

Dietary Avocado-Derived Mannoheptulose Results in Increased Energy Expenditure After a 28 Day Feeding Trial in Cats

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ABSTRACT

The effect of dietary avocado-derived mannoheptulose (MH; 8 mg/kg BW) treatment was investigated in 20 cats (*Felis catus*; 3 yr \pm 5 mo, 4 \pm 2 kg). Cats randomly received a control (-MH) and test (+MH) dietary treatment for 28 d in a crossover design with a 14 d washout period between each treatment leg. Two 22-h indirect calorimetry studies were conducted after cats began to receive dietary treatment on day 0 and 21. Blood samples were collected after a 24 hr fast on day 1 and 22. On day 28 cats were subjected to an intravenous glucose tolerance test (IVGTT) following an oral dose of water or dissolved MH (8 mg/kg BW) depending on dietary treatment. Cats were capable of digesting dietary MH as evidenced by significant effects of diet on fasted plasma MH and plasma MH during the IVGTT ($p < 0.05$). There were no effects of MH on body weight, respiratory quotient in the fasted and post-prandial states ($p > 0.05$). There was no overall effect of MH on fasted energy expenditure (EE;

kcal/hr); however, there was a trend for MH to increase 22 hr total EE ($p = 0.05$). There were no significant effects of MH on fasted blood parameters including: glucose, insulin and free fatty acids ($p > 0.05$). Furthermore, an oral MH dose during an IVGTT did not impact glucose and insulin as there were no time point differences ($p > 0.05$). Overall, the cat, an obligate carnivore, demonstrated some metabolic response to dietary MH treatment. Further investigation on whether certain physiological parameters and dietary parameters change the effects of dietary MH are warranted.

INTRODUCTION

Calorie restriction (CR) is the most vigorous and reproducible intervention to inhibit the physiological effects of aging, to delay the commencement of most pathologies (including cancer and diabetes) and to extend mean and maximum lifespan by 20 to 40% (McCay et al., 1935; Weindruch and Sohal, 1997; Weindruch and Walford, 1988). However, there are concerns associated with the feasibility of implementing such CR regimes for long periods of time. Calorie restriction mimetics (CRM) have been studied as an alternative to CR and to avoid some of the

negative effects associated with the implementation of CR regimens (Ingram et al., 2004, 2006; Ingram and Roth, 2011). The objectives of CRM strategies are to produce the same pro-longevity effects that CR provides without reducing caloric intake. Since the pro-longevity strategies of CR influence systems involved in energy sensing, and regulation of metabolism, the initial targets of CRM focused on metabolites that modify glucose metabolism. Glucose anti-metabolites, such as mannoheptulose (MH), are believed to inhibit the glycolytic pathway. Specifically, MH, a seven carbon sugar found in avocados, acts as a hexokinase inhibitor that prevents the phosphorylation of glucose therefore blocking flux through the glycolytic pathway. Glucose anti-metabolites mimic some of the beneficial physiological effects of CR including: reducing body weight, plasma insulin, body temperature, delayed tumour growth, and elevation of circulating glucocorticoid hormones (Roth et al., 2001).

Reducing body weight with CRM strategies has large potential application as greater than 50% of cats are overweight (APOP, 2012). Consequentially, diabetes mellitus is increasing in prevalence in the domestic cat population with an estimated incidence of 2.45 cases/1000 cat years-of-risk (Panciera et al., 1990; Scarlett and Donoghue, 1998). The high incidence rate of obesity and diabetes exemplifies the need for a strategy to control obesity and the associated metabolic effects.

The objectives of the present study were to measure the influence of MH supplementation at 8 mg/kg BW/d in a moderately high fat diet and to measure the effects of dietary MH treatment on the physiology of adult cats. Measures included: fasting and fed mannoheptulose, indirect calorimetry measures of energy expenditure, fat and carbohydrate oxidation, fasted blood samples and an intravenous glucose tolerance test (IVGTT). We hypothesize that dietary MH treatment will cause: 1) an increase in serum MH concentrations, 2) greater energy expenditure (EE) due to a shift from carbohydrate

oxidation to fat oxidation, 3) lower fasted plasma glucose and insulin and 3) improved insulin sensitivity during an IVGTT.

MATERIALS AND METHODS

All procedures were reviewed and approved by Procter and Gamble's Institutional Animal Care and Use Committee in accordance with IACUC guidelines.

Animals: Twenty reproductively sterile cats (N=20) of similar age (3 yr \pm 5 mo), and split 10 females and 10 males, were randomly separated into two treatment groups balanced by both sex and body condition (weight and total body fat (kg)). Cats were provided from Pet Health and Nutrition Center (PHNC) at Procter and Gamble-Pet Care, Mason, Ohio. Standard veterinarian evaluation (physical exam, chemical and CBC blood analysis) of overall health was completed prior to the initiation of the study and all cats entered the study healthy.

All cats were previously acclimated to respiration chambers and associated environment. Acclimation success was assessed using the Cat-Stress-Score (CSS; Kessler and Turner, 1997), feed intake, fearfulness (response to novel stimuli) and elimination behaviours (Gooding et al., 2012). Cats were considered successfully acclimated when they demonstrated behaviours similar to those observed in a free living environment where they are permanently housed, as well as behaviours indicative of low stress and fear response.

