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ORIGINAL ARTICLE

A preliminary report on the feeding of cynomolgus monkeys (*Macaca fascicularis*) with a high-sugar high-fat diet for 33 weeks

James N. Mubiru[1,2], Magdalena Garcia-Forey[1], Paul B. Higgins[2], Peggah Hemmat[3], Nicole E. Cavazos[3], Edward J. Dick Jr[1], Michael A. Owston[1], Cassondra A. Bauer[1], Robert E. Shade[1], Anthony G. Comuzzie[1,2] & Jeffrey Rogers[1,4]

1 Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX, USA
2 Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX, USA
3 St. Mary's University, One Camino Santa Maria, San Antonio, TX, USA
4 Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA

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Correspondence
James N. Mubiru, Southwest National Primate Research Center, Texas Biomedical Research Institute, PO Box 760549, San Antonio, TX 78245-0549, USA.
Tel.: +1 210 258 9744;
fax: +1 210 670 3323;
e-mail: jmubiru@TxBiomed.org

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Abstract

Background The metabolic syndrome is common in populations exposed to a typical Western diet. There is a lack of an animal model that mimics this condition.
Methods We fed 15 cynomolgus monkeys *ad libitum* a high-sugar high-fat (HSHF) diet for 33 weeks. Body weight, body composition, serum lipids, and insulin were measured at baseline and at 33 weeks.
Results The animals tolerated the HSHF diet very well. In the intervention group, total serum cholesterol and low-density lipoprotein cholesterol were 3- and 5-fold higher, respectively, at 33 weeks as compared with their baseline levels. Serum high-density lipoprotein cholesterol and triglycerides were not significantly affected. Dual-energy X-ray absorptiometry (DXA) analysis of the intervention group indicated that the trunk fat mass increased by 187% during this period.
Conclusions Cynomolgus monkeys should be a useful model for investigating the interactions of diet and other factors such as genetics in the development of the metabolic syndrome.

Background

The prevalence of the metabolic syndrome, characterized by increased adiposity and adversely altered lipid and glucose regulation, is increasing in populations exposed to a typical Western diet [5]. It is estimated that 25% of the US population is affected by the metabolic syndrome [11]. Numerous studies have found that the metabolic syndrome is associated with increased risk of type 2 diabetes, coronary heart disease, and certain cancers [6, 16, 26, 39]. Although weight, genetics, age, stress, and sedentary lifestyles are known contributing factors, the exact etiology of the metabolic syndrome is not completely known [3, 12, 17, 23, 24]. Observations of humans and studies in animal models have suggested that exposure to Western diets, particularly those with high intakes of sugar-sweetened beverages, contributes to its development [4, 8, 10, 36]. However, the mechanisms underlying the contribution of such dietary factors to the metabolic syndrome have yet to be fully elucidated.

One major problem that has restricted our understanding of the pathogenesis of the metabolic syndrome is the lack of animal models that fully mimic the human condition. The development of clinically relevant animal models will greatly enhance efforts to study the mechanisms underlying dietary effects on the metabolic syndrome and the interaction of diet with genetic traits.

Although rodents have been used as animal models of the metabolic syndrome, their lipid metabolism and lipid profiles are different from those of humans, making them less than desirable as models [1]. Non-human primates are phylogenetically closer to humans and have similar lipid metabolism and lipid profiles as humans [30]. Several non-human primate species have been used extensively to study the various aspects of the metabolic syndrome [18, 31]. Rhesus monkeys develop spontaneous metabolic syndrome, and this model has provided a system for the longitudinal studies for this disease [13, 14]. Rhesus monkeys have also been used extensively in caloric restriction studies. They have shown that this dietary regimen results in improvements in many factors related to the metabolic syndrome, including decreased body weight and fat mass, and improved glucoregulatory function and lipid profile compared with *ad libitum* fed controls [9, 21, 25].

