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Pilot Study to Determine Peripheral Blood Leukocyte mRNA Expression Profile of Energy *Homeostasis* Genes to Identify Potential Predictive Biomarkers of Obesity in Dogs

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Abstract: Peripheral Blood Leukocytes (PBL) continually interact with virtually every organ and tissue in the whole body. A remarkable concordance (80%) of gene expression profiles between peripheral blood mononuclear cells and different tissues has been previously demonstrated in humans. As such, gene expression responses of circulating PBL can therefore, potentially provide early warning of any abnormalities they discover. Weight alterations (increase or decrease) when associated with obesity has been reported to lead to alterations to *PBL* gene expression, especially those related to insulin and adiponectin signaling genes and even genes involved with energy homeostasis. As such, a pilot study involving PBL profiles of the following genes involved in energy homoestasis (5' Adenosine Monophosphate-Activated Protein Kinase (AMPK)-α1 and 2, -β1 and 2 and -γ1 and 2); Glucose-6-Phosphate Dehydrogenase (G6PDH) and Malate Dehydrogenase (MDH), lipogenesis (Fatty Acid Synthase (FAS) and insulin signaling Adiponectin Receptor (ADIPOR) (-1 and 2); Insulin Receptor Substrates (IRS) (-1 and 2); Phosphatidylinositol-3 Kinase (PI3-K) were evaluated between lean and overweight dogs in an attempt to identify possible PBL biomarkers for assessing obesity in dogs. As compared to lean dogs, overweight dog PBL demonstrated reduced mRNA expression of IRS-1, IRS-2, FAS, *G6PDH* and *AMPK β1* genes. Overall, these findings suggest that dysregulation of energy metabolism, associated with obesity in overweight dogs may carry over with alterations in *PBL* gene expression of genes involved in energy homeostasis and sterol metabolism. As such, *PBL* gene expression profiles may aid in early detection of PBL biomarkers for assessing obesity in dogs.

Key words: Dog, obesity, peripheral blood leukocyte, biomarker, mRNA

INTRODUCTION

Excessive bodyweight (overweight and obesity) is a very common nutritional disorder in the current dog population (German, 2006; German *et al*., 2010). Overweight and obese dogs in particular, suffer from canine Obesity-Related Metabolic Dysfunction (ORMD) (Tvarijonaviciute *et al*., 2012) which shares components of metabolic syndrome in humans, such as hypoadiponectinaemia (Tvarijonaviciute *et al*., 2012), insulin resistance (German *et al*., 2009), hyperinsulinemia (Kim *et al*., 2003) and hypertriglyceridemia (Mori *et al*., 2011).

Gene expression analysis affords us the opportunity to study genetic contribution and patterns of altered gene expression related to obesity and type 2 DM. However, tissue sampling is a limitation in particular with companion animal studies since, it is difficult to obtain permission from pet owners to use tissue samples, such as liver, adipose and muscle for genetic studies. As such, peripheral blood can be a convenient of cells. In particular, Peripheral Blood Leukocytes (PBL) are increasingly considered for gene expression studies because they can be easily and repeatedly collected in sufficient quantities compared with the more invasive sampling of adipose, muscle and liver tissues.

Gene expression analysis of PBL in particular Peripheral Blood Mononuclear Cells (PBMC) has previously been used for investigating the molecular mechanisms underlying several human diseases as surrogates for predicting potential effects in tissues that are not easily accessible (Eady *et al*., 2005). Interestingly, a remarkable concordance (80%) of gene expression profiles between PBMC and different tissues has been previously demonstrated (Liew *et al*., 2006). Moreover, PBMC have demonstrated sensitivity to changes in gene expression of genes involved in energy homeostasis (Caimari *et al*., 2010a) and sterol metabolism

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(Caimari *et al*., 2010b) resulting from acute changes in feeding conditions of rats. Lastly, PBMC enzymes are considered to be a useful marker to evaluate the energy metabolic condition of animals (Magori *et al*., 2005; Takeguchi *et al*., 2005).

Therefore, the aim of this pilot study was to assess whether differences exist in PBL gene expression profiles of genes related to energy homoeostasis (ADIPOR1, ADIPOR2, AMPK (-α1 and 2, -β1 and 2 and -γ1 and 2), IRS-1, IRS-2, PI3-K, MDH, G6PDH) and sterol metabolism (FAS) at the mRNA level by RT-PCR between lean (BCS<3/5) and overweight (BCS = 3/5) dogs. If so PBL can serve as an easily accessible cell type, for possibly detecting overweight status and subsequent obesity risk by indirectly monitoring for alterations in gene expression of genes related to energy homeostasis and sterol metabolism.