Housing: Cats were housed in a free-living group environment with indoor/outdoor access during the day (0800- 1500 h). Access to an outdoor screened area was restricted at night (1500-0800 h). Room environmental enrichment included perches, beds, toy houses, scratching posts, toys and climbing apparatus. All cats were socialized daily for a minimum of 60 min. Cats were maintained on a 12 hour lighting schedule with the lights turning on at 0630 h and turning off at 1830 h. The room temperature was maintained at 22°C and relative humidity was 50%-60%, outdoor temperature averaged 25°C with a relative humidity of

70%. Room surfaces were cleaned daily, and disinfected weekly with Nolvasan disinfectant (Allivet®, St. Hialeah, FL, USA). Water was provided ad libitum from automatic waterers.

Respiration calorimetry chambers (Qubit Systems®, Kingston, ONT, Canada) were made of Plexiglass and measured 53.3 x 53.3 x 76.2 cm. Each chamber contained a shelf, feeder, water bowl, hammock, litter box, toy and a free area with a fleece bed. Water was provided ad libitum from water bowls. The chamber was designed to allow sufficient separation of feeding, sleeping and elimination areas. Chambers and water bowls were disinfected, and litter, litter boxes, toys, hammocks and fleece beds were removed, cleaned and replaced daily.

Diet: To effectively test the effects of MH on energy metabolism, food intake, intended to maintain weight, were provided equally between animals on a body weight basis; therefore, each cat was provided 45 kcal ME/kg BW/d (females) and 50 kcal ME/kg BW/d (males). Diets were presented in kibble form and cats were fed individually at 7:00 am and permitted 60 minutes to eat during food offerings. All remaining feed was collected and weighed to account for total (grams) feed refusal. The control diet was Iams® Original Chicken and the test diet was Iams® Original Chicken + MH (Table 1).

The avocado-derived mannoheptulose was produced using commercially available

avocados (Hass variety). Frozen, whole avocados comprised of the flesh, peel and pit were ground prior to suspension in water (1:3 w/w). The resultant slurry was centrifuged to remove non-aqueous solids. A series of microfiltration (de-oiling), ultra-filtration (10kDa) and nanofiltration (100 kDa) was used to produce the MH-enriched fraction. Lyophilization was used to form the final crystalline powder yielding 18 % MH (Massimino et al., 2005).

Experimental Design: For two weeks prior to the initiation of the study cats were fed Iams® Original Chicken formula as the control diet. At the end of the first washout period, cats were randomly allocated to either the control group (-MH) or test (+MH) group. On the first day of the study (day 0) half the cats continued to receive the control diet without MH treatment (0 mg/kg BW) and the remaining half were fed the control diet with MH treatment (8 mg/kg BW). Each cat was fed their respective diet for a total of 28 days. For 14 days (washout), after the first 28 day dietary treatment, all cats were returned to the control diet without MH treatment. Following the second washout period cats were fed the alternate diet for an additional 28 day period.

Body Weight and Composition: Body weight was measured weekly and food intake measured daily. Body composition was measured via Dual Energy X-ray Absorptimetry (DXA) and BCS analysis on day -2 and on day 28 for all cats. For DXA, animals were anesthetized according to the

Table 1: Analyzed nutrient (%) and metabolizable energy content of control (-MH) and test (+MH) diets on an as-fed basis.

	Iams® Original- Control (-MH)	Iams® Original- Test (+MH)
Moisture %	6.04	6.3
Protein %	32.9	33.4
Fat %	15.96	15.9
Ash %	6.57	6.79
NFE %	34.26	35.25
MH	0 ppm	787 ppm
Predicted ME (Kcal/kg)*	3775	3751

*ME Calculated using Modified Atwater Equation (ME (kcal/kg) = (3.5*kg NFE)+(8.5*kg fat)+(3.5*kg protein)

protocol of an (IM) injection of Dexdomitor (0.013 mg/Kg; Pfizer; Orion Corp. Espoo, Finland) and Butorphanol (0.25 mg/Kg IM; Fort Dodge Animal Health, Iowa, USA) with Propofol (1-4.4 mg/Kg IV; Hospira Inc, Illinois, USA) if needed. Anesthetic reversal was achieved with the administration of Antisedan (Pfizer; Orion Corp. Espoo, Finland), at an equal to volume of Dexdomitor IM. Three DXA scans using infant software provided by Hologic Inc. (Model Delphi A with QDR® for Windows®, Hologic Inc. Bedford, MA, USA) were completed to measure body composition after an adequate plane of anesthesia was reached. Cats were placed in a sternal position and cranial aspect of ante brachium on the table with the phalanges facing caudally, hind limbs were bent slightly upward towards the abdomen while the tail curved just below the left rear. Whole body composition is the sum of the regions and segmented by: bone mineral content (kg), fat (kg), lean (kg), lean+ bone mineral content (kg), total mass (kg), and fat (%). Scans were reviewed while the cat was still on the DXA scanner to ensure that the scan acquisition was acquired properly. Once the scans were completed, cats were removed from the unit and placed in a recovery area and an IM injection of Antisedan (Orion Pharma, Finland; distributed by Pfizer Corp, NY, USA) was administered in order to reverse the pharmacological effects of Dexdomitor. All three scans were combined to obtain an average.

Indirect Calorimetry: To assess the effect of length of dietary MH adaptation on energy metabolism, four separate indirect calorimetry analyses were conducted with cats fed the control or test (MH) diet. Oxidation studies occurred on day 0 and day 21. To determine whether these effects changed during the fed and fasted state, oxidation studies were 22 hr in length and included fasted (0-3 hr), fed (3-9 hr), post prandial (9-15 hr), and return to fasting state measurements (15 -22 hr).

