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Effect of *trans*-fat, fructose and monosodium glutamate feeding on feline weight gain, adiposity, insulin sensitivity, adipokine and lipid profile

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Abstract

The incidence of obesity and type 2 diabetes mellitus (T2DM) is increasing, and new experimental models are required to investigate the diverse aspects of these polygenic diseases, which are intimately linked in terms of aetiology. Feline T2DM has been shown to closely resemble human T2DM in terms of its clinical, pathological and physiological features. Our aim was to develop a feline model of diet-induced weight gain, adiposity and metabolic deregulation, and to examine correlates of weight and body fat change, insulin homeostasis, lipid profile, adipokines and clinical chemistry, in order to study associations which may shed light on the mechanism of diet-induced metabolic dysregulation. We used a combination of partially hydrogenated vegetable shortening and high-fructose corn syrup to generate a high-fat–high-fructose diet. The effects of this diet were compared with an isoenergetic standard chow, either in the presence or absence of 1·125 % dietary monosodium glutamate (MSG). Dual-energy X-ray absorptiometry body imaging and a glucose tolerance test were performed. The present results indicate that dietary MSG increased weight gain and adiposity, and reduced insulin sensitivity ($P<0·05$), whereas high-fat–high-fructose feeding resulted in elevated cortisol and markers of liver dysfunction ($P<0·01$). The combination of all three dietary constituents resulted in lower insulin levels and elevated serum β-hydroxybutyrate and cortisol ($P<0·05$). This combination also resulted in a lower first-phase insulin release during glucose tolerance testing ($P<0·001$). In conclusion, markers of insulin deregulation and metabolic dysfunction associated with adiposity and T2DM can be induced by dietary factors in a feline model.

Key words: Adiposity: Dyslipidaemia: Glucose tolerance test: High-fructose corn syrup: Insulin: Monosodium glutamate: *Trans*-fatty acids

The numbers of obese and diabetic patients are increasing year by year, and new experimental models are required to investigate the diverse aspects of these polygenic diseases, which are intimately linked in terms of aetiology. The pathogenesis of type 2 diabetes mellitus (T2DM) involves a progressive development of insulin resistance both in the peripheral tissues and in the liver, resulting in impaired insulin secretion from the pancreatic β-cells and hyperglycaemia[1]. T2DM is preceded by the metabolic syndrome[2], which is a group of conditions including dyslipidaemia, hyperglycaemia and obesity. Excessive accumulation of abdominal fat is a risk factor for T2DM[3] and is associated with dyslipidaemia and a rise in serum cortisol levels[4]. In order to improve our understanding of the mechanisms involved in the onset of obesity and insulin resistance, various rodent models have been developed based upon genetic susceptibility or chemical induction, for example[5]. Dietary manipulations in rodents have included high fat and/or carbohydrate administered either to the rodent itself[6,7] or to its mother during pregnancy[8,9]. However, despite their widespread popularity and ease of induction, there are well-documented differences between the physiological changes induced by various different rodent T2DM models, such as differences in the pathogenesis of islet degeneration and the plasma insulin profile[10]. Care must be taken in the extrapolation of the results from rodent models to humans[5], and larger animal models may be appropriate if they are shown to more accurately resemble the human pathology of obesity and type 2 diabetes.

Feline diabetes mellitus has been shown to closely resemble human T2DM in terms of clinical, pathological and physiological features[11,12]. In common with humans, T2DM tends to develop spontaneously in middle-aged or older

Abbreviations: AUC, area under the curve; B-HBA, β-hydroxybutyrate; HFCS, high-fructose corn syrup; IGF-1, insulin-like growth factor 1; *K*, disappearance rate; MSG, monosodium glutamate; T2DM, type 2 diabetes mellitus.

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cats. Moreover, obesity is a risk factor for feline diabetes[13], and as with humans, overweight cats are becoming increasingly prevalent[14]. A sedentary lifestyle, together with a highly energetic diet, is believed to fuel the human obesity epidemic[15], and domestic cats increasingly occupy an indoor sedentary environment in which carbohydrates are consumed in the form of commercial cat diets[11]. Further similarities between feline and human diabetes include the development of insulin resistance, hyperglycaemia and pancreatic islet cell lesions, together with partial loss of pancreatic β-cells[11,12,16].

Many studies of diet-induced obesity, T2DM and liver disease have focused on specific components common to the human diet, such as fructose[17], high-fructose corn syrup (HFCS) or equivalent[18,19], *trans*-fat[19–21] and the food flavour enhancer monosodium glutamate (MSG)[18,20,22]; all of which regularly occur in the so-called Western-style human diet. However, data on the effects of these dietary components on feline lipid homeostasis, plasma metabolite levels and glucose tolerance are relatively scarce. Thiess *et al.*[23] showed that a diet containing 29·2 % lard caused a significant rise in feline plasma TAG, NEFA, β-hydroxybutyrate (B-HBA) and cholesterol, together with a slightly elongated glucose clearance and reduced insulin response during a glucose tolerance test. Cats given a 40 % carbohydrate diet, however, did not show a similar pattern. A high-protein diet led to elevated postprandial amylin concentrations in cats, compared with high-carbohydrate or high-fat diets[24]. To our knowledge, there are no published studies on the effect of dietary fructose, *trans*-fat or MSG in cats, despite the fact that cats express ketohexokinase, the enzyme that metabolises dietary fructose in the liver[25]. Furthermore, diabetic cats have recently been reported to have a twelvefold increase in the neuronal accumulation of fructose compared with normal felines[26], making them a suitable model for the study of diabetic neuropathy.