MATERIALS AND METHODS

Animal selection: The 39 animals (17 females (11 spade)) and 22 (12 neutered) males; 1-14 years old) which presented themselves at three different private veterinary clinics throughout Tokyo, Japan for vaccine between April and May, 2012 were selected for the study.

About 20 animals (9 males, 11 females) served as the lean group (BCS≤3) whereas the remaining 19 dogs (13 males, 5 females) served as the overweight group (BCS>4). None of the selected animals had any evidence of acute or chronic disease (except for overweight/obesity) based on physical and clinical examination of routine hematologic and biochemical analysis. Dog breeds include: Beagle, Chihuahua, Chu-Tora, Irish Settler, Jack Russell Terrier, Labrador Retriever, Maltese, Miniature Daschund, Mixed, Miniature Bull Terrier, Miniature Pinscher, Papilon, Pekingese, Pug, Shelti, Shi Tzu, Shiba, Shiba Inu and Toy-Poodle. Further details of all dogs used in the study have been provided in Table 1. Owner consent was obtained for all animals used in the study. The Body Condition Score (BCS) of each dog was assessed using a 5-point scale (Burkholder and Toll, 2000) independently by three different veterinarians, working at each clinic, respectively, using the amount of fat covering the rib area as judged by visual inspection and palpation. Ethical approval for this research was granted by the NVLU Animal Research Committee.

Blood collection: Blood samples (≥4 h postprandial) (4-5 mL) were collected from the cephalic vein of animals without the aid of sedation. About 3 mL of this blood was collected into PAXgene Blood RNA V.2 kit tubes (PreAnalytiX GmbH) for RNA stabilisation, preservation and sample transport. Tubes were inverted 10 times, maintained at room temperature for 2 h, frozen at -20°C overnight and subsequently moved to -80°C for storage until further use. The remainder of blood was collected into heparinised plastic tubes, for immediate centrifugation at 1200 g for 10 min at 4°C to obtain plasma which was immediately stored at -80°C until required.

Table 1: Details of lean and overweight dogs selected for study

Age (year)	Breed	Gender	Neuter/Spay Status	BCS	BW (kg)
Healthy					
1	Toy-Poodle	Male	Neuteured	2	3.8
1	Shelti	Male	Intact	3	15
1	Siba Inu	Male	Intact	3	8.6
1	Mix	Female	Intact	3	9.1
3	Shi-Tzu	Male	Neuteured	3	5.7
4	Chu-Tora	Female	Spade	3	15.4
4	Mix	Male	Intact	3	17.66
6	Miniature Dachshund	Female	Intact	3	14
7	Miniature Dachshund	Male	Neuteured	3	5.9
7	Shih Tzu	Female	Spade	3	7.8
7	Miniature Dachshund	Male	Intact	3	3.56
7	Pekingese	Male	Intact	3	6.0
8	Miniature Dachshund	Female	Spade	3	4.46
8	Miniature Dachshund	Female	Spade	3	5.82
8	Shiba	Female	Intact	3	10.2
13	Beagle	Female	Spade	3	11.7
13	Irish Setter	Female	Spade	3	23
14	Mix	Male	Intact	3	8.9
14	Shelti	Female	Spade	3	6.7
14	Pug	Female	Spade	3	10.72
Overweight					
1	Miniatiure Bull Terrier	Male	Neutered	4	11.4
4	Maltese	Male	Neutered	4	5.7
4	Labrador Retriever	Female	Spade	4	24.8
5	Miniature Dachshund	Male	Neutered	4	7.46
5	Miniature Dachshund	Female	Intact	4	5.28
5	Shi-Tzu	Male	Intact	4	5.3
6	Beagle	Male	Neutered	4	10.5
6	Miniature Dachshund	Male	Neutered	4	8.5
8	Chihuahua	Male	Neutered	4	4.3
8	Miniature Dachshund	Female	Intact	4	5.4
8	Labrador Retriever	Male	Neutered	4	29.8
9	Jack Russell Terrier	Female	Spade	4	7
9	Miniature Dachshund	Male	Intact	4	6.62
10	Shi-Tzu	Male	Intact	4	10.15
10	Shi-Tzu	Male	Neutered	4	7.3
11	Shi-Tzu	Male	Intact	4	8.4
12	Mix	Male	Neutered	4	22.68
12	Mix	Female	Intact	4	N/A
14	Shi-Tzu	Female	Spade	4	7.5