Cynomolgus monkeys have also been used to study dietary effects on various aspects of the metabolic syndrome such as atherosclerosis, vascular responses, and glucose sensitivity [33, 34]. Most recently another non-human primate, the baboon, has also been used to study the effects of exposure to a high-sugar high-fat (HSHF) diet on adiposity and metabolic markers [15]. The results in the baboon model have shown direct effects of a HSHF diet on components of the metabolic syndrome as indicated by increased body fat and triglyceride concentrations, altered adipokine concentrations, and evidence of altered glucose metabolism [15]. Veterinary experience indicates that different primate species tolerate and thrive on different types of dietary regimes and that there is no obvious way to predict the success of any given diet intervention in a particular species. In a recent study, rhesus monkeys exposed to a high-fat high-cholesterol diet failed to gain weight and also failed to increase or otherwise change their daily caloric intake [30].

The aims of this study were to investigate (i) whether the cynomolgus monkey would tolerate long-term exposure to a HSHF diet, and (ii) whether feeding cynomolgus monkeys such a diet alters body composition and serum metabolic biomarkers.

Methods

Animals

We selected 15 male cynomolgus monkeys (*Macaca fascicularis*) 5–7 years of age from the colony at the Southwest National Primate Research Center (SNPRC), Texas Biomedical Research Institute (Texas Biomed). Animals in the intervention group were fed a HSHF diet and housed individually in a temperature- and humidity-controlled environment with a 12-hour light to dark cycle to maintain normal circadian rhythms. Ten additional age-matched male control animals remained in outside group housing and were fed a low-sugar low-fat (LSLF) baseline diet. All animals were sexually mature as determined by testis size. While the ideal experimental design would be to house the two groups in the same way, resources did not permit identical housing. The LSLF group was included mainly to help in interpretation of the results.

For the animals in the intervention group, body weight and dual-energy X-ray absorptiometry (DXA) scans were recorded at baseline and after 33 weeks of dietary challenge. For the DXA scans, after a 12-hour overnight fast, the animals were sedated with ketamine and anesthesia maintained with isoflurane during the whole scanning procedure. DXA body composition scans were undertaken using a Lunar Prodigy densitometer (GE Healthcare, Madison, WI, USA). Animals were placed in the supine position on the DXA bed, and extremities were positioned within the scanning region. Scans were analyzed using Encore2007 software version 11.40.004 (GE Healthcare). Total body, trunk region (torso), and limb (arm + leg) region compositions were determined. For the age-matched control animals, body weight was measured and blood collected at 0 and 33 weeks after a 12-hour fast and under ketamine sedation, but DXA measurements were not performed because resources did not permit.

All procedures were approved by the Texas Biomed Institutional Animal Care and Use Committee (IACUC). Texas Biomed is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Diet

The LSLF diet is a commercially available solid feed (5LE0; LabDiet, PMI, St. Louis, MO, USA) high in complex carbohydrates and low in fat. The energy composition of the HSHF diet corresponded to that of a typical human fast-food diet, which is high in saturated fat and simple carbohydrates. The diet was originally developed to induce obesity and related metabolic dysregulation in the baboon (*Papio hamadryas*) [15]. It was prepared using 73% Purina Monkey Chow 5038, 7% lard, 4% vegetable oil (Crisco®; Sysco, Houston, TX, USA), 4% coconut oil, 10.5% high-fructose corn syrup, and 1.5% water. The food was flavored with artificial fruit flavors (Kool Aid®) and baked to form palatable pellets. Before the animals were put on the HSHF diet, their daily feed consumption was monitored for

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2 weeks. After the animals were started on the HSHF diet, food consumption was recorded daily. The nutrient composition of the two diets is shown in Table 1.

Serum lipid profile, insulin, and other metabolic assays

Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, and glucose were assayed using ACE® reagents on an ACE® clinical chemistry machine (Alfa Wassermann Diagnostic Technologies, LLC, West Caldwell, NJ, USA). Insulin was assayed using a human insulin ELISA kit (Millipore, Billerica, MA, USA). The homeostasis model assessment (HOMA-IR = [insulin (μU/ml) × glucose (mmol/l)]/22.5 was used to determine insulin resistance. This model has been used before in cynomolgus monkeys [28, 29].