Our aim was to compare the effects of four isoenergetic diets on weight gain, body fat, lipid profile, insulin sensitivity, adipokine, hormone and metabolite profile, together with glucose tolerance, in a feline model of diet-induced metabolic disturbance. We used a combination of partially hydrogenated vegetable shortening and HFCS to generate a high-fat–high-fructose diet. This diet was compared with an isoenergetic standard chow, either in the presence or absence of 1·125 % dietary MSG. We bred our study animals from female cats that were previously established on these diets for 3 weeks before mating, similar to our rodent models[18,20], since some of the effects of MSG are believed to occur only during the neonatal period[27–29], and because developmental programming of the metabolic syndrome may be affected by maternal nutritional balance[8,30]. Exposure to these diets occurred throughout the study, in order to mimic as closely as possible the situation that occurs in humans. In addition, our second aim was to examine the correlates of diet-induced weight and body fat change, lipid profile, cortisol and markers of liver dysfunction, in order to study early associations which may shed light on the mechanism of diet-induced metabolic dysregulation in a feline model.

Materials and methods

Cats and diets

A total of eight female domestic cats were used to breed eighteen male animals used in the present study. The breeding and care of the animals were in accordance with the protocols approved by the Animal Care and Use Committee of the King Faisal Specialist Hospital and Research Centre, and by the Institutional Animal Care Committees of Guelph University and CanCog Technologies. Commercially acceptable incandescent lighting was provided for the cats which were placed on a natural light cycle (lights on at 07.00 hours). Heating and cooling were electronically controlled and were set to maintain the room in a temperature range from 18 to 25°C. The room ventilation was designed to provide approximately fifteen filtered air changes per hour. Pen allocations for the cats provided each cat with a minimum of 0·56 m^2 of personal space. The female breeders were between 2·0 and 5·0 years old and healthy based on physical examination and routine clinical laboratory data. Cats had free access to water and were fed twice daily. We used a combination of partially hydrogenated vegetable shortening and HFCS to generate a high-fat–high-fructose diet. The following four isoenergetic formulated diets (TestDiet®; Test Diets Purina, Richmond, IL, USA) were used in the present study: a standard chow (control) diet (catalogue no. 5003); diet A consisting of control diet with 1·125 % MSG (diet A: catalogue no. 5C1J); diet B containing 20 % partially hydrogenated vegetable shortening (8·6 % *trans*-fat) and 20 % HFCS (high-fat–high-fructose diet B, catalogue no. 5B4K); diet C containing 20 % partially hydrogenated vegetable shortening, 20 % HFCS and 1·125 % MSG (high-fat–high-fructose and MSG diet C, catalogue no. 5C1H). The diet composition is given in Table 1. The cats were fed twice per d for about 1 h. They were fed to meet their maintenance energy requirements (MER) estimated by the formula $MER = 110\,cal/d \times (BW \times 0{\cdot}75)$[31], where BW is the body weight (kg). Average MSG consumption as part of diets A and C was 201·4 (SEM 18·65) mg/kg body weight. A preliminary diet acceptability study was performed on the breeder animals over a period of 1 week, to ascertain that the diets were palatable. Food consumption was monitored at every feed, and body weight was recorded at the beginning and end of the acceptability study. Female breeders (two animals per diet group) were established on each of the four different diets for 3 weeks before mating, and pregnant females continued on these diets throughout the gestation and weaning periods. Male animals bred for the study continued on these respective diets throughout the study and were fed twice daily. Food consumption was monitored at every feed, and body weight was monitored approximately every 14 d. Female kittens not included in the present study were adopted.

Body composition and biochemical measurements

Total body scans were performed using dual-energy X-ray absorptiometry using the Lunar DPX-IQ densitometer (Lunar Corporation, Madison, WI, USA) to determine body

Table 1. Composition of the experimental diets

	Control	Diet A	Diet B	Diet C
Protein (%)	31·50	30·70	31·50	31·50
Fat (%)	11·50	12·10	19·00	19·00
TFA (%)	0·32	0·32	6·65	6·65
Carbohydrate (%)	37·00	38·40	30·10	30·20
Fructose (%)	0·13	0·13	13·12	13·12
Glucose (%)	0·13	0·13	10·82	10·82
Fibre (%)	2·50	2·30	1·40	1·40
MSG (%)	0·00	1·13	0·00	1·13
Energy (kJ/g)	16·90	16·53	17·45	17·46