BCS = Body Condition Score; BW = Body Weight; HS = Hock to stifle joint length; PC = Pelvice Cirumference

Analysis of plasma metabolites and hepatic enzymes: Plasma glucose, Blood Urea Nitrogen (BUN), Creatinine (CRE), Total Cholesterol (T-Cho), Total Protein (TP) and Triglyceride (TG) concentrations as well as Lactate Ddehydrogenase (LDH), Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined using an autoanalyser (AU680, Beckman-Coulter) using the manufacturer's reagents. Nonesterified Fatty Acids (NEFA), plasma adiponectin and Immunoreactive Insulin (IRI) concentrations were measured using commercial kits as earlier described (Mori *et al*., 2009). Nonesterified Fatty

Acids (NEFA) was measured using a NEFA-C test (Wako Pure Chemical Industries) commercial kit. Plasma adiponectin and IRI concentrations were measured using commercial ELISAs (mouse/rat (Otsuka) and Llbis Dog (Shibayagi), respectively).

Quantitative real-time PCR analysis of peripheral blood leucocyte mRNA: Total leukocyte RNA from the blood samples was extracted and isolated using a *PAX* gene Blood RNA V.2 kit and a QIAamp RNA blood mini kit according to the manufacturer's instructions. The concentration of RNA was assessed by using a Malcom ES-2 (E-spect) micro UV-VIS fluorescence spectrophotometer (Tokyo, JP) whereas the presence of isolated RNA was assessed by native agarose gel electrophoresis on a 0.8% agarose gel. Quantitative RT-PCR reactions, using SYBR Green fluorescent dye for genes of interest (IRS-1, IRS-2, PI3K p85 α, MDH, G6PDH, FAS, AdipoR1, AdipoR2, AMPK-α1, -α2, -β1, -β2, -γ1, -γ2) were performed in triplicate, with 18s rRNA serving as internal standards, using an ABI 7300 RT-PCR sequence detection system. Primers for the genes are listed (Table 2).

Table 2: Primer sequences for quantitative real-time PCR

Probe	PCR product length (bp)	Primer type	Primer sequences (5'-3')	GenBank Acc. No.
		Forward	acctgcgttcaaggaggtctg	
IRS-1	81	Reverse	cggtagatgccaatcaggttc	XM_543274
		Forward	tggcaggtgaacctgaagc	
IRS-2	177	Reverse	gaagaagaagctgtccgagtgg	XM_542667
		Forward	gcattaaaccagacctcattcagc	
PI3K p85α	132	Reverse	gcgagtattggtcttcagtgttctc	AB436616
		Forward	cttctactgctccccacagc	
AdipoR1	247	Reverse	catcacagccatgaggaaga	XM843263
		Forward	tccacaaccttgcttcatct	
AdipoR2	146	Reverse	tgattccactcagaccaagg	XM534929
		Forward	cctcaagcttttcaggcatc	
AMPK α1	110	Reverse	aatcaaatagctctcctcctgaga	XM_536491
		Forward	aaggatgccacctcttatagca	
AMPK α2	225	Reverse	attttcacataattgccagtcac	XM_546691
		Forward	cccaagatcctgatggacag	
AMPK β1	112	Reverse	ggggctttatcattcacttcc	XM_543421
		Forward	agatcatggtggggagtacg	
AMPK β2	122	Reverse	ctgtgtgggctttacggagt	XM_845060
		Forward	cacaggctgccagttattga	
AMPK γ1	258	Reverse	gcgcccttctcgtctacta	XM_543685
		Forward	aagactcagagagtggtgtttacat	
AMPK γ2	71	Reverse	actcgtgggaacgatgtca	XM_532769
		Forward	ggtgcagccttggagaaatatg	
MDH	82	Reverse	cagtcaggcagttggtattgg	XM_531844
		Forward	gctacttcgatgaatttgggatc	
G6PDH	139	Reverse	cactttaacaccttgaccttctcg	XM_538209
		Forward	tactggaggggccagtgcatca	
FAS	151	Reverse	gtcccgagatggtcactgtgtc	AB436619
		Forward	gtaacccgttgaaccccatt	
18S	151	Reverse	ccatccaatcggtagtagcg	NW_003729148.1

