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Serum prostate specific antigen changes in cynomolgus monkeys (*Macaca fascicularis*) on a high sugar high fat diet

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Abstract

BACKGROUND—An inverse relationship between serum prostate specific antigen (PSA) levels and body mass index (BMI) has been reported in men but not in any animal model.

METHODS—Serum PSA in a colony of cynomolgus monkeys was assayed and correlated to body weight, prostate weight and age. In addition 15 animals were selected and fed a high sugar high fat (HSHF) diet for 49 weeks to increase their BMI and correlate it to PSA

RESULTS—Serum PSA levels were positively correlated to prostate weight ($r=0.515$, $p=0.025$) and age ($r=0.548$, $p=0.00072$) but was not significantly correlated to body weight ($r=-0.032$, $p=0.419$). For the animals on the HSHF diet, body weight, lean mass, fat mass and BMI were significantly higher at 49 weeks than at baseline ($p<0.01$). PSA was not significantly correlated to body weight and insulin at both baseline and 49 weeks. PSA was negatively correlated to BMI and insulin resistance (HOMA-IR) at 49 weeks but not at baseline. In addition we observed hepatic steatosis and increases in serum liver enzymes.

CONCLUSIONS—Increases in BMI in cynomolgus monkeys as a result of consuming a HSHF diet resulted in PSA changes similar to those in humans with increased BMI. Cynomolgus monkeys are a useful model for investigating the relationship between obesity, diabetes and PSA changes resulting from prostate gland pathology.

Keywords

Diabetes; insulin resistance; BMI

BACKGROUND

Prostate cancer is the most common non-cutaneous cancer in American men. It is estimated that more than 200,000 cases are diagnosed annually resulting in >32,000 deaths [1]. If detected early, prostate cancer can be treated effectively and this has led to intensive searches for biomarkers for screening. The prostate specific antigen (PSA) blood test is

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COMPETING INTERESTS

The authors declare that they have no competing interests.

widely used for screening, diagnosing and monitoring of prostate cancer, although it is also elevated in other disorders. The PSA test has clinical limitations as a screen for prostate cancer due to its low sensitivity and specificity. A number of factors have been shown to affect serum PSA levels. Obesity has been shown to create uncertainty in the interpretation of the PSA test in men and studies have reported an inverse relationship between serum PSA and body mass index BMI [2–5]. The prevalence of obesity in United States remains high; national survey data for 2007–2008 show that 33.8% of adults are obese [6]. In order to make better use of PSA as a diagnostic tool, we must develop a better understanding of the non pathological causes of individual variation in PSA levels. Most of the epidemiology and experimental data on PSA have been obtained from human subjects. However, serum PSA data obtained in humans is confounded by uncontrollable environmental factors like diet, indolent prostate cancer and medications (both prescribed or over the counter) making it difficult to disentangle the environmental effects on PSA levels from those of prostate cancer. It is therefore important that the PSA findings in humans are validated in an animal model whose environmental conditions can be controlled. The PSA gene is only found in primates and our preliminary studies have shown that, among the common nonhuman primate species used in biomedical research, macaque species are the best models in which to study PSA biology and also prostate hyperplasia has been reported in both cynomolgus and rhesus macaques [7].

The main aim of this study was to investigate the effect of body mass index (BMI) on serum PSA levels in cynomolgus monkeys. This was achieved by fattening cynomolgus monkeys with a high sugar high fat (HSHF) diet combined with a sweetened drink to achieve high caloric intake.

METHODS

Animals

To obtain baseline data on serum PSA levels, we used archived serum from 50 adult cynomolgus monkeys (> 4years) of different ages. For correlation between serum PSA levels and prostate weight we used 54 prostate tissues that were collected opportunistically from animals that were presented for necropsy. For the investigation of the effect of BMI on serum PSA levels, we challenged 15 male cynomolgus monkeys (*Macaca fascicularis*) 5–7 years of age with a high sugar high fat diet for 49 weeks. This subset of animals was from the colony at the Southwest National Primate Research Center (SNPRC), Texas Biomedical Research Institute (Texas Biomed). All animals were sexually mature as determined by body weight, testis size and age [8]. All animals were housed individually in a temperature- and humidity-controlled environment with a 12-hour light to dark cycle to maintain normal circadian rhythms.

Diet

The high sugar high fat (HSHF) diet corresponded to a typical human fast-food diet that is high in saturated fat and simple carbohydrates. The diet was prepared using 73% Purina Monkey Chow 5038, 7% lard, 4% vegetable oil (Crisco® Orrville, OH), 4% coconut oil, 10.5% high fructose corn syrup, 1.5% water. This diet was originally developed to induce obesity and related metabolic dysregulation in the baboon (*Papio hamadryas*) [9]. The nutrient composition of the baseline and the HSHF diet is shown in Table I. The food was flavored with artificial fruit flavors (Kool Aid®) and baked to form palatable pellets. During the last 16 weeks the animals were also provided a sweetened drink *ad libitum* made of water, high fructose corn syrup and Kool-Aid® (440 kcal/liter) to increase caloric intake. The amounts of solid food and sweet drink consumed were recorded daily.

