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Physical training and weight loss in dogs lead to transcriptional changes in genes involved in the glucose-transport pathway in muscle and adipose tissues

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ABSTRACT

Obesity is a worldwide problem in humans and domestic animals. Interventions, including a combination of dietary management and exercise, have proven to be effective for inducing weight loss in humans. In companion animals, the role of exercise in the management of obesity has received relatively little attention. The aim of the present study was to investigate changes in the transcriptome of key energy metabolism genes in muscle and adipose tissues in response to diet-induced weight loss alone, or combined with exercise in dogs.

Overweight pet dogs were enrolled on a weight loss programme, based on calorie restriction and physical training (FD group, $n = 5$) or calorie restriction alone (DO group, $n = 7$). mRNA expression of 12 genes and six microRNAs were investigated using quantitative real-time PCR (qPCR). In the FD group, *FOXO1* and *RAC1* were expressed at lower levels in adipose tissue, whereas *ESRRA* and *AKT2* were more highly expressed in muscle, when compared with the DO group. Comparing expression before and after the intervention, in the DO group, nine genes and three microRNAs showed significant altered expression in adipose tissue (*PPARG*, *ADIPOQ* and *FOXO1*; $P < 0.001$) and seven genes and two microRNAs were significantly downregulated (*NRF2*, *RAC1*, *ESRRA*, *AKT2*, *PGC1a* and mir-23; $P < 0.001$) in muscle. Thus, calorie restriction causes regulation of several metabolic genes in both tissues. The mild exercise, incorporated into this study design, was sufficient to elicit transcriptional changes in adipose and muscle tissues, suggesting a positive effect on glucose metabolism. The study findings support inclusion of exercise in management of canine obesity.

Introduction

Obesity is a multifactorial disease caused by a combination of genetic and lifestyle factors. It is considered to be an epidemic not only in humans but also in companion animals (Klimentidis et al., 2011). Obesity in companion animals shares many similarities with human obesity, including co-morbidities, such as insulin resistance and hyperlipidaemia, as well as increasing susceptibility to pancreatitis, osteoarthritis and cancer (de Godoy and Swanson, 2013). Systemic insulin resistance is manifested by decreased insulin-stimulated glucose transport and metabolism primarily in adipocytes and skeletal muscle (Kahn and Flier, 2000). Insulin stimulated glucose uptake into various tissues is an essential element of glucose homeostasis in healthy individuals (Oliveira et al., 2014). Glucose transporter 4 (GLUT4) is the main insulin-responsive hexose transporter in muscle and adipose tissues, and is therefore important for maintenance of normoglycaemia (Minokoshi et al., 2003).

Designing a weight loss programme for management of obesity usually has the complication that lean body mass is lost in conjunction with a reduction in adipose tissue. In humans, a combination of physical exercise and introduction of a low-calorie, high-protein diet is generally recommended, so that lean body mass can be preserved, rather than calorie restriction alone (Shaw et al., 2006; Amorim Adegboye and Linne, 2013). In dogs, increased physical activity has also been shown to preserve lean body mass during weight loss programmes (Vitger et al., 2015). Other studies in obese dogs have shown the benefits of incorporating exercise in weight management programmes, with a focus on weight loss rate (Chauvet et al., 2011) or on dietary energy intake (Wakshlag et al., 2012). However, there is limited published research on the specific metabolic response triggered by exercise, combined with calorie restriction in companion animals.

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Table 1
Study population. Signalment, bodyweight (BW), weight loss, body composition assessed by body condition scoring (BCS, 9 point scale), and changes in lean body mass based on measurements of dual energy x-ray absorptiometry in overweight dogs enrolled in a 12-week weight reduction intervention on diet combined with exercise (FD) or diet alone (DO).

Group	Breed	Sex	Age (months)	BW start (kg)	BW end (kg)	Weight loss (%)	BCS start	BCS end	Change in lean mass[a] (%)
FD	Australian Shepherd	FS	120	26.7	22.3	16.5	7	6	–3.6
FD	Australian Shepherd	FS	36	27.3	23.9	12.5	7	7	0.4
FD	Labrador Retriever	FS	97	39.6	33.9	14.4	7	6	4.2
FD	Mixed breed	FS	83	25.5	22.3	12.5	7	6	9.7
FD	Bernese Mountain Dog	FS	67	49.7	42.1	15.3	8	7	20.2
DO	Mixed breed	F	66	48.7	40.4	17.0	8	6	–2.4
DO	Labrador Retriever	MN	74	52.3	48.1	8.0	8	8	–1.4
DO	Labrador Retriever	M	43	44.0	39.5	10.2	6	5	–2.9
DO	Labrador Retriever	FS	43	34.1	29.0	15.0	7	5	–3.5
DO	Bernese Mountain Dog	F	60	48.9	41.1	16.0	8	6	–6.6
DO	Basset Griffon Vendeen	F	80	19.7	17.4	11.7	7	7	–3.9
DO	Nova Scotia Duck Tolling Retriever	FS	107	25.0	21.1	15.6	8	7	2.3

[a] Positive results for change in lean mass occurred when lean mass was gained during weight loss.
F, female; FS, female spayed; M, male; MN, male neutered.

The negative health effects of obesity and a sedentary lifestyle are closely associated with changes in glucose metabolism and insulin sensitivity. In humans, weight loss and physical exercise have been shown to protect against these changes (Fogelholm and Stallknecht, 2006), although the biological mechanisms are not fully understood. Identification of the key genes involved in such processes is crucial for a better understanding of the molecular events occurring in metabolically active tissues, in response to calorie restriction and exercise. The present study was designed to evaluate changes in the transcriptome of adipose tissue and skeletal muscle from overweight dogs before and after weight loss programmes, based on caloric restriction alone or in combination with increased physical activity. Candidate genes and microRNAs were selected for the study based on their involvement in glucose transport and metabolism.