TFA, *trans*-fatty acids; MSG, monosodium glutamate.

composition, percentage of total body fat and bone mineral content at 3 months of age and, again, at 9 months. Pre-anaesthesia consisted of intramuscular atropine, and full anaesthesia induction was accomplished with intravenous propofol. Anaesthesia was maintained by intubation and isoflurane gas. Monitoring procedures during and after anaesthesia consisted of direct evaluation of respiration, heart rate, mucous membranes and intermittent auscultation. Total body scans on cats were performed in the paediatric small mode (Lunar Pediatric Software version 4.7e; GE Lunar, Madison, WI, USA). Body weight, percentage of fat, weight change and percentage of change in body fat were recorded. Body length was measured from the tip of the nose to the base of the tail in anaesthetised animals laid ventral side down, using a cloth tape. Clinical chemistry profiles, serum lipids, alkaline phosphatase, alanine transaminase, B-HBA and fasting serum insulin and glucose were assessed at 9 months of age. Samples for clinical chemistry and complete blood count were sent to Advance Vet Laboratories (Mississauga, ONT, Canada) for analysis. Serum TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol concentrations were measured in the serum of 9-month-old fasted cats using the Reflovet Plus instrument (Roche, F. Hoffmann-La Roche Limited, Basel, Switzerland), according to the manufacturer's instructions. NEFA were measured in cat serum using the Half Micro Test (Roche Diagnostics GmbH, Mannheim, Germany). Hormone measurements were also assessed at 9 months of age in the serum of fasted cats. Insulin was measured using the feline insulin ELISA kit from Mercodia (Uppsala, Sweden; 10-1233-01), according to the manufacturer's instructions. Leptin and adiponectin were measured by ELISA using commercial assay kits (EZCL-31K; Millipore, Bedford, MA, USA and K1001-1 Otsuka Pharmaceuticals, Tokyo, Japan, respectively). Retinol-binding protein 4 was measured using the EZHRBP4-18K RBP4 ELISA kit (Millipore). Cortisol was measured using the 900-071 Cortisol ELISA kit from Assay Designs/Stressgen Bioreagents (Ann Arbor, MI, USA). Insulin-like growth factor 1 (IGF-1) was measured using the Mediagnost IGF-1 ELISA kit (IGFBP blocked; BioVendor LLC, Candler, NC, USA). Homeostatic model assessment index values, a measure of insulin resistance, were calculated according to the established formula: (fasting serum insulin (μIU/ml)) × (fasting serum glucose (mM))/22·5[32].

Intravenous glucose tolerance test

Glucose tolerance tests were performed when the cats were 9 months of age. Food was withheld for 12 h before testing. At least 1 h before testing, a cephalic catheter (20 gauge × 3·8 cm, manufactured by BD Medical, Mississauga, ONT, Canada) was placed under light physical restraint and patency maintained with 0·5 ml heparinised saline. If the catheter became non-patent, venepuncture was performed on the cats to obtain the samples. Then, 3ml samples of blood were drawn into SST-coated collection tubes before glucose administration (50 % (w/v) glucose solution, 2 ml glucose/kg body-weight baseline value at $t = 0$ min) and at 5, 10, 20 and 40 min after glucose injection. Blood samples were placed on ice, and the serum was separated by centrifugation at 2800 rpm for 10 min and frozen for subsequent analysis of glucose, insulin and NEFA, as detailed earlier.

Statistical analysis

All analyses were performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA) and GraphPad InStat version 3 (San Diego, CA, USA). Data are presented as means with their standard errors of body weight, percentage of body fat, clinical chemistry, serum lipid profile and hormone measurements. Data were analysed by one-way ANOVA with Bonferroni's *post hoc* tests to determine statistical significance between selected diet groups. Significance was set at $P<0·05$. Pearson's correlations were calculated to evaluate the association of variables listed in Table 2.

Results

Body characteristics and growth hormone axis

Table 1 shows the diet composition. The average amount of the isoenergetic diets consumed was 71·61 (SEM 8·38) g/d, with no significant differences between the four diets used in the study. At 3 months of age, there was no significant difference in adiposity and body weight (Table 2), but the percentage of weight gain and body-fat gain between 3 and 9 months of age in diet group A cats was 140 % higher than control levels ($P<0·05$; Fig. 1(c) and (d) and Table 2). Percentage of fat and body-weight change in diet groups B and C were not significantly different from the control. There was a significant variation in body length at 9 months, with animals in diet group B obtaining 9 % less body length than the control; however, there was no apparent difference in bone mineral content and IGF-1 levels between the three diet groups compared with the control (Table 2).

