

## General Summary

A number of health problems purported to be associated with or exacerbated by obesity in dogs and cats. Early warning signs of energy metabolism dysregulation in overweight animals could be detected by the presence of insulin and adiponectin signaling genes via PBL qRT-PCR method, and may be valuable in early diagnosis. In addition, better understanding of these mechanisms might assist in the prevention of the development of obesity-related metabolic dysfunction or diabetes in obese animals.

### 1. Using body fat percentage assessing overweight status in dogs

Currently, 5 point body condition scoring (BCS) is commonly used by veterinarians and clinicians to assess adiposity in dogs in Japan. However, assigning a BCS score is subjective in nature, and most clinicians do not score with half points, instead preferring to round off values, thereby rendering less accurate assessments. Therefore, we sought to determine whether assessing body fat percentage (BF%), using simple morphometric measurements, and supplementing it with 5-point BCS can render increased sensitivity for detecting increasing adiposity in overweight small breed dogs via plasma metabolite validation.

Overall, lean BF % range was determined to be 15-20% for non-neutered male dogs,

and 15-25% for female (non-spayed/spayed) and neutered male dogs. BCS categorized overweight animals displayed significantly higher levels of non-esterified fatty acids (NEFA;  $p=0.005$ ); whereas significantly higher levels of NEFA ( $p=0.006$ ), Total cholesterol (T-Cho;  $p=0.029$ ), and Triglycerides (TG;  $p=0.001$ ) were observed in BCS+BF % categorized overweight animals as compared to lean animals. The increase in sensitivity, due to BF %, for gauging alterations to plasma metabolite values, may be due to increased correlation strength. BF % positively correlated with plasma insulin ( $r=0.627$ ,  $p=0.002$ ), NEFA ( $r=0.674$ ,  $p<0.001$ ), T-Cho ( $r=0.825$ ,  $p<0.0001$ ), TG ( $r=0.5823$ ,  $p<0.005$ ), Blood Urea Nitrogen ( $r=0.429$ ,  $p<0.05$ ), creatinine ( $r=0.490$ ,  $p=0.021$ ), and Total Protein ( $r=0.737$ ,  $p<0.0001$ ) levels which all tend to increase as a result of increasing adiposity.

In conclusion, BF % supplementation to 5 point BCS, appears to increase the likelihood of validating overweight status in small breed dogs, by detecting alterations in plasma metabolite values, especially lipid metabolites, induced as a result of increasing adiposity.

## 2. Plasma lipoprotein profiles and malondialdehyde in hyperlipidemia dogs

The aim of this Chapter is to compare metabolic parameters, malondialdehyde (MDA) as a lipid oxidation marker, and lipid profiles between dogs with untreated hyperlipidemia and hyperlipidemia with treatment, in order to examine the usefulness of MDA and lipid profiles as diagnostic parameters at early stages of hyperlipidemia. Dog samples were collected from clinics which were separated into three groups: control, untreated hyperlipidemia based on temporally screening, and hyperlipidemia with current anti-hyperlipidemic (statins and fibrates) treatment. TG levels of untreated hyperlipidemia dogs were significantly higher than those of control dogs. ALT levels of hyperlipidemic dogs with treatment were the highest among three groups. VLDL and LDL of both cholesterol and triglyceride of untreated hyperlipidemia dogs were the highest among three groups. HDL1 levels in triglyceride of hyperlipidemia dogs with treatment were significantly higher than those of control and untreated hyperlipidemia dog. MDA concentrations of untreated hyperlipidemia dogs were significantly higher than those of control and hyperlipidemic dogs with treatment. The results indicated that dogs with untreated hyperlipidemia clearly showed abnormal lipid status, whereas hyperlipidemic dogs under anti-hyperlipidemia treatment showed more normal lipid status suggesting the effectiveness of the therapy. Anti-hyperlipidemics (statins and fibrates) for dogs are also effective in relieving elevated

levels of lipids and lipid oxidation. Plasma lipid (triglyceride and cholesterol) profiles and MDA are useful diagnostic tools for identifying early stages of untreated hyperlipidemia in dogs.

### 3. Insulin and adiponectin gene expression of PBL in obese Miniature Dachshunds

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. Alteration to weight, when associated with obesity, has been reported to lead to alterations to PBL gene expression, especially those related to insulin and adiponectin signaling genes.

