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Changes in Different Insulin Sensitive Tissues Gene Expression of Cat Fed on High-fat Diet

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ABSTRACT

The objective of this study was to determine whether Peripheral Blood Leukocytes (PBL) are sensitive enough to detect early signs of diet induced obesity related changes occurring in insulin sensitive tissues, such as abdominal omental and subcutaneous adipose, liver and skeletal muscle, by comparing transcriptome profiles of insulin signaling (IRS-1, IRS-2 and PI3-K p85 α), adiponectin signaling (AdipoR1 and AdipoR2), energy homoeostasis (G6PDH and MDH) and sterol metabolism (FASN) genes as determined by RT-PCR in cats fed on High Fat (HF) diet. Regarding PBL concordance, using a HF diet induced obesity cat model, out of seven genes examined, concordance was observed with ~60% (5 out of 8) of them (IRS-1, IRS-2, Adipo-R1, Adipo-R2 and MDH) between PBL and tissue transcriptomes. HF diet cat PBL IRS-1 and IRS-2 mRNA expression were both reduced, when compared to control diet which was in concordance with reduced IRS-1 and IRS-2 mRNA expression in both abdominal and subcutaneous adipose of HF diet cats. Similar to IRS-1 and IRS-2, AdipoR1 and AdipoR2 mRNA expression in HF diet cats was also reduced, when compared to control diet which was in concordance with reduced AdipoR1 and AdipoR2 in liver and skeletal muscle, respectively of HF diet cats. Lastly, PBL MDH mRNA expression was reduced and was concordant with reduced mRNA expression in liver and skeletal muscle. Overall, our results demonstrate that PBL are sufficiently sensitive to high fat diet induced alterations to transcriptomes of insulin sensitive tissues and can serve as surrogate tissue for various insulin sensitive tissues.

Key words: HF diet, mRNA expression, obese cats, transcriptome profiles

INTRODUCTION

Obesity has a detrimental effect on general health and is a significant risk factor for Type 2 diabetes mellitus (T2DM), a common endocrinopathy in humans and cats (Burkholder and Toll, 1997). The prevalence of overweight or obesity incidence has been steadily rising in cats, with a reported figure of 35% as of 1995 in the United States (Lund *et al*., 2005). Similarly to humans, the increase in feline obesity can also be related to two main causes: Changes occurring in diet, comprising of excessive intake of high-fat and carbohydrate foods and a lack of or reduced level of physical activity (Backus *et al*., 2007). As such, cats have recently been proposed as a valuable animal model for studying human obesity and may provide additional insights into the

pathogenesis of T2DM in humans (Osto *et al*., 2012). Feline T2DM shares many features of human T2DM with respect to its pathophysiology, underlying risk factors and treatment strategies (Henson and O'Brien, 2006).

In order to explore energy (glucose and lipid) metabolism in obese cats, it is important to develop molecular tools to investigate transcriptional changes which may be occurring in insulin sensitive tissues. Alterations to gene expression may be a good indicator of metabolic changes occurring in the body (De Godoy and Swanson, 2013), especially genes associated with obesity and diabetes risk. For example, the molecular mechanism of insulin action is directed though a complex signaling network (Kollias *et al*., 2011; Cheatham and Kahn, 1995) with IRS-1, IRS-2 and PI3-K P-85α, in particular, being important downstream players (De Mello *et al*., 2008; White, 1998). In addition, adiponectin also plays a significant role in the regulation of insulin resistance and energy homeostasis (Lee *et al*., 2011, 2012b; Berg *et al*., 2001; Friedman, 2000) mediating its glucose-lowering and/or anti-inflammatory effects, through two cell membrane receptors: AdipoR1 and AdipoR2. FASN is a multi-enzyme protein catalyzing fatty acid synthesis. With respect to energy homeostasis, G6PDH is the rate-limiting enzyme of the pentose phosphate pathway that provides the majority of NADPH required for lipid biosynthesis. As such, G6PDH overexpression has been implicated in insulin resistance, hyperlipidemia and increased oxidative stress in animals (Cheatham and Kahn, 1995; White, 1998; Wang *et al*., 2012; Park *et al*., 2005). Lastly, MDH is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH. This reaction is a part of many metabolic pathways including the citric acid cycle and gluconeogenesis and can indirectly be used to gauge energy homeostasis.