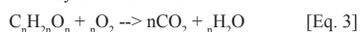
Indirect calorimetry was conducted by measuring respiratory gases for 5 minutes

every 30 minutes. Concentrations of O₂ and CO₂ in the respiratory chambers were measured with O₂ and CO₂ gas analyzers (Qubit Systems®, Kingston, ON, Canada). The calorimeter is an open circuit, ventilated calorimeter with the room air being drawn through at a rate of ~5-8 L/min. Airflow was set at 5 or 8 L/min, depending on cat BW, and actual rate was measured with the use of a mass flow meter to enable total volume calculation. Gas analyzers and mass flow meters were calibrated prior to each individual oxidation study and at least every 6 h during a study, or when a drift of greater than 1% was observed. Calibration was performed using standard gas mixtures at two concentrations. Respiratory quotient, fat and CHO oxidation, and EE were calculated as follows (Weir, 1949):

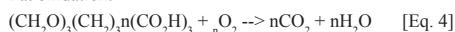
$$\text{Respiratory Quotient (RQ)} = \frac{\text{litres CO}_2 \text{ produced/ litres of O}_2 \text{ consumed}}{\quad} \quad [\text{Eq. 1}]$$

$$\text{EE (kcal)} = 3.94 \times \text{litres O}_2 \text{ consumed} + 1.11 \times \text{litres CO}_2 \text{ produced} \quad [\text{Eq.2}]$$

Carbohydrate oxidation:



Fat oxidation:



Fed energy expenditure, fat and CHO oxidation post feeding was calculated as least square mean ± SEM for all respiratory collections that occurred post feeding. Twenty-four hour energy expenditure, fat and CHO oxidation post feeding was calculated as least square mean ± SEM multiplied by 24 for each dietary treatment and exposure.

Blood Analyses: All blood samples (2.5 mL) were collected via jugular venipuncture using the Vacutainer® system with sampling from the left or right jugular vein. Blood samples were taken in the fasted state following completion of oxidation measurements and 4 hours postprandial on day 1 and 22. Samples were then placed on ice for 1 h. After clotting, blood samples were centrifuged at 3,000 rpm for 15 min at -4°C, and serum was decanted and stored at -20°C or -70°C for later analyses. Serum was measured for glucose, non-esterified fatty acids (NEFA), insulin and MH. Analysis

Table 2: Body weight, total body fat and lean body mass in adult domestic short hair cats during the consumption of the washout/control diet (-MH, Day -2) and after 28 days of exposure to either the test (+MH) or control (-MH) diet^{1,2}.

	Test		Control		P-value
	Day -2	Day 28	Day -2	Day 28	
Body Weight ²	4.22 ± 0.2	4.26 ± 0.2	4.25 ± 0.2	4.27 ± 0.2	0.7433
Body Fat ²	0.560 ± 0.1	0.603 ± 0.1	0.596 ± 0.1	0.571 ± 0.1	0.1002
Lean Body Mass ²	3.81 ± 0.1	3.74 ± 0.1	3.76 ± 0.1	3.77 ± 0.1	0.1858

¹Values are least-square means ± SEM, n = 20. Means compared across day (Day -2 vs. Day 28) within diet without a common superscript (*) differ; P < 0.05. Means compared between diet (Control vs. Test) within day without a common superscript (letter) differ; P < 0.05. NS, P ≥ 0.05. P-value presented refers to the diet*exposure effect of treatment.

²Body weight, body fat and lean body mass are presented on a kg basis.

of glucose and NEFA was completed using the Beckman Coulter AU480 automated chemistry analyzer which uses colorimetric measurements (Beckman Coulter Inc.; Indianapolis, IN, USA). Analysis of insulin was completed using a feline ELISA kit (Mercodia Inc., Winston Salem, NC, USA). MH was analyzed using LC/MS/MS (Sciex API4000 Q-TRAP, AB SCIEX, Framington, MA, USA)

Intravenous Glucose Tolerance Test: An intravenous glucose tolerance test (IVGTT), based on the assumption that glucose concentration decreases exponentially with time following a loading dose, was conducted on day 28 of the study. Following DXA analysis, catheters were implanted under anesthesia to permit frequent blood sampling. Once full recovery from anesthesia had occurred (5 hrs, based on internal unpublished data), cats in the treatment group were provided a MH dose dissolved in water orally using a syringe. The control group was provided 0 mg/kg MH as only water was given orally via a syringe. This ensures that all handling practices were consistent between groups. Two hours following MH administration cats were injected intravenously with 800 mg/kg BW glucose (50% w/v; Butler Schein Animal Health, Dublin, OH, USA). Blood samples were drawn after MH administration at times of -10, -5, -1, and after MH/glucose administration at 2, 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min post-injection of glucose. Following the last blood sample, catheters were removed

and cats were fed their daily food ration. Samples were analyzed for plasma glucose, insulin and MH.

Statistical Analyses: A crossover design with repeated measures was used for this experiment. There were two dietary factors tested (+MH or -MH) and additionally, length of dietary exposure to each treatment was included in the model. A mixed linear model with cat as a random variable using PROC MIXED was used (SAS, version 9.1; SAS Institute Inc., 2002-2003, Cary, NC, USA). The model used was: $Y_{ij} = W_i + \epsilon_i$; in which Y_{ij} = the dependent variable, W_i = dietary treatment (control (-MH) or test (+MH)), and ϵ_i = random residual error. Diet (control (-MH) or test (+MH)) was considered a fixed effect. Treatment least square means were compared using the pdiff multiple comparison procedure. Differences were considered significant when $P < 0.05$ and all data are expressed as least square mean ± SEM. For all measures a trend was observed at $P < 0.10 - P > 0.05$.

RESULTS

Body Weight and Composition: There was no effect of MH supplementation on body weight, body fat or lean body mass (Table 2; $P > 0.05$).