Primer sequences used in quantitative RT-PCR analysis of peripheral blood leukocyte mRNA from lean and overweight dogs; ADIPOR = Adiponectin Receptors; AMPK = 5' Adenosine Monophosphate-activated Protein Kinase subunits; FAS = Fatty Acid Synthase; G6DPH = Glucose-6-Phosphate Dehydrogenase; IRS = Insulin Rceptor Substrates; MDH = Malate Dehydrogenase; PI3-K = Phosphatidylinositol-3 Kinase; 18s, 18s ribosmal RNA

Relative gene expression values were calculated using the comparative C_T method for quantification as described by Livak and Schmittgen (2001). All target genes were normalised to 18s rRNA and subsequently compared to lean data to determine relative n-fold differences.

Statistical analysis: Plasma metabolite values were expressed as median ±min/max values. qRT-PCR relative expression values were expressed as median with minimum and maximum values. The Mann-Whitney U-test was used to assess significance between groups, set at $p<0.05$. Analysis was performed using Sigmaplot (Version. 11.2, Build 11.2.0.5, Systat Software Inc., San Diego, CA).

RESULTS

Analysis of plasma metabolites and hepatic enzymes: Clinical characteristics and median values of general plasma metabolites and hepatic enzymes are presented in Table 3. Overweight status resulted in a significant increase ($p<0.05$, Mann-Whitney U-test) only in plasma insulin as compared to lean animals.

Quantitative RT-PCR comparative analysis of peripheral blood leukocyte mRNA between lean and overweight dogs: Comparison of *PBL* gene expression trends at the mRNA level, between overweight and lean dog PBL are presented in Fig. 1. With regards to insulin signaling activity and glucose metabolism, overweight dog PBL demonstrated a significant ($p<0.05$, Mann-Whitney U-test) median reduction in IRS-1 (~40%) and IRS-2 (~30%) mRNA expression as compared to lean dog

Table 3: Clinical characteristics and plasma metabolite concentrations

Parameters	Healthy (n = 20)	Overweight (n = 19)
Physical indexes		
Age (years)	7.0 (1.0, 14.0)	8.0 (2.0, 24.0)
Body condition score (1-5)	3.0 (2.0, 3.0)	4.0 (4.0, 5.0)*
Body weight (Kg)	8.2 (3.6, 23.0)	7.5 (4.3, 29.8)
DM		
Glucose (mg dL^{-1})	102.5 (64.0, 162.0)	107.0 (69.0, 126.0)
Insulin (ng mL^{-1})	0.65 (0.05, 2.28)	1.32 (0.2, 10.6)*
Obesity		
Adiponectin (mg mL^{-1})	25.6 (0.02, 63.9)	20.9 (2.56, 79.5)
Non-estrified fatty acids (mEq L^{-1})	0.63 (0.28, 2.43)	0.94 (0.34, 3.15)
Total cholesterol (mg dL^{-1})	205.0 (86.0, 436.0)	215.0 (128.0, 316.0)
Triglycerides (mg dL^{-1})	45.5 (12.0, 290.0)	65.0 (10.0, 193.0)
Hepatic and renal injury		
Alanine aminotransferase (U L^{-1})	47.0 (17.0, 160.0)	53.5 (20.0, 204.0)
Alkaline phosphatase (U L^{-1})	119.5 (47, 2150)	134.4 (82.0, 1811.0)
Aspartate aminotransferase (U L^{-1})	25.4 (11.8, 74.4)	35.3 (16.4, 268.4)
Blood urea nitrogen (mg dL^{-1})	14.5 (7.0, 35.1)	13.1 (7.0, 33.5)
Creatinine (mg dL^{-1})	0.76 (0.55, 1.25)	0.67 (0.33, 1.31)
Lactate dehydrogenase (U L^{-1})	62.0 (30.0, 525.0)	106.0 (48.0, 672.0)
Total protein (g dL^{-1})	6.5 (4.1, 7.1)	6.8 (5.6, 8.6)