Unfortunately, tissue sampling is a limitation, in particular in human and companion animal studies due to ethical considerations. A number of human studies have demonstrated that transcriptomic changes occurring in peripheral blood can serve as biomarkers of pathological changes occurring in other internal tissues not easily accessible (Staratschek-Jox *et al*., 2009; Mohr and Liew, 2007; Umbright *et al*., 2010; Eady *et al*., 2005; Todorova *et al*., 2012). As such, Peripheral Blood Leukocytes (PBL) have been purported to be a convenient, easy to access and readily available source of cells for sampling, with multiple studies reporting that PBL can potentially provide early warning of obesity related disorders in cats and humans (Kollias *et al*., 2011; De Mello *et al*., 2008; Lee *et al*., 2011, 2012b). However, there is a lack of studies examining and determining the concordance of gene expression trends in PBL with those found in other tissues in cats.

As such, the objective of this preliminary study was to determine whether PBL are sensitive enough to reflect upon diet induced (high fat) influenced changes, occurring with obesity related genes in tissues, by examining for concordance between PBL and tissue transcriptome profiles. In order to meet this objective, PBL transcriptome profiles of insulin signaling (IRS-1, IRS-2 and PI3-K p85 α), adiponectin signaling (AdipoR1 and AdipoR2), energy homoeostasis (G6PDH and MDH) and sterol metabolism (FASN) genes were determined by RT-PCR in various insulin sensitive tissues (liver, skeletal muscle, subcutaneous fat, visceral fat, peripheral blood leukocytes) in cats fed with a high fat diet and compared to tissue transcriptomes of lean control cats. PBL and tissue transcriptome concordance would be determined by examining for similar mRNA expression trends. If PBL are sensitive enough, they might serve as an easily accessible cell type for possibly detecting early signs of diet induced (HF) obesity related changes occurring in insulin sensitive tissues.

MATERIALS AND METHODS

Animals and diet: Ten intact, unrelated, cross-bred female cats (1-2 years old) were used for our study. The cats were determined to be at optimal weight and diagnosed to be healthy without any clinical manifestations as determined by one veterinarian. All cats were individually housed and maintained for upto two months (8 weeks) at AQS Co. Ltd., (Narita, Japan).

Cats were randomly divided into two groups of 5 animals. One group was designated normal diet while the other one was designated high fat diet. During the examination period, the normal diet group was fed on a commercial diet (Zoo animal diets ZN for cats, Oriental Yeast Co. Ltd., Tokyo, Japan), whereas, the high fat diet group was fed on a custom made to order high-fat diet (Nippon Pet Food, Inc., Tokyo, Japan). The composition of both the commercial and high fat diets is shown in Table 1. Coincidentally, prior to this study, all cats were consuming the same commercial diet (Zoo animal diets ZN for cats, Oriental Yeast Co. Ltd., Tokyo, Japan). Both groups were fed on their diets *ad libitum* for their Daily Energy Requirement (DER) from 9:00 am to 8:30 am of the next day, for a period of 8 weeks. DER is generally calculated as 1.1-1.8×Resting Energy Requirement (RER), depending on the disease or severity of injury (Ramsey, 2012). The RER of cats, dogs and other mammals may be predicted using the equation RER (kcal day^{-1}) = 70×BW0.75, in which BW = body weight in kg (Ramsey, 2012). The DER for cats used in our study was calculated at 1.4×RER, with RER being calculated for each cat on the basis of its BW before the meal at 9:00 am.