Indirect Calorimetry: There were no effects of dietary MH treatment or duration of MH exposure on fed or fasted RQ (Table 3; $P > 0.05$). There was no effect of diet on fasted EE (kcal/kg^{0.67}/d); however, there was a trend towards an effect of diet

Table 3: Energy metabolism in adult domestic cats after acute (Day 0) and chronic (Day 21) exposure to a control (-MH) and test (+MH) diet ¹.

		Test		Control		P-Value
		Day 0	Day 21	Day 0	Day 21	
Fasted	RQ	0.77 ± 0.005	0.79 ± 0.005	0.78 ± 0.005	0.78 ± 0.005	0.3498
	EE ²	76.5 ± 4.8*	82.7 ± 4.2**	77.8 ± 4.8	78.7 ± 4.2	0.1591
Fed	RQ	0.84 ± 0.003	0.84 ± 0.005	0.84 ± 0.003	0.84 ± 0.005	0.8278
	EE ²	62.6 ± 2.2*	66.1 ± 1.6**	62.8 ± 2.2	62.0 ± 1.6	0.0548

¹Values are least-square means ± SEM, n = 20. Means compared across day (Day 0 vs. Day 21) within diet without a common superscript (*) differ, P < 0.05. Means compared between diet (Control vs. Test) within day without a common superscript (letter) differ, P < 0.05. NS, P ≥ 0.05. P-value presented refers to the diet*exposure effect of treatment.

²Fasting and fed EE are represented on a kcal/kg^{0.67}/d.

on EE in the fed state as cats consuming the test diet (+MH) had elevated EE (Table 3; p=0.05). Furthermore, there was an effect of exposure on EE in the fasted and fed states for cats consuming the test (+MH) diet as EE was higher on day 21 versus day 0 (p=0.02). There were no individual time point differences over the 22 hr respiratory gas sampling period for RQ and EE on day 21 (P>0.05). When AUC for EE was compared between dietary treatment on day 0 and day 21 during the post-prandial (0-3 hrs), fed (3-9 hrs), return to fasting (9-15 hrs) and fasted (15-22 hrs) states no effect of dietary treatment was observed (p=0.48; data not shown).

Fasted and Fed Blood: Serum MH concentrations were different between control and MH test groups in both the fasted and fed state (P<0.001) and fasted serum MH increased with length of exposure to the test diet (p=0.04). There were no effects of dietary MH treatment on fasted and fed insulin, glucose or NEFA. There was a trend for increased glucose to insulin ration (G:I) with dietary MH treatment (p<0.10; Table 4). Fed glucose increased significantly from day 1 to day 22 during exposure to the test (+MH) and control (-MH) diet (P<0.05). In addition, NEFA increased with prolonged exposure to the control diet (P=0.01).

Intravenous Glucose Tolerance Test: There were no differences in time-course effects between treatments on plasma glucose and insulin during the IVGTT following

a glucose bolus at time 0 (Figure 1; A, B; P>0.05). However, cats receiving the oral MH dose (8 mg/kg BW) had greater plasma MH concentrations at all time points when compared with cats receiving the oral saline dose during the IVGTT (Figure 1C; P<0.05).

DISCUSSION

Overall, orally administered MH is digested and absorbed, as demonstrated by plasma MH changes in the adult domestic short-hair cat fed a MH-containing diet. There were no significant effects of dietary MH treatment on body weight, body fat or lean body mass. Further, there was no effect of MH on RQ and thus, relative amounts of fat and carbohydrate oxidation; however, MH caused a numerical increase in total 22 hr post-feeding EE. There were no effects of MH on blood plasma metabolites. To our knowledge, no one has sought to understand the practical application of diets containing a CRM on feline metabolism, which is markedly different than other mammals due to the greater need for protein vs. carbohydrate.

All blood metabolites are similar to those previously reported in healthy, adult domestic short-hair cats (Appleton et al., 2001; 2004; Coradini et al., 2011; Hoenig et al., 2011). Plasma MH concentration was greater in cats fed dietary MH treatment or when MH was orally delivered during IVGTT. The presence of MH in serum indicates cats can absorb dietary/orally administered MH. MH has also been demonstrated to be biologically available in other species

Table 4: The effects of MH and length of exposure on blood plasma metabolites in adult domestic cats consuming a control (-MH) and test (+MH) diet after acute (Day 1) and chronic (Day 22) exposure.¹

		Test		Control		P-Value
		Day 1	Day 22	Day 1	Day 22	
Fasted	Glucose ²	76.3 ± 3	82.0 ± 3	79.5 ± 2	82.6 ± 2	0.5973
	Insulin ²	5.4 ± 0.8	5.5 ± 0.8	4.6 ± 0.9	6.2 ± 0.9	0.2494
	G: I	0.43 ± 0.05	0.51 ± 0.06	0.54 ± 0.05	0.46 ± 0.06	0.6153
	NEFA ²	0.44 ± 0.2	0.45 ± 0.2	0.48 ± 0.3	0.46 ± 0.3	0.5591
	MH ²	175.2 ± 19 ^{***a}	198.1 ± 19 ^{***a}	NS ^b	NS ^b	<.0001
Fed (4 hr Post-Prandial)	Glucose	87.7 ± 4*	100.4 ± 4**	89.7 ± 4*	100.7 ± 4**	0.7747
	Insulin	9.3 ± 2	9.3 ± 2	9.1 ± 2	9.5 ± 2	0.8628
	G: I	0.40 ± 0.06	0.46 ± 0.07	0.32 ± 0.06	0.41 ± 0.07	0.0577
	NEFA	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.02*	0.28 ± 0.02**	0.1129
	MH	2719.5 ± 126 ^a	2915.0 ± 126 ^a	NS ^b	NS ^b	<.0001

¹Values are least-square means ± SEM, n = 20. Means compared across day (Day 1 vs. Day 22) within diet without a common superscript (*) differ; P < 0.05. Means compared between diet (Control vs. Test) within day (Day 1 or Day 22) without a common superscript (letter) differ; P < 0.05. NS, P ≥ 0.05. P-value presented refers to the diet*exposure effect of treatment. ²Glucose (mg/dL), insulin (uIU/mL), NEFA (mEq/L) and MH (mg/dL).