Materials and methods

Animals and study design

Twelve privately owned adult dogs of different breeds were recruited into the study. All dogs were determined to be overweight based on their body condition of ≥6 on a 9 point scale (LaFlamme, 1997). The study was approved by the National Animal Research Council (Approval number 2011/561-80; date of approval, 24 October 2011).

The study was designed as a prospective non-randomised clinical trial, as described in detail by Vitger et al. (2015). Briefly, all dogs were enrolled in a 12 week weight loss programme and allocated to either a fitness/exercise and diet (FD) group ($n = 5$) or to a diet-only (DO) group ($n = 7$), based on owner preference (Table 1). Dogs in the FD group were required to exercise three times a week at the University Hospital for Companion Animals, University of Copenhagen. The training programme consisted of 30 min in an underwater treadmill and 30 min on a land treadmill. In addition, owners were encouraged to increase their dog's physical activity at home. Owners of dogs in the DO group were instructed not to change their daily exercise routines during the study period. The level of the dogs' physical activity at the start of the study and during the intervention was monitored by accelerometry (described in more details by Vitger et al., 2015). Briefly, accelerometer counts were obtained for baseline activity over 1 week and again on alternate weeks during the intervention, six times for nine dogs, five times for two dogs and four times for one dog.

During the study period, dogs were fed a commercial low-fat high-protein dry diet (Satiety support, Royal Canin Denmark), rationed to achieve weight loss in both groups of approximately 1.5% per week (mean 62 ± 2 kcal per unit of metabolic target bodyweight; $kg^{0.75}$). The dogs were weighed on alternate weeks and the food allocation was adjusted accordingly. Before and after the intervention, body composition was assessed by fan-beam dual-energy X-ray absorptiometry (Lunar Prodigy, GE Healthcare, Munich, Germany), with all dogs in ventral recumbency. Data were analysed using a purpose-designed computer software (enCORE 13.60, GE Healthcare).

Tissue samples

Biopsies were collected from each animal under anaesthesia before and after the intervention (Vitger et al., 2015). In the FD group, biopsies were taken 1–5 days after the end of the last exercise session. Muscle biopsies were collected from the adductor muscle, approached through clipped and disinfected skin from the caudal thigh using a Tru-Core II 14 gauge Biopsy Instrument (inter-V, Medical Device Technologies). Subcutaneous adipose tissue was obtained surgically from the abdomen, caudal to the umbilicus. All tissue biopsies were snap-frozen in liquid nitrogen and stored at –80 °C until use.

RNA extraction and cDNA synthesis

Total RNA was isolated from muscle biopsies using Tri Reagent (Molecular Research Centre) following the manufacturer's instructions. Total RNA from adipose tissue was isolated as described previously (Cirera et al., 2014). Genomic DNA contamination was minimised using RNAse-free DNAse set (Qiagen). The integrity of the RNA samples was assessed by use of an Experion device (BioRad Laboratories). Muscle samples with an RNA quality indicator (RQI) ≥ 7 and adipose tissue samples with RQI ≥ 6 were considered acceptable for downstream analysis.

cDNA synthesis was performed in duplicate for all the samples according to the method described elsewhere (Nygard et al., 2010). cDNA synthesis for microRNAs was performed according to the method described by Balcells et al. (2011). All samples were diluted (eight fold) before being used in quantitative real-time PCR (qPCR).

Primer design

Candidate genes, involved in energy metabolism specifically relating to glucose transport into the cell (Klip et al., 2014), together with relevant microRNAs, were selected. Primers were designed using Primer3Plus software[1] for 12 genes: peroxisome proliferator-activated receptor gamma (*PPARG*), forkhead box O1 (*FOXO1*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PGC1a*), solute carrier family 2 (facilitated glucose transporter) member 4 (*GLUT4*), nuclear respiratory factor 1 (*NRF1*), nuclear factor erythroid 2-like 2 (*NRF2*), oestrogen-related receptor alpha (*ESRRA*), TBC1 domain family member 4 (*TBC1D4*), ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) (*RAC1*), v-akt murine thymoma viral oncogene homolog 2 (*AKT2*), pyruvate dehydrogenase kinase isozyme 4 (*PDK4*) and adiponectin *C1Q* and collagen domain containing (*ADIPOQ*). Primers for the reference genes were according to Brinkhof et al. (2006), namely hypoxanthine-guanine phosphoribosyltransferase (*HPRT*), β_2 microglobulin (*B2M*), 60S ribosomal protein L8 (*RPL8*), 40S ribosomal protein S5 (*RPS5*) and 40S ribosomal protein S19 (*RPS19*). Primer sequences, amplicon length and qPCR efficiency are presented in the Appendix: Supplementary Table S1.

Primers for 10 canine microRNAs genes (six target microRNAs and four reference microRNAs): miR-21, miR-23a, miR-93, miR-103, miR-107, miR-223, let-7a, miR-16, miR-26a and miR-24 were designed using the publicly available software MirPrimerDesign 3 (Busk, 2014), according to the design rules previously described (Balcells et al., 2011; Cirera and Busk, 2014). Candidate microRNAs were selected based on studies showing their dysregulation in response to exercise and weight loss in humans or those targeting genes involved in glucose homeostasis, lipid metabolism or the immune system (Safdar et al., 2009; Russell et al., 2013; Nielsen et al., 2014). Primer sequences and qPCR efficiency are presented in the Appendix: Supplementary Table S2.

[1] See: www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi (accessed 20th May 2015).