Cats were housed in individual cages and provided with water *ad libitum*. The animal room was maintained at 24±2°C and at 55±10% relative humidity on a 12:12 h light: dark cycle (light on 8:00 am to 8:00 pm). Living conditions for the cats were similar before, during and after our experiment. Approval for this work was given by the Nippon Veterinary and Life Science University Animal Research Care and Ethics Committee.

Blood sampling and collection of tissue samples: At the conclusion of the 8 week feeding schedule, fasted blood (5 mL) was withdrawn from the jugular vein of cats into heparinized tubes. Any surplus diet food was removed at 4:00 pm of the previous day to starve the animals to ensure fasted blood collection. Out of the 5 mL of fasted blood collected, 3 mL was deposited into PAXgene Blood RNA V.2 kit tubes (PreAnalytiX GmbH) for RNA stabilization, preservation and sample transport. Tubes were inverted ten 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 the blood was collected into heparinized plastic tubes, for immediate centrifugation at 1700 g for 10 min at 4°C to obtain plasma which was immediately stored at -80°C until required.

Table 1: Composition of the experimental diets

Parameters	Control diet	High-fat diet
Crude protein (%)	33.6	32.6
Crude fat (%)	16.0	23.9
Crude fiber (%)	3.5	0.9
Crude ash (%)	5.5	5.5
Moisture (%)	5.5	7.0
Nitrogen free extract (%)	35.9	29.9
Caloric content (kcal kg^{-1})	4,210	4,660

Regarding tissue sample collection, 2 cats were randomly chosen from each group (normal and high fat diet) mainly due to ethical reasons as allowed by the University Research Animal Care and Ethics Committee. The animals were fasted overnight and tranquilized with 0.05 mg kg^{-1} b.wt. of acepromazine malate (Tech America, KS, US), before being anesthetized with isoflurane. Liver, muscle and adipose tissues samples (2-3 g) were collected and removed from anesthetized animals by laparotomy and all procedures were performed under minimal stress conditions to the animals. Visceral fat was collected from near the jejunum, subcutaneous fat was collected from the inguinal area and skeletal muscle was collected from the biceps femoris muscle. Samples were flash-frozen in liquid nitrogen and stored on dry ice until being transferred to -80°C where they were stored until further analysis.

Plasma metabolite and enzyme analysis: Plasma glucose, Blood Urea Nitrogen (BUN), Creatinine (CRE), Total Cholesterol (T-Cho), Total Protein (TP) and Triglyceride (TG) concentrations, as well as Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH) activities were determined using an autoanalyser (Monolis Corporation, Tokyo, Japan) using the manufacturer's reagents. Non-esterified Fatty acids (NEFA), plasma adiponectin and plasma immunoreactive insulin concentrations were measured using commercial kits: NEFA-C test (Wako Pure Chemical Industries, Tokyo, Japan) kit, mouse/rat adiponectin ELISA kit (Otsuka, Tokyo, Japan) and Llbis cat IRI ELISA kit (Shibayagi, Shibukawa, Japan), respectively, as previously described (Staratschek-Jox *et al*., 2009).

Quantitative real-time PCR analysis of tissue and PBL mRNA: Total leukocyte RNA from blood samples was extracted and isolated using a PAX gene Blood RNA V.2 kit (Qiagen, Düsseldorf, Germany) and a QIAamp RNA Blood Mini Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions. Total RNA from liver and muscle samples was extracted by homogenization of liver and muscle samples (50-150 mg) in TRIZOL reagent (Invitrogen, Tokyo, Japan). Total RNA from adipose tissue was extracted and isolated by RNeasy Lipid Tissue Mini Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions. RNA concentration was assessed by using a Malcom ES-2 (e-spect) micro UV-VIS fluorescence spectrophotometer (Tokyo, Japan). Total RNA (1 μg) was reverse-transcribed using a QuantiTect Reverse Transcription kit (Qiagen, Düsseldorf, Germany) after inactivation of reverse transcription by heating at 95°C for 3 min. The cDNA product was subjected to real-time PCR according to the user instructions for the Real-Time PCR System 7300 (Applied Biosystems, Foster City, CA). qRT-PCR was performed at 95°C for 5 sec and 60°C for 34 sec in 20 μL buffer containing SYBR premix ExTaq II and ROX Reference Dye (Takara Bio, Shiga, Japan) and 0.2 μM each of the primers (Table 2). Absolute quantification, using the standard curve method by establishing a linear amplification curve from serial dilutions of plasmid DNA containing each cDNA, was performed to analyze RT-PCR results. Expression levels of studied genes were normalized to the expression of β-actin, a normal housekeeping gene by expressing a ratio of test gene copy number/β-actin copy number.