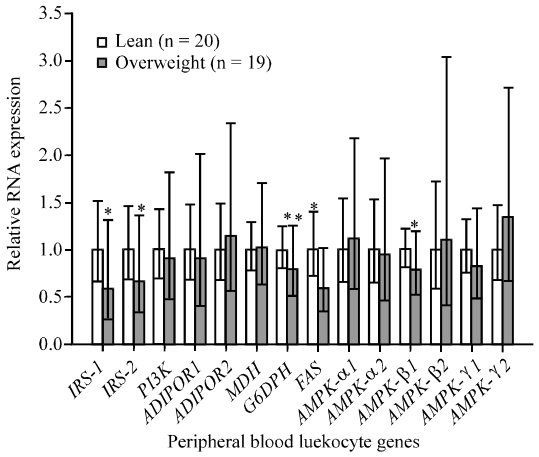

Fig. 1: Comparison of gene expression in peripheral blood leukocytes of overweight (n = 19) and lean (n = 20) dogs. Results are expressed as relative mRNA expression in median arbitrary units ±minimum/maximum range; The Mann-Whitney U-test was used to assess significance between groups, set at $p<0.05$; *Significantly lower when compared against corresponding lean group whereas double asterisk denotes tendency to be lower when compared against corresponding lean group; Bars indicate upper and lower range values; ADIPOR = Adiponectin Receptor; AMPK = 5' Adenosine Monophosphate-activated Protein Kinase; FAS = Fatty Acid Synthase; G6PDH = Glucose-6-Phosphate Dehydrogenase; IRS = Insulin Receptor Substrates; MDH = Malate Dehydrogenase; PI3-K = Phosphatidylinositol-3 Kinase

PBL. However, no accompanying difference in mRNA expression level was observed with PI3K p85α in overweight dog PBL. In addition, there was a tendency ($p = 0.063$, Mann-Whitney U-test) for reduced G6PDH (~20%) mRNA expression in overweight dog PBL when compared against lean dog PBL.

With respect to lipid synthesis and adiponectin signaling, overweight dog PBL demonstrated a significant ($p = 0.005$, Mann-Whitney U-test) median decrease in FAS (~40%) mRNA expression, as compared to lean dog PBL. No significant difference in either ADIPOR1 or R2 mRNA expression trends was observed between overweight and lean dog PBL.

Lastly, regarding energy homeostasis, overweight dog PBL demonstrated a significant ($p = 0.021$, Mann-Whitney U-test) median decrease in AMPK-α1 (~20%) mRNA expression, as compared to lean animal PBL. No significant difference in the mRNA expression level of *AMPK-α1*, *AMPK-α2*, *AMPK-β1*, *AMPK-β1* and *AMPK-γ2* genes was observed between overweight and control dog PBL.

DISCUSSION

Leukocytes continually interact with virtually every organ and tissue in the whole body. Therefore, the gene expression responses of circulating Peripheral Blood Leukocytes (PBL) can potentially provide early warning of any abnormalities they discover (Halvatsiotis *et al*., 2010; Shen *et al*., 2007; Visvikis-Siest *et al*., 2007). Alteration to weight when associated with obesity has been reported to lead to alterations to *PBL* gene expression (De Mello *et al*., 2008b; Ghanim *et al*., 2004), especially those related to insulin (De Mello *et al*., 2008a; Mori *et al*., 2009) and adiponectin (Kollias *et al*., 2011) signaling genes. IRS-1, IRS-2 and PI3-K are important downstream players of insulin (White, 1998) and have been implicated with the incidence of insulin resistance and diabetes (Kerouz *et al*., 1997; Rondinone *et al*., 1997). Lower protein levels of IRS-1, IRS-2 and PI3-K p85α have been reported in human patients suffering from insulin resistance (Friedman *et al*., 1999; Rondinone *et al*., 1997). In the study, qRT-PCR revealed a significant reduction in IRS-1 and IRS-2 mRNA expression of overweight dog PBL, as compared to lean animals which is supported by a previous study with cats, conducted by the laboratory (Mori *et al*., 2009). Coincidentally, increased circulating insulin concentration (~2x median increase) was also observed in overweight as compared to lean dogs which would corroborate with the reduction of insulin signaling gene mRNA expression observed. It has previously been shown that dogs with chronic, naturally occurring obesity compensate for obesity-induced insulin resistance by secreting more insulin after a glucose challenge (Bergman *et al*., 2001).