Statistical analysis

The mean values at 33 weeks for the intervention group were compared with each animal's mean value at baseline using the two-tailed paired samples *t*-test (Microsoft Office Excel). The mean values for the intervention group after 33 weeks were compared with those of the age-matched control group using the unpaired *t*-test. A *P*-value <0.05 was considered significant.

Table 1 Composition of the low-sugar low-fat (LSLF) and high-sugar high-fat diets (HSHF)

	LSLF diet (5LE0)[1]	HSHF diet[2]
Fuel value (kcal/g)	3.34	4.33
Protein (%)	15.3	11.4
Fat (%) acid hydrolysis	5.9	18.5
Fiber (%)	5.8	3.1
Total carbohydrate (%)	57.7	55.2
Ash (%)	6.2	4.9
Vitamin C (mg/100 g)	54	233
Cholesterol (mg/100 g)	4.9	127
Kilocalories from fat (%)	12.6	38.6
Kilocalories from protein (%)	18.3	10.5
Kilocalories from total carbohydrate (%)	69.1	51.0
Kilocalories from simple sugars (%)	3.13	10.7
Protein/energy ratio (%/kcal/g)	4.58	2.6
Minerals/energy ratio (%/kcal/g)	1.85	1.13
Simple sugar profile (%)		
Glucose	0.29	3.5
Fructose	0.32	5.1
Sucrose	1.85	2.2
Lactose	0.15	0.7
Maltose	–	0.1

[1]From chemical analysis of micronutrient composition provided by the manufacturer (5LE0; LabDiet, PMI, St. Louis, MO, USA, August 23, 2006).
[2]From chemical analysis of nutrient composition from Covance Laboratories (Madison, WI, USA).

Results

Diet consumption

The animals tolerated the HSHF diet very well, and none of them displayed adverse clinical signs during the experimental period. Average caloric intake during the experimental period is shown in Table 2. By week 33, animals in the intervention group were consuming an average of 731 kcl per day, an increase of 59% over baseline calorie intake ($P < 0.05$).

Body weight and body composition changes in animals fed HSHF diet

Animals consuming the HSHF diet gained body weight as compared with baseline weights (Table 3). The major contributor to changes in body weight was fat deposition. Total lean mass increased by 11% ($P < 0.01$), while total body fat mass increased by 140% ($P < 0.05$; Table 3). Most of the body fat mass was deposited in the trunk regions. Trunk fat mass increased by 187% while lean mass increased by only 15% (Table 3).

Serum lipid profile, and insulin and glucose levels in animals fed HSHF diet

Total cholesterol and LDL-C concentrations increased 3- and 5-fold, respectively, as compared with baseline values ($P < 0.01$; Table 4). However, serum HDL-C and triglyceride concentrations did not change significantly, although both did increase ($P = 0.2$, 0.157, respectively; Table 4).

After 33 weeks, the animals fed the HSHF diet had greater fasting insulin concentrations ($P < 0.01$) and greater HOMA-IR ($P < 0.01$) as compared with base-

Table 2 Caloric intake (mean ± SD) of cynomolgus monkeys on the high-sugar high-fat (HSHF) diet during the experimental period[1]

Time point	Caloric intake (kcal/day)	Body weight (kg)
Baseline	457.19 ± 140.09	5.48 ± 1.09
8 weeks	704.71 ± 101.61*	5.89 ± 1.35[ns]
16 weeks	676.28 ± 106.32*	5.99 ± 1.44*
33 weeks	731.34 ± 149.82*	6.51 ± 1.77*

ns, not significant.
[1]One-way repeated measures analysis of variance was used to compare caloric intake and body weight at different time points. All pairwise multiple comparisons were performed by the Tukey test.
*$P < 0.05$ as compared with baseline values.