As such, PBL mRNA expression profiles of genes involved in insulin signaling (ADIPOR (-1 and 2); IRS (-1 and 2); PI3-K) lipogenesis (FAS) and energy homeostasis (G6PDH; MDH) were carried out on lean Miniature Dachshund and compared against similar profiles of breed and age matched overweight Miniature Dachshund in an attempt to identify possible PBL biomarkers for assessing obesity in dogs.

Overweight status resulted in a significant increase in plasma NEFA, T-Cho,

triglycerides and ALT, and a significant decrease in plasma adiponectin, as compared to lean Miniature Dachshund. Overweight dogs PBL demonstrated reduced mRNA expression of IRS-1 and -2; PI3-K, ADIPOR1 and FAS 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 insulin and sterol metabolism. As such, PBL gene expression profiles may aid in early detection of PBL biomarkers for assessing obesity in dogs.

#### 4. High-fat diet cats gene expression in PBL and insulin sensitive tissues

Alterations to gene expression, especially transcriptional changes, occurring in insulin sensitive tissues, may be a good indicator of metabolic changes occurring in the body. The objective of this Chapter is to determine whether PBL can serve as an easily accessible cell type for possibly detecting obesity and subsequent obesity risk in cats.

Regarding insulin signaling activity, high-fat diet cats had a significantly reduced IRS-1 mRNA expression in abdominal fat and peripheral leukocytes, with a significantly increased IRS-1 mRNA expression in liver as compared to control cats. Moreover, in high-fat diet cats, a significant reduction in IRS-2 mRNA expression in subcutaneous and visceral fat,

and a significant increase in PI3K p85 $\alpha$ mRNA expression in liver and skeletal muscle with a significant reduction in PBL was observed as compared against control cats. With respect to lipid synthesis and adiponectin signaling, high-fat diet fed cats' abdominal adipose demonstrated a significant median increase in ADIPOR1 mRNA expression, with reduced ADIPOR1 mRNA expression in liver and PBL being observed as compared to control cats. In addition, subcutaneous and visceral adipose demonstrated a significant median increase in ADIPOR2 mRNA expression, and FAS mRNA expression was significantly higher in all tissues except PBL as compared to control cats. Lastly, in high-fat diet fed cats, G6PHD mRNA expression was significantly higher in liver and skeletal muscle, but significantly lower in PBL as compared to control cats. In addition, abdominal and subcutaneous adipose demonstrated a significant median increase, while liver and PBL demonstrated a significant reduction in MDH mRNA expression as compared to control cats.

Overall, our results demonstrate that PBL can serve to act as surrogate tissue for various insulin sensitive tissues, depending on 1) the genes of interest, 2) the degree of pathology associated with the insulin sensitive tissue, and 3) the disease condition. Although the expression pattern of the aforementioned genes examined was not completely uniform, there was some correlation between PBL and various tissues. The response to obesity is

largely tissue specific with numerous commonly activated pathways suggesting a coordinated attempt by tissues to limit metabolic perturbations occurring in early-stage obesity.

#### 5. Insulin and adiponectin gene expression of PBL in short- and long-term obese cats

Naturally occurring obesity is more representative of the true clinical picture than experimental short-term dietary manipulation in cats. The aim of this preliminary study was to compare plasma metabolite and PBL mRNA transcriptome profiles of genes mainly involved with energy homeostasis, insulin and adiponectin signaling, in short-term high-fat diet induced and long-term naturally occurring obese cats.

Plasma metabolite profiling highlighted the inherent aberrations associated with different types and exposure time of obesity. In addition, PBL transcriptome profiles were very consistent regarding the genes used in our study, highlighting the sensitivity of PBL to the effects of obesity regardless of being acute or long term, on a host.

Overall, firstly, present studies have showed that BCS supplementing it with body fat percentage (BF%) provided more accurate assessments for dogs. Secondly, the present investigation indicated that plasma lipid profiles and MDA are most likely useful parameters for identifying early stages of obesity with mild hyperlipidemia in dogs. Thirdly, insulin and

adiponectin gene expression responses of circulating PBL can potentially provide early warning of any abnormalities, when associated with obesity in both dogs and cats. Lastly, dogs and cats have been proposed as a valuable animal model for studying human obesity, especially naturally occurring obesity animals.