Statistical analysis: Plasma metabolite values were expressed as Mean±SD values and significance between groups was assessed using the Mann-Whitney U-Test set at $p<0.05$. QRT-PCR expression values were expressed as median with minimum and maximum values of expression

Table 2: Primer sequences for quantitative RT-PCR

Probe	PCR product length (bp)	Primer type	Primer sequences (5'-3')	GenBank accession No.
IRS-1	81	Forward	acctgcgttcaaggaggtctg	XM_543274
		Reverse	cggtagatgccaatcaggttc	
IRS-2	177	Forward	tggcaggtgaacctgaagc	XM_542667
		Reverse	gaagaagaagctgtccgagtgg	
PI3K p85α	132	Forward	gcattaaaccagacctcattcagc	AB436616
		Reverse	gcgagtattggtcttcagtgttctc	
AdipoR1	212	Forward	cttctactgctccccacagc	XM843263
		Reverse	ccactgtggtggccttga	
AdipoR2	102	Forward	tccacaaccttgcttcatct	XM534929
		Reverse	cctcgatactgaggggtagc	
MDH	82	Forward	ggtgcagccttggagaaatatg	XM_531844
		Reverse	cagtcaggcagttggtattgg	
G6PDH	139	Forward	gctacttcgatgaatttgggatc	XM_538209
		Reverse	cactttaacaccttgaccttctcg	
FASN	151	Forward	tactggaggggccagtgcatca	AB436619
		Reverse	gtcccgagatggtcactgtgtc	
β-actin	129	Forward	gccaaccgtgagaagatgact	AF021873
		Reverse	cccagagtccatgacaataccag	

ratios (ratio of test gene copy number/β-actin copy number). The Mann-Whitney U-Test was used to assess significance between groups, set at $p<0.05$. Analysis was performed using Sigma plot (Version. 11.2, Build 11.2.0.5, Systat Software Inc., San Diego, CA).

RESULTS

Analysis of plasma metabolites and enzymes: Clinical characteristics, general plasma metabolites and enzymes of both animal groups are presented in Table 3. HF diet feeding resulted in a significant increase ($p<0.05$, Mann-Whitney U-test) concentrations in body weight as compared to control cats only. None of the other plasma metabolites or enzymes activities concentrations in HF diet fed animals significantly differed from control cats.

Quantitative RT-PCR gene expression profile between control and high-fat diet fed cats in various insulin sensitive tissues: Comparison of different insulin sensitive tissues gene expression trends at the mRNA level, between HF diet and control cats are presented in Fig. 1. With regards to insulin signaling activity and glucose metabolism, HF diet cats had a significantly lower ($p<0.05$, Mann-Whitney U-Test) IRS-1 mRNA level in abdominal fat and peripheral leukocytes as compared to control cats. However, HF diet cats had a significantly increased IRS-1 mRNA level in liver as compared to control cats. HF diet fed cats' omental and subcutaneous adipose demonstrated a significant median reduction in IRS-2 mRNA expression when compared against control cats. PI3K p85αmRNA expression was significantly increased in liver and skeletal muscle, significantly reduced in PBL when compared against control cats.

With respect to lipid synthesis and adiponectin signaling, high-fat diet cats' abdominal adipose demonstrated a significant median increase in AdipoR1mRNA expression, as compared to control cats. In contrast, significantly lower levels of AdipoR1mRNA expression in liver and PBL in high-fat diet cats. HF diet cats' subcutaneous and visceral adipose demonstrated a significant median increase in AdipoR2 mRNA expression as compared to control cats. HF diet cats FASN mRNA expression was significantly higher ($p<0.05$, Mann-Whitney U-test) in all the tissues except PBL than control cats.

