophosphocholine	1.42	1.16	2.04	0.72	0.35			
	2-docosahexaenoylglycerophosphocholine	1.27	1.08	1.82	4.02	2.2	0.5413	0.6706	0.6048
Cofactors and Vitamins									
Ascorbate and aldarate metabolism	ascorbate (Vitamin C)	1.27	1.55	2.23	17.83	8	0.4988	0.5858	0.7208
	dehydroascorbate	0.94	0.68	1.2	22.3	18.59	0.7094	0.7117	0.7476
	threonate	0.9	1.12	1.23	0.07	0.05	-0.5637	-0.5281	-0.5988
Xenobiotics									
Drug	hydroquinone sulfate	1.05	0.95	1.07	0.63	0.59			
Food component/Plant	indoleacrylate	0.86	0.59	1.03	0.4	0.39			
	stachydrine	0.96	1.03	1.23	0.5	0.41			
	equol sulfate	0.67	0.4	0.74	0.37	0.49		-0.6325	-0.5786

[1] For each metabolite, mean value is the group mean of re-scaled data to have median equal to 1.
[2] Mean values in shaded cells were significant with p- and q-values <0.05. p values were calculated from one way ANOVA; q-values were used to estimate the false discovery rate (FDR) in multiple comparisons. Fold change values >1.0 were increased; fold change values <1.0 were decreased.
[3] Correlation coefficients in shaded cells were significant with p- and q-values <0.05.

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