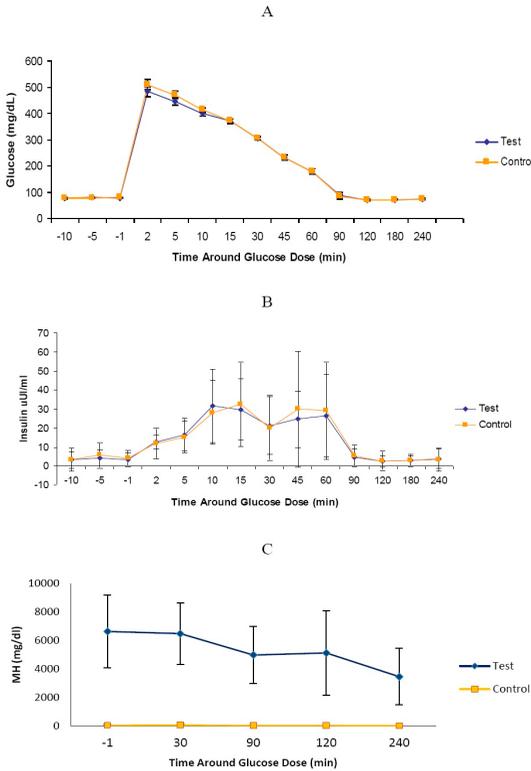
including rabbits, humans, rats and dogs (Roe and Hudson 1936; Blatherwick et al., 1940; Koh and Berdanier, 1974; Issekutz et al., 1977). Cats are obligate carnivores and possess lower digestive and absorptive capacity for dietary carbohydrates relative to other mammals due to the insufficient production of salivary amylase, pancreatic amylase and intestinal disaccharides (Meyer and Kienzle, 1991). Despite these limitations, previous studies have suggested that cats are capable of digesting dietary sugars; however, their capacity to cope with large amounts dietary carbohydrates may be limited (Kienzle, 1993, 1994). In the present study, cats fed MH did not exhibit any signs of MH indigestibility and MH had no untoward effects on serum biochemistry or plasma insulin. The present study adds the seven carbon sugar, mannoheptulose, to our understanding of tolerable and digestible carbohydrates for cats.

We did not observe any change in body weight or body composition in the present study. However, it should be noted that no change in maintenance caloric intake was imposed during the study. True CR regimes often cause significant reductions in BW and

fat mass (Colman et al., 1998; Cefalu et al., 1997). Though some CRM strategies impact energy sensing pathways, dietary CRM supplementation does not necessarily lead to a reduction in body weight or body fat as subjects are fed to energy requirements or ad libitum (Wan et al., 2003; Mamczarz et al., 2005). However, Lane et al., (1998) reported that three doses (0.2%, 0.4% or 0.6%) of the glycolytic inhibitor, 2-deoxyglucose (2-DG) resulted in an initial decline in food intake and body weight in rats. After several weeks of feeding, body weight of rats dosed with 0.2 and 0.4% 2DG were not different from controls; however, the 0.6% supplementation caused rats to maintain the lower weight. However, it should be noted that the 0.6% dose was reported to be toxic based on cardiac issues in the rats (Minor et al., 2010). In the present study, cats fed 8 mg/kg BW did not differ in BW compared with cats fed the control diet. The absence of a BW change is not surprising considering the equal caloric intake for the cats on the study.

Dietary MH treatment affected EE (kcal/hr) in the fasted and post-prandial states. However, there were no specific time-point

Figure 1: Plasma glucose (A), insulin (B) and MH (C) in adult domestic cats following the consumption of either a control (-MH) or test (+MH) diet during an IVGTT on day 28 with and without MH treatment¹.



¹Values are least-square means (n=20), means within time points having different superscripts (*) are significantly different (P<0.05).

differences over the 22-hr respiratory sampling period despite cats fed the test (+MH) diet having greater EE than cats fed the control diet. To date, there is no consensus in the scientific literature if CR and CRM decrease or increase respiration rates in mammals. However, it may be necessary to standardize EE findings by correcting for differences in body weight and body composition for CR and control subjects due to reduced BW and percent body fat in CR animals. It has been reported that EE adjusted for body weight and fat mass declines with CR (Dulloo and Girardier, 1993; Gonzalez-Pachero et al., 1993; Rothwell and Stock, 1982; Santos-Pinto et al., 2001). In contrast, others report no change in EE with CR particularly if EE