Table 3: Clinical characteristics and plasma metabolite concentration in cats

Clinical parameters	Control diet (n = 5)	High-fat diet (n = 5)
Age (months)	12.0±0.00	13.6±1.70
Pre-study body weight (kg)	2.4±0.30	2.6±0.30
Post-study body weight (kg)	2.5±0.30	3.4±0.50*
Glucose (mg dL^{-1})	75.6±3.40	84.4±9.20
Insulin (ng mL^{-1})	1.4±0.20	1.6±0.20
Adiponectin (mg mL^{-1})	5.6±2.40	6.4±2.20
Triglycerides (mg dL^{-1})	51.8±7.80	51.8±32.4
Total cholesterol (mg dL^{-1})	106.0±7.00	115.4±27.0
Non-esterified fatty acids (mEq L^{-1})	0.6±0.30	0.5±0.20
Aspartate aminotransferase (U L^{-1})	30.8±2.10	34.0±4.10
Alanine aminotransferase (U L^{-1})	61.8±9.30	62.0±11.5
Alkaline phosphatase (U L^{-1})	114.6±36.4	134.6±58.8
Blood urea nitrogen (mg dL^{-1})	22.2±1.30	22.0±2.60
Creatinine (mg dL^{-1})	0.88±0.1	1.0±0.20
Lactate dehydrogenase (U L^{-1})	122.8±33.6	120.6±30.1
Serum total protein (g dL^{-1})	6.9±0.40	6.8±0.40

Values are Mean±SD, *Significance when compared to control (Mann-Whitney U-test)