The 5' Adenosine Monophosphate-activated Protein Kinase (AMPK) is a metabolic sensor of energy status that maintains cellular energy homeostasis, as well as whole-body levels (Hardie, 2011; Srivastava *et al*., 2012). Mammalian AMPK exists as a heterotrimeric complex consisting of 3 subunits: Catalytic α-subunits (α1/α2), regulatory β- (β1/β2) and γ-subunits (γ1-γ3), occurring as multiple isoforms and encoded by seven different genes. Interestingly, the individual subunits are generally unstable in the absence of their binding partners. As such, this can be a useful feature because when any one subunit is overexpressed, it acts as a dominant mutant, replacing the endogenously expressed subunit by competing for binding to the other two (Mu *et al*., 2001; Hawley *et al*., 2010).

Alterations in AMPK activity have been hypothesized to be a factor, predisposing the development of obesity (Ruderman *et al*., 2003), specifically a lack of AMPK α2 (Villena *et al*., 2004). AMPK α1 knockout mice have no apparent metabolic defects whereas AMPK α2 knockout mice exhibited insulin resistance (Viollet *et al*., 2003). As a result, some studies have speculated that AMPK α2 may be the major mediator of adiponectin function for fatty acid oxidation in muscle cells (Chen *et al*., 2005; Yoon *et al*., 2006) and hepatic glucose production (Andreelli *et al*., 2006) in humans. qRT-PCR revealed a significant reduction in AMPK-α1 mRNA expression of PBL originating from overweight dogs as compared to lean animals. No significant difference in mRNA expression levels of any of the other AMPK subunits were observed between overweight and lean dog PBL in the study.

Early warning signs of energy metabolism dysregulation in overweight dogs could be detected by PBL qRT-PCR results observed with Fatty Acid Synthase (FAS) and Glucose-6-Phosphate Dehydrogenase (G6PDH). G6PDH is a cytosolic enzyme in the pentose phosphate pathway which supplies reducing energy to cells by maintaining the level of NADPH which can be used by tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissue and the adrenal glands. PBL G6PDH mRNA expression was significantly reduced in overweight as opposed to lean counterparts which corroborates with reduced FAS mRNA expression in overweight dog PBL. FAS is involved in fatty acid synthesis and has been shown to be reduced in high fat feeding studies involving mice and cats (Kim *et al*., 2003; Lee *et al*., 2011). Both G6PDH and FAS mRNA expression trends correspond with the altered energy metabolic needs of overweight animals. For example with an excess amount of plasma NEFA, tissues need not synthesize fatty acids; absorbing them from circulation instead.

This study has a number of limitations. First although, a high concordance rate (>80%) of gene expression between PBL and other tissues has been shown in humans and some other species (Liew *et al*., 2006), it is currently unknown whether:

- This concordance is applicable to only housekeeping genes or others as well
- This concordance holds true for cats and dogs

Future studies involving PBL should also include various tissue samples (liver, muscle and adipose) if possible for confirmation of degree of concordance of PBL mRNA expression between PBL and tissues. Second, since only mRNA expression was gauged for gene expression and not protein levels, expression trends and results should be interpreted with care since mRNA expression trends do not necessarily translate over to the protein level. Third, the number of animals used in this study was not large. A larger animal sample size should be used in the future to determine reproducibility and increase statistical power of the results. Lastly in future studies, breed, age or gender matching should be attempted to eliminate possible influences of each aforementioned factor on PBL expression profiles.

CONCLUSION

In the study , alterations to energy homeostasis (AMPK β1, G6PDH), insulin signaling (IRS-1, IRS-2) and sterol metabolism (FAS) gene expression were detected using qRT-PCR in PBL of overweight dogs as compared to lean counterpart PBL. The majority of the PBL altered mRNA expression trends, observed in this study, corroborate with many other studies focusing on obesity influenced mRNA expression trends in tissues, such as adipose, muscle or liver. As such, peripheral blood can be a convenient source of cells. In particular, PBL are increasingly considered for gene expression studies because they can be easily and repeatedly collected in sufficient quantities compared with the more invasive sampling of tissues. As such, the use of PBL may hold promise to indirectly gauge for changes, occurring with energy *homeostasis* genes involved in glucose and lipid metabolism of distal tissues which may serve as predictive biomarkers of obesity in dogs. Future studies will be carried out to determine concordance rates of gene expression between PBL and distal tissues, such as adipose, muscle and liver.

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REFERENCES

Andreelli, F., M. Foretz, C. Knauf, P.D. Cani and C. Perrin *et al*., 2006. Liver adenosine monophosphate-activated kinase-á2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. Endocrinology, 147: 2432-2441.