Clinical chemistry, hormone and lipid profile

Creatinine levels in diet groups B and C were 1·5- and 1·4-fold elevated above control animals, and serum cortisol levels were similarly elevated in these two diet groups (1·4- and 1·5-fold, respectively, $P<0·001$; Table 2). Levels of B-HBA were significantly increased in diet group C cats compared with the control (2·7-fold, $P<0·001$; Table 2). Diet group B animals had

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Table 2. Body characteristics, hormones, clinical chemistry and lipid profile in cats
(Mean values with their standard errors)

	Control		Diet A		Diet B		Diet C		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Weight (kg)									
3 months	1·31	0·16	1·00	0·05	1·00	0·04	0·92	0·13	0·09
9 months	4·06	0·13	4·38	0·15	3·24	0·17	3·75	0·57	0·09
Weight increase (%)	249·43	34·37	344·08*	28·70	221·83	14·00	306·31	7·04	0·01
Body fat (%)									
3 months	4·72	0·44	4·00	0·00	4·03	0·02	4·40	0·40	0·3
9 months	20·85	1·26	27·06†	3·09	16·20	0·97	16·70	2·05	0·01
Body-fat increase (%)	378·38	36·09	576·50*††	78·57	302·59	22·61	277·32	19·21	<0·01
Body length (cm)	55·50	0·87	57·80	1·39	50·25*	0·85	55·00	1·58	<0·01
BMC (g)	103·50	5·55	106·80	3·12	84·25	4·89	93·00	13·39	0·16
IGF-1 (nmol/l)	25·55	4·73	29·93	3·86	27·57	3·41	28·58	7·83	0·94
HOMA-IR	1·41	0·37	4·39*††	0·24	3·20	1·12	1·15	0·31	<0·01
ALP (U/l)	93·40	6·45	70·40*	7·36	120·75**†††	15·61	55·50	7·14	0·001
ALT (U/l)	79·60	12·13	55·60**	2·20	102·00*†††	13·51	50·25	6·47	0·01
Creatinine (μmol/l)	90·40	7·83	108·60**††	6·05	138·50***	5·85	128·00	5·81	<0·001
Cortisol (ng/ml)	32·82	1·83	35·29†††	1·69	45·86***†	1·51	49·82	1·72	<0·0001
B-HBA (μmol/l)	22·60	8·24	31·80**††	7·53	48·25	12·45	62·00	7·60	0·04
Leptin (ng/ml)	4·50	0·53	5·29†††	1·34	1·09***	0·42	0·58	0·09	0·001
Adiponectin (μg/ml)	10·80	2·53	8·52††	0·82	10·59	3·68	14·85	1·63	0·33
RBP4 (μg/ml)	3·29	0·54	4·75	0·70	3·92	0·49	4·73	1·40	0·53
T-CHOL (μg/ml)	162·80	9·44	154·60	11·48	147·25	7·86	162·25	9·39	0·68
HDL (μg/ml)	82·94	7·21	93·70	4·57	76·10	7·92	88·65	6·44	0·31
LDL (μg/ml)	51·05	6·82	44·42	11·99	51·08	3·51	51·54	5·61	0·91
TAG (mmol/l)	0·94	0·17	1·26	0·29	0·65	0·16	0·54	0·04	0·09

BMC, bone mineral content; IGF-1, insulin-like growth factor 1; HOMA-IR, homeostatic model assessment index; ALP, alkaline phosphatase; ALT, alanine transaminase; B-HBA, β-hydroxybutyrate; RBP4, retinol-binding protein 4; T-CHOL, total cholesterol.
Mean values were significantly different from those of the control: *$P<0·05$, **$P<0·01$, ***$P<0·001$.
Mean values were significantly different from those of diet C: †$P<0·05$, ††$P<0·01$, †††$P<0·001$.

twice the levels of serum alkaline phosphatase and alanine transaminase than diet group C ($P<0·001$) animals, suggesting an impairment of liver function. Fasting insulin levels and homeostatic model assessment index values in diet group A cats were 2·4- and 3·1-fold greater than the control, suggesting the development of insulin resistance in these animals ($P<0·05$ and $P<0·01$; Tables 2 and 3, respectively). Serum leptin levels decreased by approximately fourfold in diet groups B and C, and diet group C animals had lower leptin and baseline fasting insulin levels than diet group A ($P<0·05$; Tables 2 and 3, respectively) animals. Serum lipids were all within the normal range and did not differ significantly (Table 2). Other clinical chemistry markers are indicated in Table S1 of the supplementary material (available online at http://www.journals.cambridge.org/bjn).

Intravenous glucose tolerance test

Fig. 2 (a)–(c) shows mean serum glucose concentrations, insulin and NEFA levels, respectively, during a glucose tolerance test administered to the animals at 9 months of age. Areas under the curve (AUC), disappearance rates (K) and half-life ($T_{1/2}$) values are tabulated in Table 3. There was no difference in baseline (fasted) glucose levels (Fig. 2(a) and Table 3); however, after 40 min of intravenous glucose challenge, cats in diet groups B and C had significantly elevated serum glucose levels compared with the control ($P<0·05$; Fig. 2(a)). The clearance rate of glucose ($K_{glucose}$) was 158% that of the control in diet group A cats, but only 44% that of the control in diet group B cats ($P<0·05$), suggesting a trend towards glucose intolerance in high-fat- and fructose-fed cats. Baseline insulin concentrations in diet group A cats were twice as high as controls and remained significantly elevated after 20 min of glucose challenge ($P<0·05$; Fig. 2(b)). The AUC for insulin was 140% that of the control in diet group A cats, but only 65% that of the control values in diet group C cats ($P<0·0001$; Table 3), suggesting impairment of first-phase insulin release in these animals. Cats in diet groups B and C also had fasting NEFA levels, which were 152% elevated above the control (overall significance $P=0·02$; Table 3). The AUC for NEFA were also greater in these two diet groups compared with the control.