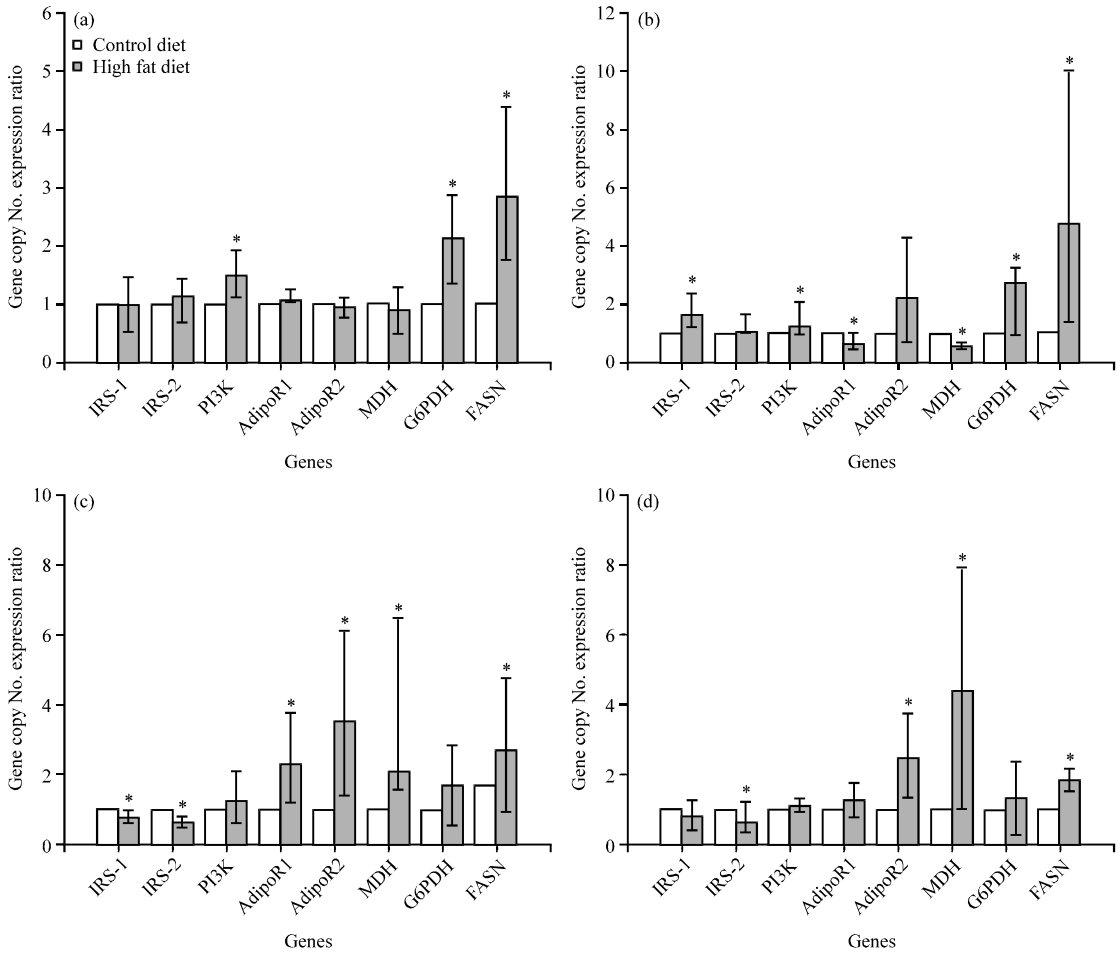

Fig. 1(a-e): Continue

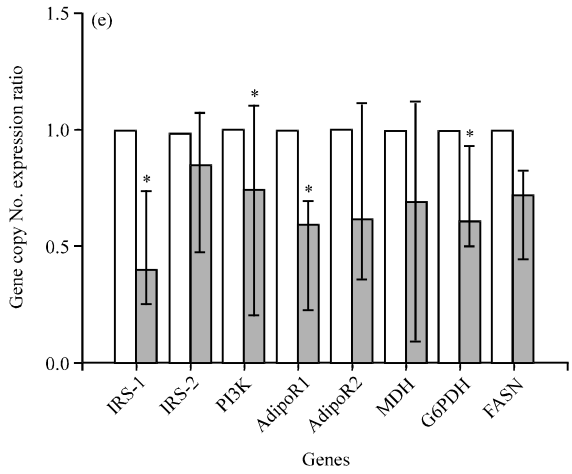

Fig. 1(a-e): Transcriptome comparison of various insulin sensitive tissues, (a) Skeletal muscle, (b) Liver, (c) Abdomin adipose, (d) Subcutanious adepose and (e) Peripheral blood leukocytes of high-fat diet and control diet fed cats. Transcriptomes of various insulin sensitive tissues were determined by RT-PCR of genes involved in insulin signaling (IRS-1, IRS-2 and PI3-K genes), adiponectin signaling (AdipoR1 and AdipoR2), lipid metabolism (FASN and G6PDH) and energy metabolism (MDH). Results are expressed as median gene copy No. expression ratios. Control diet cat gene copy No. expression ratio = (Control diet gene copy No./control diet β-actin copy No.)/(Control diet gene copy No./control diet β-actin copy No.) serves to act as a reference for each gene to be compared to high-fat diet. High-fat cat gene copy No. expression ratio = (High fat diet gene copy No./High fat diet β-actin copy No.)/(Control diet gene copy No./Control diet β-actin copy No.). *Significant difference when compared to control diet cats ($p<0.05$, Mann-Whitney U-test). Bars indicate upper and lower range values. AdipoR: Adiponectin receptors, FASN: Fatty acid synthase, G6PDH: Glucose-6-phosphate dehydrogenase, IRS: Insulin receptor substrates, MDH: Malate dehydrogenase, PI3-K: phosphatidylinositol-3 kinase

Lastly, regarding energy homeostasis, HF diet cats' 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. In addition, HF diet cats G6PHD mRNA expression was significantly higher in liver and skeletal muscle but significantly lower in PBL as compared to control cats.