is adjusted for body composition (Keesey and Corbet, 1990; Even and Nicolaidis, 1993; Ballor, 1991; Masoro et al., 1982; McCarter et al., 1985; McCarter and Palmer, 1992; Ramsey et al., 2000) while others report increased EE when adjusted for changes in body composition at 40% CR in rats (Selman et al., 2005). There are limited data regarding the effect of CRMs on EE and the data available is contradictory. Dark et al., 1994, reported that hamsters subjected to a 1,500 mg of MH/kg caused them to enter into a state of torpor which was hypothesized to be a consequence of reduced glucose availability. IGF-1 knockout mice, designed to have reduced signaling through the IGF-1 and insulin pathways, have been shown to have an increased lifespan and improved insulin sensitivity with no changes in energy metabolism, including EE, compared to controls (Shimokawa et al., 2002). In our study, cats had greater EE when fed MH containing diets in the fed state. The greater EE may have been attributed to decreased glucose availability for energy and a metabolic shift to fat oxidation to supply energy. Unfortunately, a shift in RQ was not observed in this study to support this hypothesis. An alternative hypothesis may be SIRT1 up-regulation. SIRT1 is a regulator of mitochondrial biogenesis and MH-induced changes in its gene expression may lead to increased mitochondrial function and EE (Guarente, 2006). Differences in results of the effects of CR on EE may be attributable to methods used to determine EE; for instance, doubly-labeled water versus calorimetry, the state in which the measures of EE were taken (resting versus active) and whether or not changes in body size and composition were

accounted for. Further research is warranted to determine if there is indeed a modification in metabolic rate when animals are fed CRMs; however, the enhanced EE observed with MH treatment after 21 days presents a novel opportunity for such CRMs to be used for weight control in cats.

There were no effects of dietary MH on RQ or relative amount of fat and CHO oxidation in either the fasted or fed states. This result was unexpected since MH supplementation typically causes a shift in macronutrient oxidation from a principle reliance on glucose to an increased emphasis on fatty acid oxidation to meet energy demands (Bruss et al., 2010). Increased fatty acid (FA) oxidation and decreased FA synthesis and glucose oxidation are hypothesized to be the underlying metabolic adaptations to CR (Bruss et al., 2010). Similar results are observed in CRM strategies, like MH, designed to impact energy sensing pathways and competitively inhibit cellular usage of glucose. Koh and Berdanier, 1974, studied the effects of a 20 mg dose of MH on the hepatic synthesis of FA in rats and found that FA oxidation increased with MH treatment. The additional FA oxidation is likely a consequence of increased release of NEFA into plasma by adipose tissue and enhanced hepatic uptake of FA for oxidation as has been observed in MH treated rats (Mitzkat and Meyer, 1970; Simon et al., 1972). Several other groups have reported that dietary MH supplementation reduces glucose oxidation in isolated islet cells of the mouse pancreas (Hedekov et al., 1972; Ashcroft et al., 1970; Matschinsky et al., 1971). The inhibitory effect of MH on glucose oxidation has further been supported by Sener et al., 1998, who noted pancreatic islet cells incubated with MH (1.0 mmol/L) had decreased glucose utilization in addition to glucose oxidation. Scruel et al., 1998, noted that other organs (liver and parotid cells) were less impacted than pancreatic islet cells on functional responses to glucose. Overall, more research is needed to elucidate the effects of MH on fat and carbohydrate oxidation in cats. Similarly, the collection of fecal and urine

nitrogen is warranted for nitrogen correction to provide a more accurate assessment of the relative amounts of fat and carbohydrate oxidation.

There were no significant MH effects on fasted and fed glucose and insulin; however, there was a trend towards a higher G:I ratio with MH. These results support those of previous findings that MH administration typically causes a decline of serum insulin leading to a hyperglycemia that is routinely observed in diabetics (Viktora et al., 1969). While increase plasma glucose was observed with the test (+MH) diet, and more unexpectedly with the control (-MH) diet, plasma insulin level numerically increased with the control (-MH) diet but not the MH diet. These results contributed to the trend for higher G:I ratio with MH dietary treatment. No change in plasma NEFA concentration was noted for the MH treatment; however, plasma NEFA concentration increased with prolonged exposure to the control (-MH) diet. While reduced plasma NEFA levels are indicative of healthy body weight and composition, plasma NEFA concentrations typically decline with increased glucose oxidation. As such, we expected increased fat oxidation with MH treatment. Elevated insulin also decreases plasma NEFA concentration (Jensen, 1998); therefore, the numerical higher insulin levels associated with the consumption of the control (-MH) diet (Jensen, 1998) would have been expected to reduce NEFA concentration for cats fed the control (-MH) versus the test (+MH) diets. Overall, the cat demonstrated some reactivity to MH treatment based on the post-prandial elevations of plasma G:I; however, the elevated NEFA concentrations observed during control feeding are unexpected and further analysis of fatty acid metabolism during MH treatment is warranted to gain additional understanding.

In conclusion, avocado-derived mannoheptulose appears to affect some biomarkers of glucose metabolism and resulted in greater EE in the fed state. It is plausible the differences in EE and glucose metabo-

lism may impact body weight, composition and glucose/insulin profiles associated with long-term MH feeding and warrants further investigation. In addition, the overall responsiveness of cats to MH may provide additional understanding of the nutritional and metabolic idiosyncrasies of the feline once the MH mechanisms of function has been further elucidated.

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Abbreviations: MH, mannoheptulose, CR, calorie restriction, CRM, calorie restriction mimetic, FA, fatty acid, NEFA, non-esterified fatty acids, CHO, carbohydrate, IVGTT, intravenous glucose tolerance test, BW, body weight.