Correlates of body characteristics, lipid and hormone profiles

Pearson's correlation analysis was performed on the variables detailed in Table 2. The percentage of body-fat increase was strongly correlated with body-weight increase, TAG and leptin (r 0·84, 0·79 and 0·76, respectively, $P<0·001$; Table 4). Serum leptin showed an inverse relationship to creatinine, cortisol and NEFA. Cortisol correlated with B-HBA, NEFA, creatinine and adiponectin (Table 4), and IGF-1 showed positive associations with 9-month body weight, bone mineral content and fasting insulin.

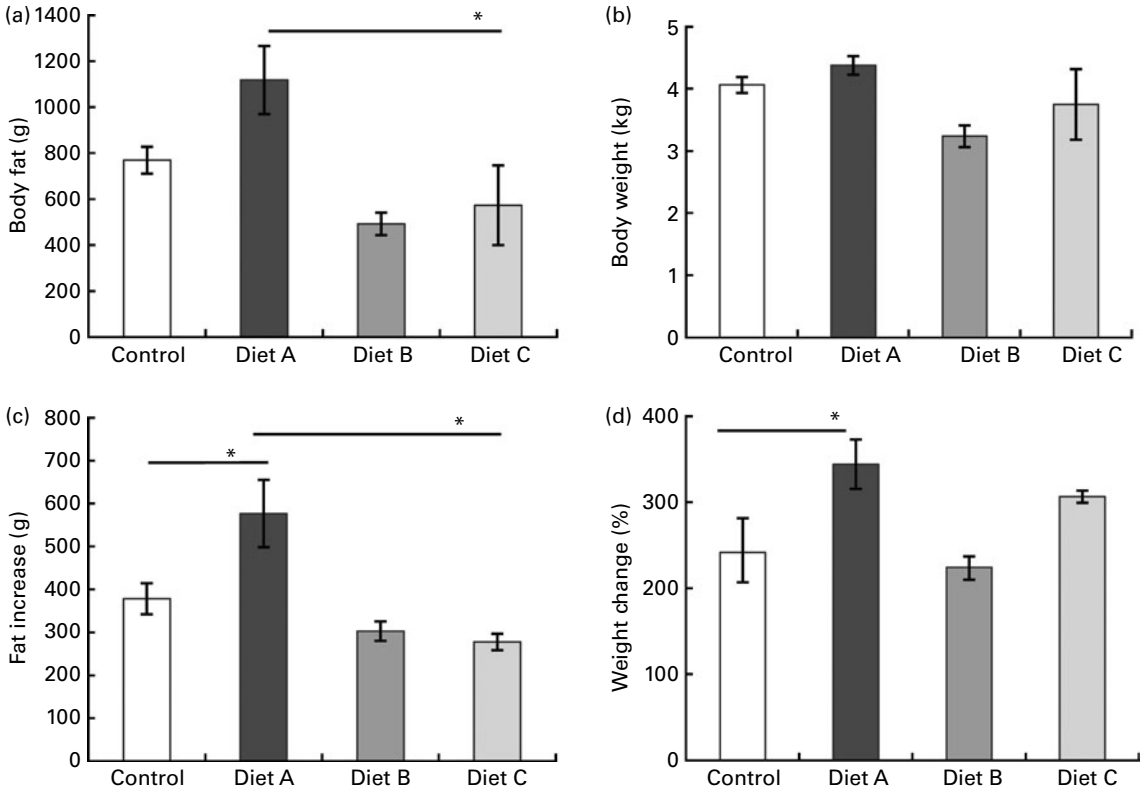

Fig. 1. Effect of diet on body composition. (a) Body fat (g), (b) body weight (kg), (c) percentage of increase in body fat from 3 to 9 months of age and (d) percentage of change in body weight between 3 and 9 months of age in cats from the control diet (□, *n* 4), diet A (monosodium glutamate (MSG): ■, *n* 5), diet B (high fat–high fructose: ▩, *n* 4) and diet C (high fat–high fructose and MSG: ▨, *n* 4) groups. Values are means, with standard errors represented by vertical bars. * Mean values were significantly different ($P<0·05$).