DISCUSSION

The use of Peripheral Blood Leukocyte (PBL) has been advocated for exploring glucose and lipid metabolism in obese cats (Lee *et al*., 2012b; Mori *et al*., 2009), with multiple studies reporting that PBL transcriptomes can potentially provide early warning of obesity related disorders in cats and humans (Kollias *et al*., 2011; De Mello *et al*., 2008; Lee *et al*., 2012b), especially when a high concordance rate (>80%) of gene expression between PBL and other tissues has been shown in humans and some other species (Mohr and Liew, 2007).

In our study, when comparing Plasma Metabolite Profiles (PMP) of HF diet and control cats, HF diet cats demonstrated only a significant increase in body weight (~35% greater) without being accompanied by any significant alterations to biochemical parameters commonly associated with

obesity risk, such as NEFA, TG, total cholesterol, insulin, or glucose concentration, when compared to control cats, One possible explanation for the lack of significant alterations to biochemical parameters is that daytime restricted feeding (e.g., food is provided *ad libitum* for 3-5 h at the same time every day, usually during daytime) can attenuate the disruptive effect that diet-induced obesity has on circadian expression of metabolic factors (Sherman *et al*., 2012). Disruption of circadian rhythms can lead to obesity and metabolic disorders. High-fat feeding modifies behavioral and molecular circadian rhythms in mice leading to metabolic abnormalities mimicking the human metabolic syndrome, including obesity and insulin resistance (Kohsaka *et al*., 2007; Mendoza *et al*., 2008). To counter this, timed restricted feeding provides a time cue and resets the circadian clock, leading to better health. Sherman *et al*. (2012) demonstrated that a timed restricted feeding HF diet leads to increased insulin sensitivity and fat oxidation and decreased body weight, fat profile and inflammation contrary to HF-diet-fed mice but comparable to Low Fat (LF)-diet-fed mice.

Another explanation for a lack of perturbations observed in plasma metabolites of HF diet cats is that cats used in our study were all intact and did not under gonadodectamies. Backus *et al*. (2007) provided evidence that intact animals, as opposed to those who underwent gonadectomies, seem more resilient to gain body weight and a congruent increase in insulin due to a high fat diet. Only when a threshold level was met, did intact cats given the highest-fat diet (fat = 64% of Metabolisable Energy (ME) in a purified diet of constant protein: ME ratio) gain a significant amount of body weight (17±5% greater). Nguyen *et al*. (2004) also observed similar results between intact and neutered cats. As such, intact animals may be more resilient to high-fat diet induced serum perturbations. Therefore, an insufficient feeding time period for animals to reach a steady state, in conjunction with an insufficient amount of dietary fat to exceed required threshold levels may and two different groups of animals may have compounded the ability of a high fat diet to induce disturbances in plasma metabolites. The use of two different groups of animals and not the same animals before and after weight gain could have also been a factor.

Regarding PBL concordance, using a high-fat diet induced obesity cat model, out of seven genes examined, concordance was observed with ~60% (5 out of 8) of them (IRS-1, IRS-2, AdipoR1, AdipoR2 and MDH) between PBL and tissue transcriptomes in HF diet fed cats. HF diet cat PBL IRS-1 and IRS-2 mRNA expression were both reduced, when compared to control diet which was in concordance with reduced IRS-1 and IRS-2 mRNA expression in both abdominal and subcutaneous adipose of HF diet cats. Similar to IRS-1 and IRS-2, AdipoR1 and AdipoR2 mRNA expression in HF diet cats was also reduced, when compared to control diet which was in concordance with reduced AdipoR1 and Adipor2 in liver and skeletal muscle, respectively of HF diet cats. Lastly, PBL MDH mRNA expression was reduced and was concordant with reduced mRNA expression in liver and skeletal muscle. Surprisingly however, the remaining three genes in which no concordance in mRNA expression was observed (PI3-K, G6DPH and FASN) between PBL and any of the sampled tissues demonstrated very uniform and polar expression trends between PBL and tissues. For example, all tissues (liver, skeletal muscle, abdominal omental and subcutaneous adipose) demonstrated increased PI3-K, G6DPH and FASN mRNA expression trends in HF diet, as compared to diet control cats. Alternately, HF diet cat PBL demonstrated reduced mRNA expression trends for the aforementioned genes. Therefore, overall, PBL sensitivity to a high fat diet appears great enough to lead to some degree of transcriptome concordance with insulin sensitive tissues.