Table 3 Body weight and dual-energy X-ray absorptiometry scans of animals fed a high-sugar high-fat diet taken at baseline and at 33 weeks (Mean ± SD)[1]

	Baseline	33-week
Body weight (kg)	5.48 ± 1.09	6.51 ± 1.77*
Total body lean mass (g)	4572 ± 968	5076 ± 1295*
Total body fat mass (g)	289 ± 264	699 ± 696**
Total body tissue fat (%)	5.57 ± 3.81	10.51 ± 8.99**
Region fat (%)	5.33 ± 3.6	10.2 ± 8.84**
Trunk fat mass (g)	174.67 ± 199.83	501 ± 553.75**
Trunk lean mass (kg)	2.48 ± 0.54	2.85 ± 0.67*
Limb fat mass (g)	83.6 ± 54.37	140.73 ± 119.49*
Limb lean mass (kg)	1.64 ± 0.38	1.72 ± 0.549^{ns}

ns, not significant.
[1]The means at 33 weeks were compared with baseline by two-tailed paired *t*-test.
*$P < 0.01$ as compared with baseline values, **$P < 0.05$ as compared with baseline values.

Table 4 Metabolic parameters of animals fed a high-sugar high-fat diet taken at baseline and at 33 weeks (Mean ± SD)[1]

	Baseline	33-week
Total cholesterol (mg/dl)	122.73 ± 35.73	417.60 ± 161.10*
High-density lipoprotein (mg/dl)	56.47 ± 13.44	62.64 ± 23.18^{ns}
Low-density lipoprotein (mg/dl)	57.40 ± 27.46	324.8 ± 192.4*
Triglycerides (mg/dl)	44.40 ± 13.67	77.6 ± 87.93^{ns}
Serum insulin (μU/ml)	22.47 ± 17.36	76.90 ± 55.37*
Fasting glucose (mg/dl)	65.67 ± 9.41	72.8 ± 21.08^{ns}
HOMA-IR	3.34 ± 3.37	14 ± 9.9*

ns, not significant.
[1]The means at 33 weeks were compared with baseline by two-tailed paired *t*-test.
*$P < 0.01$ as compared with baseline values.

line values; however, fasting glucose levels were not significantly different between baseline and 33 weeks (Table 4).

Body weight and metabolic variables of HSHF-fed animals compared with LSLF-fed animals

Total cholesterol and LDL-C were significantly higher in animals fed the HSHF diet as compared with those fed the LSLF diet, while there were no significant differences in HDL-C, triglyceride and fasting glucose (Table 5).

Discussion

Cynomolgus monkeys are native to Southeast Asia, Indonesia, and the Philippines. Fruits and seeds make up a large proportion of their dietary intake, but they are also known to eat birds, lizards, frogs, and fish.

Table 5 Body weights and metabolic parameters of animals fed the low-sugar low-fat (LSLF) diet and those fed the high-sugar high-fat (HSHF) diet for 33 weeks (Mean ± SD)

	LSLF (n = 10)	HSHF (n = 15)
Body weight at baseline[1]	6.06 ± 1.70	5.48 ± 1.77
Body weight at 33 weeks (kg)	6.36 ± 1.64	6.51 ± 1.77
Serum parameters[2]		
Total cholesterol (mg/dl)	111.45 ± 24.09	417.60 ± 161.10*
High-density lipoprotein (mg/dl)	53.18 ± 12.96	62.64 ± 23.18^{ns}
Low-density lipoprotein (mg/dl)	50.09 ± 16.78	347.78 ± 177.00*
Triglyceride (mg/dl)	40.8 ± 12.48	77.6 ± 87.92^{ns}
Fasting glucose (mg/dl)	60.27 ± 13.94	72.8 ± 21.81^{ns}

ns, not significant.
[1]For body weight, the two-way repeated measures ANOVA was used to compare the means of the two diets at baseline and at 33 weeks. Pairwise multiple comparisons were performed with the Holm-Sidak method. Body weight on the HSHF diet, week 33 vs. baseline, $P < 0.001$. Body weight on the LSLF diet, week 33 vs. baseline $P = 0.187$.
[2]For the serum parameters the means of the HSHF diet at 33 weeks were compared with those of the LSLF diet by the student's *t*-test. *$P < 0.05$, HSHF compared with LSLF.