Discussion

The present results suggest that dietary manipulation prenatally and over the first 9 months of life can markedly affect normal metabolism. MSG invoked a phenotype of increased weight gain, adiposity and elevated insulin levels, together with a significantly higher $AUC_{insulin}$. A combination of high-fat and -fructose feeding resulted in elevated cortisol and markers of liver dysfunction, whereas the combination of all three dietary constituents resulted in significantly lower insulin levels, together with markers of metabolic ketosis. The present results with MSG are interesting when compared with previous observations in rodents, which show that neonatal administration of MSG, usually by injection shortly after birth, results in an obese phenotype in adulthood, accompanied by hyperinsulinaemia, consistent with the development of insulin resistance[27–29]. The mechanism in rodents

Table 3. Glucose, insulin and NEFA parameters during an intravenous glucose tolerance test
(Mean values with their standard errors)

	Control		Diet A		Diet B		Diet C		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Baseline glucose (mM)	4·00	0·14	5·33	0·56	5·18	0·23	5·12	0·44	0·21
$AUC_{glucose}$ (mmol/l × min)	1001·44	70·01	984·88	52·55	1209·64	105·05	1118·71	93·85	0·02
$K_{glucose}$ (%/min)	2·21	0·26	3·49*†††	0·39	0·97*	0·26	1·20	0·10	<0·001
Glucose $T_{1/2}$ (min)	32·90	4·18	20·77	2·57	107·07	47·24	59·28	4·92	0·07
Baseline insulin (pmol/l)	55·56	14·66	134·21*†	15·60	100·99	39·92	34·46	9·27	0·02
$AUC_{insulin}$ (pmol × min)	5538·56	366·39	7774·16**††	465·71	5591·85†	321·53	3613·29	231·19	<0·001
$K_{insulin}$ (%/min)	2·59	0·16	3·18	0·41	2·21	0·80	2·50	0·55	0·55
Insulin $T_{1/2}$ (min)	27·05	1·71	23·06	3·14	91·43	65·05	31·65	6·10	0·38
Baseline NEFA (mM)	0·42	0·06	0·39	0·07	0·62	0·05	0·64	0·08	0·02
AUC_{NEFA} (mm × min)	10·80	0·31	8·40*†††	0·34	13·35*	0·63	15·11	0·90	<0·001
K_{NEFA} (%/min)	3·63	1·17	2·15	0·58	1·98	0·54	1·40	0·76	0·27
NEFA $T_{1/2}$ (min)	52·95	37·55	56·59	28·46	52·61	23·06	88·94	25·53	0·79

AUC, area under the curve; *K*, clearance rate; $T_{1/2}$, half-life.
Mean values were significantly different from those of the control: *$P<0·05$, **$P<0·01$.
Mean values were significantly different from those of diet C: †$P<0·05$, ††$P<0·01$, †††$P<0·001$.

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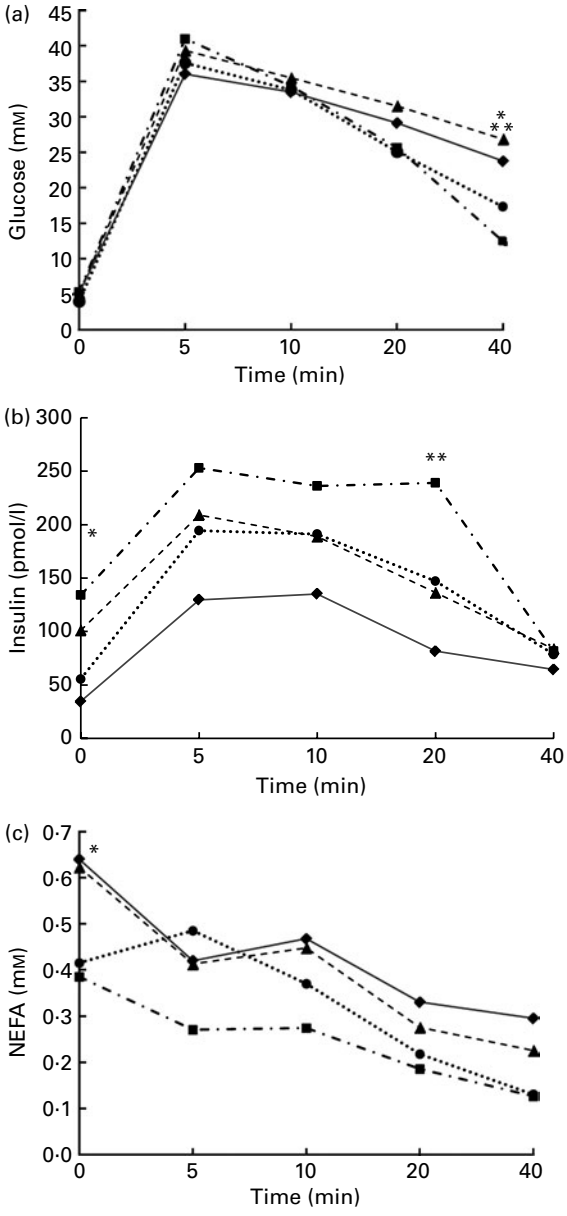