In our study, the concordance observed tended to more likely occur with skeletal muscle and liver as opposed to adipose (4 out of 5 concordant genes) which would suggest for a possible bias in tissue representation perhaps, depending on disease or pathological condition. For example, two

other diet induced obese studies were conducted by our laboratory, whereby control and obese animals were fed on the same balanced diet, except that obese animals were given 2x their daily RER for upto 6 weeks, in order to see the effect of obesity and not diet perse; PBL transcriptome concordance was significantly higher with tissues (5 out of 5; IRS-1, IRS-2, PI3-K, MDH, G6DPH) (Ramsey, 2012), (8 out of 8 genes; IRS-1, IRS-2, PI3-K, MDH, G6DPH, SREP1-c, FASN and ACL; [unpublished data]. The higher level of concordance in the other studies may have been attributed to the fact that the influence of obesity and not diet was key since animals were receiving more energy than required, whereas in this study, animals were receiving greater amounts of dietary fat in their diet, making up a greater portion of their caloric intake which was still regulated to their daily RER. As such, PBL sensitivity to obesity versus diet induced alterations in tissue may be greater, thereby leading to a more uniform concordance in transcriptome trends. However, the more important question which begs our attention is what implication would PBL transciriptome concordance have if it only represents or reflects what may be happening in certain tissues and not others?

The lack of uniformity in gene expression pattern between tissues is a given and expected, since different insulin sensitive tissues accordingly respond differently from one another regarding diet induced obesity (Lee *et al*., 2012a). The response of individual genes to obesity is distinct and largely tissue specific and a systems approach shows numerous commonly activated pathways, suggesting a coordinated attempt by tissues to limit metabolic perturbations occurring in early-stage obesity. Therefore, tissues showing commonly activated pathways would be more likely to show transcriptome concordance, as opposed to others lacking them. As such, for example, some mRNA expression trends were more likely to be similar between liver and skeletal muscle (IRS-1, IRS-2, MDH) as opposed to abdominal and omental adipose (AdipoR1 and AdipoR2) in our study. Alternately, some mRNA expression trends were uniform across all tissues examined (PI3-K, G6DPH and FASN). Therefore, any concordance observed between PBL and tissue transcriptomes needs to be cautiously interpreted.

This was a preliminary study and due to ethical reasons, the tissue group size was small (n = 2 for control and HF Diet) paranthesis and could not be increased. This small number reduced the power of the study and may have precluded other significant differences from emerging with respect to PBM profiles between control and HF diet cats, in addition to PBL transcriptome concordance patterns with tissues. In addition, we acknowledge that caution is required with our results since any study in the future with a much larger sample group may not render similar trends or patterns observed in our study. We hope to be able to increase the numbers of samples in future studies, after receiving clearance from our animal review committee, in order to repeat and validate our results on a larger scale. Lastly, because mRNA expression may change rapidly in tissue and can only provide a snapshot of the metabolic processes occurring at that particular time, mRNA expression trends do not necessarily translate over to the protein level. Therefore, careful interpretation of mRNA expression trends is required taking into account that corresponding protein levels have not been measured.

CONCLUSION

Our results demonstrate that PBL are sufficiently sensitive to detect diet influenced transcriptomic changes occurring in insulin sensitive tissues and 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. Unfortunately,

the expression pattern of the genes examined in this study was not uniform between all the tissues examined and therefore, the PBL pattern did not match any one particular tissue resulting from a HF diet per se. Instead, based on what genes were concordant with tissues, PBL transciptome profile appeared to be similar to liver and skeletal muscle for 3 out 5 genes.

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