Bergman, R.N., G.W. Van Citters, S.D. Mittelman, M.K. Dea, M. Hamilton-Wessler, S.P. Kim and M. Ellmerer, 2001. Central role of the adipocyte in the metabolic syndrome. J. Invest. Med., 49: 119-126.

Burkholder, W.J. and P.W. Toll, 2000. Obesity. In: Small Animal Clinical Nutrition, Hand, M.S. (Ed.). 4th Edn., Mark Morris Institute, Topeka, pp: 401-430.

Caimari, A., P. Oliver, J. Keijer and A. Palou, 2010a. Peripheral blood mononuclear cells as a model to study the response of energy homeostasis-related genes to acute changes in feeding conditions. OMICS: J. Integr. Biol., 14: 129-141.

Caimari, A., P. Oliver, W. Rodenburg, J. Keijer and A. Palou, 2010b. Feeding conditions control the expression of genes involved in sterol metabolism in peripheral blood mononuclear cells of normoweight and diet-induced (cafeteria) obese rats. J. Nutr. Biochem., 21: 1127-1133.

Chen, M.B., A.J. McAinch, S.L. Macaulay, L.A. Castelli and P.E. O'brien *et al*., 2005. Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese Type 2 diabetics. J. Clin. Endocrinol. Metab., 90: 3665-3672.

De Mello, V.D.F., M. Kolehmainen, L. Pulkkinen, U. Schwab and U. Mager *et al*., 2008a. Downregulation of genes involved in NFκB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: The GENOBIN study. Diabetologia, 51: 2060-2067.

De Mello, V.D.F., M. Kolehmainen, U. Schwab, U. Mager and D.E. Laaksonen *et al*., 2008b. Effect of weight loss on cytokine messenger RNA expression in peripheral blood mononuclear cells of obese subjects with the metabolic syndrome. Metabolism, 57: 192-199.

Eady, J.J., G.M. Wortley, Y.M. Wormstone, J.C. Hughes and S.B. Astley *et al*., 2005. Variation in gene expression profiles of peripheral blood mononuclear cells from healthy volunteers. Physiol. Genomics, 22: 402-411.

Friedman, J.E., T. Ishizuka, J. Shao, L. Huston, T. Highman and P. Catalano, 1999. Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. Diabetes, 48: 1807-1814.

German, A.J., 2006. The growing problem of obesity in dogs and cats. J. Nutr., 136: 1940S-1946S.

German, A.J., M. Hervera, L. Hunter, S.L. Holden, P.J. Morris, V. Biourge and P. Trayhurn, 2009. Improvement in insulin resistance and reduction in plasma inflammatory adipokines after weight loss in obese dogs. Domestic Anim. Endocrinol., 37: 214-226.

German, A.J., V.H. Ryan, A.C. German, I.S. Wood and P. Trayhurn, 2010. Obesity, its associated disorders and the role of inflammatory adipokines in companion animals. Vet. J., 185: 4-9.

Ghanim, H., A. Aljada, D. Hofmeyer, T. Syed, P. Mohanty and P. Dandona, 2004. Circulating mononuclear cells in the obese are in a proin?ammatory state. Circulation, 110: 1564-1571.

Halvatsiotis, I., P.C. Tsiotra, I. Ikonomidis, A. Kollias and P. Mitrou *et al*., 2010. Genetic variation in the adiponectin receptor 2 (ADIPOR2) gene is associated with coronary artery disease and increased ADIPOR2 expression in peripheral monocytes. Cardiovascular Diabetol., 9: 10-21.

Hardie, D.G., 2011. AMP-activated protein kinase-an energy sensor that regulates all aspects of cell function. Genes Devel., 25: 1895-1908.

Hawley, S.A., F.A. Ross, C. Chevtzoff, K.A. Green and A. Evans *et al*., 2010. Use of cells expressing gamma subunit variants to Identify diverse mechanisms of AMPK activation. Cell Metab., 11: 554-565.

Kerouz, N.J., D. Horsch, S. Pons and C.R. Kahn, 1997. Differential regulation of insulin receptor substrates-1 and-2(IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. J. Clin. Invest., 100: 3164-3172.

Kim, S.P., M. Ellmerer, G.W. Van Citters and R.N. Bergman, 2003. Primacy of hepatic Insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. Diabetes, 52: 2453-2460.