Fig. 2. Intravenous glucose tolerance test in 9-month-old cats. (a) Mean plasma glucose (b) insulin and (c) NEFA levels in response to an oral glucose load in cats from the control diet (·····●·····), diet A (monosodium glutamate (MSG), --■--), diet B (high fat–high fructose, ---▲---) and diet C (high fat–high fructose and MSG, ——◆——) groups. Mean values were significantly different: * $P<0·05$, ** $P<0·01$, *** $P<0·001$.

appears to be due to partial or complete destruction of the *N*-methyl-D-aspartate receptor-rich arcuate nucleus. It is now apparent that maternal administration of MSG may penetrate the placental barrier and distribute to the embryonic tissues of the fetus. Oral administration of MSG and ^{3}H-labelled glutamate to pregnant mice resulted in marked elevations of ^{3}H-labelled glutamate in the placenta and in the fetal brain, liver and kidney[33]. Furthermore, injections of MSG in pregnant mice using ^{3}H-labelled glutamate as a tracer have been shown to result in cytopathological damage to the fetal arcuate nucleus and ventromedial nucleus of the

Table 4. Pearson's correlation coefficients between percentage of body fat, leptin, cortisol, insulin-like growth factor 1 (IGF-1) and bone mineral content (BMC)

	r	*P*
Percentage of body-fat increase		
Weight increase (%)	0·84***	<0·0001
Body fat (%)	0·90***	<0·0001
Body length (cm)	0·54*	0·02
TAG (mmol/l)	0·79**	<0·001
Na (mmol/l)	0·57*	0·01
Urea (mmol/l)	0·81***	<0·0001
Leptin (ng/ml)	0·76**	<0·001
Leptin (ng/ml)		
Weight (kg)	0·53*	0·02
Weight increase (%)	0·50*	0·03
Body fat (%)	0·79**	<0·001
Body-fat increase (%)	0·76**	<0·001
Body length (cm)	0·54*	0·02
TAG (mmol/l)	0·79***	<0·0001
NEFA (mM)	−0·61**	<0·001
Creatinine (μmol/l)	−0·68**	0·001
Cortisol (ng/ml)	−0·64**	0·003
Erythrocytes (10^{12}/l)	−0·71**	<0·001
Cortisol (ng/ml)		
B-HBA (μmol/l)	0·75**	<0·001
NEFA (mM)	0·55*	0·02
Creatinine (μmol/l)	0·67**	0·002
Adiponectin (μg/ml)	0·46*	0·05
Leptin (ng/ml)	−0·64**	0·003
Body fat (%)	−0·48*	0·04
Weight (kg)	−0·51*	0·03
BMC (g)	−0·60**	0·008
Serum protein (g/l)	−0·49*	0·03
IGF-1 (nmol/l)		
Weight (kg)	0·57*	0·02
BMC (g)	0·67**	0·004
Insulin (pmol/l)	0·50*	0·04

B-HBA, β-hydroxybutyrate; BMC, bone mineral content; IGF-1, insulin-like growth factor 1.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$.

hypothalamus[34]. Moreover, maternal exposure to MSG has been demonstrated to result in obesity and brain lesions[35], together with behavioural changes in the offspring, including learning disabilities[36,37], which could be reversed by co-administration of sodium ferulate[38]. The ability of sodium ferulate to protect against MSG-induced apoptosis of neuronal cells appears to occur via prevention of the glutamate-induced decrease in the activity of the phosphatidylinositol 3-kinase (PI3K/Akt) and the mitogen-activated protein kinase kinase (MEK/ERK1/2) signalling pathways, and also through inhibition of the down-regulation of glutamate-induced Bcl-2 protein expression[39].

Much less is known about feline glutamate metabolism. In common with dogs and rabbits, glutamate is rapidly absorbed from the feline intestine[40]. [^{3}H]Glutamate has been shown to bind specifically to the feline central nervous system under physiological conditions of pH and temperature[41], and hypothalamic lesions were demonstrated to occur in kittens injected intravenously with glutamate[42]. Electrolytic destruction of the posterior commissure and the commissure of the inferior colliculus in cats was shown to result in hyperphagia and weight gain[43]. Conversely, bilateral lesions to the ventral

hypothalamus resulted in decreased energy intake and marked weight loss. Further experiments in cats have shown that destruction of the feline hypothalamic nucleus semilunaris accessories rendered the hypothalamic nucleus entopeduncularis neurons unresponsive to glucose and insulin[44]. In common with humans, cats with the largest percentage of body fat also had the lowest insulin sensitivity and the greatest increase in basal insulin concentrations with weight gain in a study by Appleton *et al.*[45]. The present results also showed that in common with obese cats[45], MSG-treated cats with increased adiposity also had higher AUC for insulin during an intravenous glucose tolerance test.