Therefore, this species is best considered an opportunistic omnivore. In this study we have shown that cynomolgus monkeys readily tolerate chronic feeding of a diet high in sugar (simple carbohydrates) and fat (from lard, vegetable oil and coconut oil).

Cynomolgus monkeys develop diabetes naturally with changes in plasma lipids and lipoprotein and pancreatic islet lesions similar to those that occur in human diabetics [22, 37, 38]. Lipid and lipoprotein measures are also affected similarly in both cynomolgus monkeys and humans when fed high-fat high-fructose diets [19, 33, 34].

In this study, we show that a HSHF diet fed chronically to male cynomolgus monkeys induced serum and body composition changes similar to those found in humans with the metabolic syndrome. After 33 weeks of consuming a HSHF diet, the study animals exhibited markedly elevated serum total cholesterol and LDL-cholesterol and insulin resistance, with no significant changes in either HDL-cholesterol or triglyceride levels. Similar results have been reported by others in cynomolgus monkeys as well as other non-human primates. Suzuki et al. [33] fed female cynomolgus monkeys a high-fat high-fructose diet for 28 weeks and at the end of the study found significant increases in serum total cholesterol and LDL-C but no changes in HDL-C and triglyceride. The present study differs from that of Suzuki et al. [33] in that the sex of the

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animals used was different and that body composition was measured with DXA in this study. Higgins et al. [15] fed baboons a HSHF diet for 8 weeks and observed increased body fat and triglyceride concentrations, altered adipokine concentrations, and evidence of altered glucose metabolism. It is important to note that the present study lasted much longer than that of Higgins et al. [15] and used cynomolgus monkeys instead of baboons.

In the HSHF diet the contribution of simple sugars and fat to total caloric intake was 11% and 39%, respectively. These figures are comparable to those in a typical American diet. In general the American diet is low in fiber and the contribution of simple sugar and fat to total caloric intake stand at 7% and 33%, respectively [2, 7]. The LSLF diet is a grain-based meal, while the HSHF diet has a high percentage of simple sugars, mainly from high-fructose corn syrup. The HSHF food was baked and artificial flavors were added to increase palatability. We found that the study animals significantly increased their caloric intake on this diet. Therefore, we conclude that this procedure can be used effectively in future studies with cynomolgus monkeys to investigate the physiologic effects of increased caloric intake. It was not possible to quantify the energy intake of the age-matched monkeys that were left on the LSLF diet throughout the 33-week period, but our initial 2 weeks of monitoring of food intake indicated that the daily caloric intake of the control group was less than that of the intervention group. We could not determine whether the lipid profile and body composition changes observed were because of increased total caloric intake or increased intake of simple sugars. Another confounding variable is the fact that the two diets were of different caloric densities, and therefore, the animals on the HSHF diet received less protein and probably less minerals (as a percentage of calories) compared with the LSLF diet. These questions will require further study.

It is important when interpreting the results of this study to note that there are distinct species differences among non-human primates in their responsiveness to dietary fat, sugar, and cholesterol challenges. Cynomolgus monkeys in particular respond more strongly to dietary perturbations with regard to fat, sugar, and cholesterol challenge than humans and are generally considered to be the most cholesterol-sensitive non-human primate species [20, 27, 32, 35]. Considering the quite pronounced response to dietary challenge shown by cynomolgus monkeys, we are of the view that this species could serve as a relevant model for studying genetic predictors for hyperresponsiveness to dietary fat, sugar, and cholesterol such as polymorphisms in specific enzymes or transporters.

Conclusions

The observations from the present study, together with those from other studies, demonstrate that cynomolgus monkeys can be used successfully to study the impact of dietary factors on metabolic processes and could serve as a useful model to further our understanding of the etiology of the metabolic syndrome and its relationship to overall disease risk. Recently, the cynomolgus monkey has been approved for whole-genome sequencing by the NIH National Human Genome Research Institute, and we anticipate that a great deal of new information concerning the genetics and genomics of this species will be available soon. This will provide important opportunities to investigate complex interactions between diet and genetics in the onset and progression of the metabolic syndrome.

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