Kollias, A., P.C. Tsiotra, I. Ikonomidis, E. Maratou and P. Mitrou *et al*., 2011. Adiponectin levels and expression of adiponectin receptors in isolated monocytes from overweight patients with coronary artery disease. Cardiovascular Diabetol., Vol. 10 10.1186/1475-2840-10-14

Lee, P., A. Mori, H. Takemitsu, I. Yamamoto and T. Arai, 2011. Lipogenic gene expression in abdominal adipose and liver tissues of diet-induced overweight cats. Vet. J., 190: e150-e153.

Liew, C.C., J. Ma, H.C. Tang, R. Zheng and A.A. Dempsey, 2006. The peripheral blood transcriptome dynamically reflects system wide biology: A potential diagnostic tool. J. Lab. Clin. Med., 147: 126-132.

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ method. Methods, 25: 402-408.

Magori, E., M. Nakamura, A. Inoue, A. Tanaka and N. Sasaki *et al*., 2005. Malate dehydrogenase activities are lower in some types of peripheral leukocytes of dogs and cats with type 1 diabetes mellitus. Res. Vet. Sci., 78: 39-44.

Mori, A., P. Lee, H. Takemitsu, E. Iwasaki and N. Kimura *et al*., 2009. Decreased gene expression of insulin signaling genes in insulin sensitive tissues of obese cats. Vet. Res. Commun., 33: 315-329.

Mori, N., P. Lee, K. Kondo, T. Kido, T. Saito and T. Arai, 2011. Potential use of cholesterol lipoprotein profile to confirm obesity status in dogs. Vet. Res. Commun., 35: 223-235.

Mu, J., J.T. Brozinick, O. Valladares, M. Bucan and M.J. Birnbaum, 2001. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol. Cell, 7: 1085-1094.

Rondinone, C.M., L.M. Wang, P. Lonnroth, C. Wesslau, J.H. Pierce and U. Smith, 1997. Insulin Receptor Substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. National Acad. Sci. U.S.A., 94: 4171-4175.

Ruderman, N.B., A.K. Saha and E.W. Kraegen, 2003. Minireview: Malonyl CoA, AMP-activated protein kinase and adiposity. Endocrinology, 144: 5166-5171.

Shen, Y.Y., J.A. Charlesworth, J.J. Kelly, K.W. Loi and P.W. Peake, 2007. Up-regulation of adiponectin its isoforms and receptors in end-stage kidney disease. Nephol. Dialysis Trans., 22: 171-178.

Srivastava, R.A.K., S.L. Pinkosky, S. Filippov, J.C. Hanselman, C.T. Cramer and R.S. Newton, 2012. AMP-activated protein Kinase: An emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. J. Lipid Res., 53: 2490-2514.

Takeguchi, A., S. Urabe, A. Tanaka, T. Sako and T. Washizu *et al*., 2005. Activities of enzymes in some types of peripheral leucocytes may reflect the differences in nutrient metabolism between dogs and cats. Res. Vet. Sci., 78: 21-24.

Tvarijonaviciute, A., J.J. Ceron, S.L. Holden, D.J. Cuthbertson, V. Biourge, P.J. Morris and A.J. German, 2012. Obesity-related metabolic dysfunction in dogs: A comparison with human metabolic syndrome. BMC Vet. Res., Vol. 8 10.1186/1746-6148-8-147.

Villena, J.A., B. Viollet, F. Andreelli, A. Kahn, S. Vaulont and H.S. Sul, 2004. Induced adiposity and adipocyte hypertrophy in mice lacking the AMP-activated protein kinase-alpha2 subunit. Diabetes, 53: 2242-2249.

Viollet, B., F. Andreelli, S.B. Jorgensen, C. Perrin and D. Flamez *et al*., 2003. Physiological role of AMP-activated protein kinase (AMPK): Insights from knockout mouse models. Biochem. Soc. Trans., 31: 216-219.

Visvikis-Siest, S., J.B. Marteau, A. Samara, H. Berrahmoune, B. Marie and M. Pfister, 2007. Peripheral blood mononuclear cells (PBMCs): A possible model for studying cardiovascular biology systems. Clin. Chem. Laboratory Med., 45: 1154-1168.

White, M.F., 1998. The IRS signaling system: A network of docking proteins that mediate insulin and cytokine action. Mol. Cell Biochem., 182: 3-11.

Yoon, M.J., G.Y. Lee, J.J. Chung, Y.H. Ahn, S.H. Hong and J.B. Kim, 2006. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase and peroxisome proliferator-activated receptor a. Diabetes, 55: 2562-2570.