The combination of high-fat and -fructose feeding (diet B) resulted in a reduction of serum leptin and an increase in serum creatinine and cortisol. Impaired glucose metabolism was suggested by a reduced glucose clearance rate after an intravenous glucose tolerance test. Commercial extruded cat diets are high in carbohydrates[46], usually maize-based, and cats express the hepatic enzyme necessary to metabolise fructose[25]. Impaired glucose tolerance, together with reduced adiposity and markers of liver damage, has recently been reported in *trans*-fat-fed mice[7]. High-fat and -fructose diets are frequently used to induce T2DM in animal models[47], and in rodents, it is believed that dietary fructose contributes to the development of hyperinsulinaemia[48], whereas high-fat feeding impairs rodent pancreatic insulin secretion[49], contributing to the induction of glucose intolerance. A comparison of the effect of high-fat and high-carbohydrate diets on feline glucose tolerance suggested that the high fat component was responsible for perturbations found in the clearance of glucose and was more effective in raising serum B-HBA levels[23]. Fructose- and HFCS-fed rodents have recently been demonstrated to have elevated alanine transaminase levels, suggesting the initiation of liver dysfunction[50]. High-fat and -fructose feeding has been shown to precipitate non-alcoholic fatty liver disease[51], and *trans*-fat feeding raised murine serum alanine transaminase levels[52]; however, information on the aetiology of feline hepatic lipidosis is relatively scarce.

The combination of high fat and fructose plus MSG (diet C) resulted in significantly lower fasting insulin levels, together with a lowered first-phase insulin release during the intravenous glucose tolerance test, even though glucose levels remained in the normal range. It has previously been shown in cats rendered progressively diabetic by treatment with growth hormone and dexamethasone that glucose levels during an intravenous glucose tolerance test remained in the normal range even when the first-phase insulin response had diminished by roughly fivefold[53]. Diet C also raised serum B-HBA, cortisol, alkaline phosphatase and NEFA levels, which may point to the early development of ketosis in this diet group, despite the fact that fasting glucose levels were similar to the control, and all parameters studied fell within the normal reference range for cats. The exact mechanism whereby this combination of MSG and high fat–fructose resulted in a different phenotype than either one alone remains to be fully established. One possibility is that although both diets B and C had increased fat and fructose content, resulting in higher fasting NEFA levels, the diet C combination may have altered feline metabolism, precipitating a switch in hepatic metabolism to ketogenesis in these hypoinsulinaemic animals. B-HBA is a product of fatty acid oxidation in the liver, and elevated lipid peroxidation was demonstrated in MSG-treated rodents fed a hyperenergetic diet compared with animals given the hyperenergetic diet in the absence of MSG treatment[54]. Additionally, neonatal injections of MSG were shown to alter cafeteria diet-induced thermogenesis compared with saline-treated controls fed the same energy-rich diet, resulting in a significant weight gain in the cafeteria-MSG-treated mice compared with cafeteria-saline-treated controls[55]. Diet group C cats also had low levels of serum leptin in addition to insulin. The low levels of leptin found in diabetic ketoacidotic patients have been shown to be rapidly restored upon insulin infusion[56]. Interestingly, these patients also had higher levels of cortisol, B-HBA and NEFA than the controls.

We have also examined correlations between feline body characteristics, hormones, metabolites and serum lipid components irrespective of the different dietary regimens employed. In particular, leptin correlated strongly with percentage of body fat, serum TAG and weight change, confirming previous observations that human leptin correlates with fat mass[57] and the waist:hip ratio correlates with serum TAG[58], both in children. Additionally, we have found an inverse relationship between feline leptin and cortisol levels similar to that reported in humans[59], supporting the notion that cats closely resemble humans in several aspects of physiology. In the present study, we also noted a further correlation between serum IGF-1 and insulin levels. This is in agreement with the findings of Kawachi *et al.*[60] who speculated that circulating levels of both hormones could be determined by common nutritional and other causal factors, thus allowing for the possibility that diet-induced elevations in circulating IGF-1 may lead to increased adiposity, which in turn could result in hyperinsulinaemia.

The present study has some limitations. First, the group sizes were small, which reduced the power of the study and may have precluded other differences emerging as significant. On the other hand, we were able to demonstrate statistically significant diet-induced metabolic effects between the four diet groups. Second, we did not separate the effects of high fat from those of high fructose; however, we were able to show that dietary manipulation in these cats results in the development of metabolic characteristics shared by obesity and diabetes, using this model. Third, the length of the present study may limit its potential; however, our data suggest that further research in this area is warranted, and it would be of interest to see if the metabolism of the high-fructose/fat/MSG diet groups would change if they reverted to a control diet. It would also be pertinent to ascertain whether concomitant administration of sodium ferulate might alter feline metabolism in response to these diets, similar to its effect on MSG-treated rodents[38,39].

In summary, the present study shows that dietary manipulation prenatally and over the first 9 months of life can affect feline weight gain, together with markers of insulin deregulation and metabolic dysfunction. Our analysis of the

correlations between extent of feline adiposity, leptin and other metabolic parameters suggests a close similarity between feline and human metabolism with respect to these parameters, providing further evidence that these animals may make a suitable model for the examination of metabolic deregulation.

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