REFERENCES

1. APOP. Association for Pet Obesity Prevention. Ward E, Bartages J, Budsberg S, Peterson M, editors. (2012) Big pets get bigger: latest survey shows US dog and cat obesity epidemic expanding. Calabash, NC, USA.
2. Appleton DJ, Rand JS, Sunvold GD. 2001. Insulin sensitivity decreases with obesity, and lean cats with low insulin sensitivity are at greatest risk of glucose intolerance with weight gain. *Journal of Feline Medicine and Surgery*. 2001; 4(3): 211-228.
3. Appleton DJ, Rand JS, Priest J, Sunvold GD, Vickers JR. Dietary carbohydrate source affects glucose concentrations, insulin secretion, and food intake in overweight cats. *Nutrition Research*. 2004; 24, 447-467.
4. Ashcroft SJH, Hedeskov CJ, Randle PJ. Glucose metabolism in mouse pancreatic islet. *Biochem. J*. 1970; 118, 143-154.
5. Ballor DL. Effect of dietary restriction and/or exercise on 23-h metabolic rate and body composition in female rats. *J Appl Physiol* 1991; 71:801-806
6. Blatherwick NR, Larson HW, Sawyer SD. Metabolism of d-mannohelptulose. Excretion of the sugar after eating avocado. *J. Biol. Chem.* 1940; 133, 643-650.
7. Bruss MD, Khambatta CF, Ruby MA, Aggarwal I, Hellerstein MK. Calorie restriction increases fatty acid synthesis and whole body oxidation rates. *Am J Physiol Endocrinol Metab*. 2010; 298: E108-E116
8. Cefalu WT, Wagner JD, Wang ZQ, Bell-Farrow AD, Collins J, Haskell D, Bechtold R, Morgan T. A study of caloric restriction and cardiovascular aging in cynomolgus monkeys (*Macaca fascicularis*): a potential model for aging research. *J Gerontol Biol Sci*. 1997; 52:10-19.
9. Colman RJ, Roecker EB, Ramsey JJ, Kemnitz JW. The effect of dietary restriction on body composition in adult male and female rhesus macaques. *Aging: Clin Exp Res*. 1998; 10, 83-92.
10. Coradini M, Rand JS, Morton JM, Rawlings JM. Effects of two commercially available feline diets on glucose and insulin concentrations, insulin sensitivity and energetic efficiency of weight gain. *Br J Nutr* 2011; 106, S64-S77. doi:10.1017/S0007114511005046
11. Dark J, Miller DR, Zucker I. Reduced glucose availability induces torpor in Siberian hamsters. *Am J Physiol* 1994; 267, 496-501.
12. Dulloo AG, Girardier L. 24 hour energy expenditure several months after weight loss in the underfed rat: evidence for a chronic increase in whole-body metabolic efficiency. *Int J Obes Relat Metab Disord* 1993; 17:115-123
13. Even PC, Nicolaidis S. Adaptive changes in energy expenditure during mild and severe feed restriction in the rat. *Br J Nutr* 1993; 70:421-431
14. Gooding MA, Duncan IJH, Atkinson JL, Shoveller AK. Development and validation of a behavioural acclimation protocol for cats to respiration chambers used for indirect calorimetry studies. *JAAWS*. 2012; 15(2): 144-162.
15. Guarente L. Sirtuins as potential targets for metabolic syndrome. *Nature* 2006; 444, 868-874.
16. Hedeskov CJ, Hertz L, Nissen C. The effect of mannoheptulose on glucose and pyruvate-stimulated oxygen uptake in normal mouse pancreatic islets. *Biochim. Biophys. Acta* 1972; 261, 388-397.
17. Hoening M, Jordan ET, Glushka J, Kley S, Patil A, Waldron M, Prestegard JH, Ferguson DC, Wu S, Olson DE. Effect of macronutrients, age, and obesity on 6- and 24-h postprandial glucose metabolism in cats. *Am J Physiol Regul Integr Comp Physiol* 2011; 301, R1798-R1807
18. Ingram DK, Anson RM, Decabo R, Mamczarz J, Zhu M, Mattison J, Lane MA, Roth GS. Development of calorie restriction mimetics as a longevity strategy. *Ann N Y Acad Sci* 2004; 1019, 412-423.
19. Ingram DK, Zhu M, Mamczarz J, Zou S, Lane, MA, Roth GS, de Cabo R. Calorie restriction mimetics: An emerging research field. *Aging Cell* 2006; 5, 97-108.
20. Ingram DK, Roth GS. Glycolytic inhibition as a strategy for developing calorie restriction mimetics. *Exp Gerontol*. 2011; 46, 148-154.
21. Issekutz B, Issekutz TB, Elahi D. Effect of mannohelptulose on glucose kinetics in normal and glucoorticoid treated dogs. *Life Sci*. 1977; 16, 635-643.
22. Jensen MD. Diet effects on fatty acid metabolism in lean and obese humans. *Clin Nutr*. 1998; 67, 531S-534S.
23. Kessler MR, Turner DC. Stress and adaptation of cats (*Felis Silvestris Catus*) housed singly, in pairs and in groups in boarding catteries. *Anim Welfare*. 1997; 6: 243-254.

24. Keeseey RE, Corbett SW. Adjustments in daily energy expenditure to caloric restriction and weight loss by adult obese and lean Zucker rats. *Int J Obes* 1990; 14,1079-1084
25. Kienzle E. Blood sugar levels and renal sugar excretion after the intake of high carbohydrate diets in cats. *J. Nutr.* 1994; 124, 2568S-2571S.
26. Kienzle E. Carbohydrate metabolism of the cat. 4. Activity of maltase, isomaltase, sucrase and lactase in the gastrointestinal tract in relation to age and diet. *J. Anim. Physiol. Anim. Nutr.* 1993; 70, 89-96.
27. Koh E, Berdanier CD. Effects of mannoheptulose on lipid metabolism of rats. *J. Nutr.* 1974; 104: 1227-1233.
28. Lane MA, Ingram DK, Roth GS. 2-Deoxy-D-glucose feeding in rats mimics physiological effects of calorie restriction. *J. Anti Aging Med.* 1998; 1, 327-337.
29. Mamczarz J, Bowker JL, Duffy M, Zhu M, Hagepanos A, Ingram DK. Enhancement of amphetamine-induced locomotor response in rats on different regimes of diet restriction and 2-deoxy-D-glucose treatment. *Neurosci.* 2005; 131, 451-464.
30. Masoro EJ, Yu BP, Bertrand HA. Action of food restriction in delaying the aging process. *Proc. Natl. Acad. Sci.* 1982; 79: 4239-4241.
31. Massimino SP, Niehoff RL, Sarama RJ, et al. (2005). Processes for preparing plant matter extracts and pet food compositions. USPTO, Publication 0249837A1; EPO 1773133B1.
32. Matschinsky FM, Ellerman JE, Krzanowski J, Kotler-Brajtburg J, Langraf R, Fertel R. The dual function of glucose in islets of Langerhans. *J. Biol. Chem.* 1971; 246, 1007-1011.
33. McCarter R, Masoro EJ, Byung PY. Does food restriction retard aging by reducing the metabolic rate? *Am J Physiol* 1985; 248,E488-E490
34. McCarter RJ, Palmer J. Energy metabolism and aging: a lifelong study of Fisher 344 rats. *Am J Physiol* 1992; 263:E448-E452
35. McCay CM, Crowell MF, Maynard LA. The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.* 1935; 10, 63-79.
36. Meyer H, Kienzle E. Dietary protein and carbohydrates: relationship to clinical disease. Proc. Purina International Symposium, 15 Jan 1991, Orlando, FL. pp. 13-26.
37. Minor RK, Smith DL Jr, Sossong AM, Kaushik S, Poosala S, Spangler EL, Roth GS, Lane M, Allison DB, de Cabo R, Ingram DK, Mattison JA. Chronic ingestion of 2-deoxy-d-glucose induces cardiac vacuolization and increases mortality in rats. *Toxicol Appl Pharmacol* 2010; 243, 332-9.
38. Mitzkat HJ, Meyer U. Hepatic metabolite pattern of the energy-linked metabolism in instant diabetes after mannoheptulose. *Life Sd. (II)* 1970; 9, 561-570.
39. Panciera DL, Thomas CB, Eicker SW, Atkins CE. Epizootiologic patterns of diabetes mellitus in cats: 333 cases (1980-1986). *J Am Vet Med Assoc.* 1990; 197, 1504-1508.
40. Ramsey JJ, Harper ME, Weindruch R. Restriction of energy intake, energy expenditure, and aging. *Free Radic. Biol. Med.* 2000; 29, 946-968.
41. Roe JH, Hudson CSJ. The utilization of d-mannoheptulose (d-manno-ketoheptose) by adult rabbits. *Biol.Chem.* 1936; 112, 443-449.
42. Roth GS, Ingram DK, Lane MA. Caloric restriction in primates and relevance to humans. *Ann. N. Y. Acad. Sci.* 2001; 928, 305-315.
43. Rothwell NJ, Stock MJ. Effect of chronic food restriction on energy balance, thermogenic capacity, and brown adipose tissue activity in the rat. *Biosci Rep* 1982; 2,543-549
44. Santos-Pinto FN, Luz J, Griggo MA. Energy expenditure of rats subjected to long term food restriction. *Int J Food Sci Nutr* 2001; 52:193-200
45. Scarlett JM, Donoghue S. Associations between body condition and disease in cats. *J Am Vet Med Assoc* 1998; 212:1725-1731.
46. Scruel O, Vanhoutte C, Sener A, Malaisse WJ. Interference of D-mannoheptulose with D-glucose phosphorylation, metabolism and functional effects: comparison between, liver, parotid cell and pancreatic islets. *Mol Cell Biochem* 1998; 187, 113-120.
47. Selman C, Phillips T, Staib JL, Duncan JS, Leewenburgh C, Speakman JR. Energy expenditure of calorically restricted rats is higher than predicted from their altered body composition. *Mech Ageing Dev* 2005; 126, 783-793
48. Sener A, Kadiata MM, Olivares E, Malaisse WJ. Comparison of the effects of D-mannohelutulose and it hexaacetate ester on D-glucose metabolism and insulinotropic action in rat pancreatic islets. *Diabetologia.* 1998; 41, 1109-1113
49. Shanley DP, Kirkwood TB. Calorie restriction and aging: a life history analysis. *Evolution Int J Org Evol* 2000; 54, 740-750.
50. Shimokawa I, Higami Y, Utsuyama M, Tuchiya T, Komatsu T, Chiba T, Yamaza H. Lifespan extension by reduction in growth hormone insulin-like growth factor-1 axis in a transgenic rat model. *Am. J. Pathol.* 2002; 160, 2259-2265.
51. Simon E, Frenkel C, Kraicer PF. Blockade of insulin secretion by mannoheptulose. *Israel J. Med. Sci.* 1972; 8, 743-752.
52. Viktora JK, Johnson BF, Penhos JC, Rosenberg CA, Wolff FW. Effect of ingested mannohelutulose in animals and man. *Metabolism.* 1969; 18, 87-102.
53. Wan R, Camandola S, Mattson MP. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. *FASEB.* 2003; 17, 1133-1134.
54. Weindruch R, Walford RL. The retardation of aging and disease by dietary restriction. 1988; Springfield, IL: Charles C. Thomas.
55. Weindruch R, Sohal RS. Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. *N. Engl. J. Med.* 1997; 337, 986-994.
54. Weir JBV. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949; 109, 1-9.