Body weight and DXA measurements in animals fed the HSHF diet

Body weight and dual energy X-ray absorptiometry (DXA) scans were recorded at baseline and after 49 weeks of dietary challenge. For the DXA scans, after a 12-hour overnight fast, animals were sedated with ketamine and anesthesia maintained with isoflurane during the whole scanning procedure. Dual energy X-ray absorptiometry (DXA) body composition scans were undertaken using a Lunar Prodigy densitometer (GE Healthcare, Madison, WI). 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, Madison, WI). Body mass index (BMI) was calculated as mass (kg) divided by height (m) squared.

Serum prostate-specific antigen and other metabolic assays

For large scale colony screening serum PSA was measured with the Access[®] 2 Immunoassay Systems (Beckman Coulter). This assay system has been successfully used to assay serum PSA in cynomolgus monkeys [10–11]. For the animals fed the HSHF diet serum PSA was done with the Active PSA ELISA kit (Diagnostic Systems Laboratories, Inc. Webster, TX). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured on a Unicel Dx C 600 analyzer (Beckman Coulter, Inc., Fullerton, CA). Insulin was assayed using a human insulin ELISA kit (Millipore, Billerica, MA). The homeostasis assessment model HOMA-IR = [insulin (μU/ml) x glucose (mmol/L)]/22.5 was used to determine insulin resistance. This model has been used before in cynomolgus monkeys [12–13].

Necropsy

Animals on the challenge diet were euthanized after 49 weeks and complete necropsies were performed. Samples of liver, pancreas, colon, prostate, and testis were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined by two veterinary pathologists (EJD and MO).

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

Statistical analysis

The means at 49 weeks were compared to each animal's mean at baseline using two-tailed paired samples *t*-test. Correlations between serum PSA and various variables were done using Pearson's correlation analyses (SPSS version 16.0). A *p*-value < 0.05 was considered significant.

RESULTS

Relationship of serum PSA with body weight, prostate weight and age in the colony

Random sampling of serum from animals of different ages and also using data from prostates that were collected opportunistically at necropsy we show that serum PSA was positively correlated to prostate weight ($r=0.515$, $p=0.025$) and age ($r=0.548$, $p=0.00072$), but was not correlated with body weight ($r=0.032$; $p<0.419$, Figure 1).

Diet consumption for animals fed the HSHF diet

None of the animals displayed adverse clinical signs during the experimental period. By week 49, animals were on average consuming 900 kilocalories per day and increase of 98% over baseline calorie intake.

Body weight, BMI and DXA measurements on animals fed a HSHF diet

Of the 15 animals, 11 gained weight, three animals lost weight and one maintained its original weight during the feeding period. Body weight, lean mass, and fat mass and BMI were significantly higher at 49 weeks than at baseline ($p < 0.01$, Table II). The changes in body weight were mainly due to increases in fat mass as fat mass increased by 260% while lean mass only increased by 16% (Table II).

Serum PSA, insulin and insulin sensitivity (HOMA-IR) from animals fed a HSHF diet

Average serum PSA concentration was not significantly different at 49 weeks as compared to baseline (Table II, $p = 0.063$). Fasting glucose levels were not significantly different at 49 weeks compared to baseline ($p = 0.14$). Figure 2 summarizes the relationship between body weight change and change in serum PSA at the end of the feeding period, however this relationship did not reach statistical significance ($r = 0.3063$, $p = 0.133$).

Serum PSA was negatively correlated to BMI at 49 weeks but not at baseline ($r = 0.45$, $p = 0.047$; Figure 3). A similar trend was observed for body weight although the correlation did not reach statistical significance ($r = 0.358$, $p = 0.095$).

Serum PSA was negatively correlated to insulin resistance (HOMA-IR) at 49 weeks but not at baseline ($r = 0.48$, $p = 0.04$). A similar trend was observed for fasting insulin levels although the correlation did not reach statistical significance ($r = 0.439$, $p = 0.058$; Figure 4).

Pathology and serum liver enzymes of animals fed the HSHF diet

Animals euthanized after 49 weeks on the challenge diet did not show any gross or microscopic lesions in the prostate, testis and colon. Gross and histopathologic findings were mainly confined to the liver (Figure 5). The livers of 4 animals were enlarged, yellow-tan, and friable. The most common microscopic finding was hepatic steatosis, which was present in 10 animals. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly higher at 49 weeks than at baseline (Table II).

DISCUSSION

Obesity is a growing global epidemic; in the United States 68% of adults are overweight or obese [6]. Obese men have lower PSA levels than non obese men [3,14]. The causes of this inverse relationship between obesity and PSA are not fully understood but some studies have attributed it to decreased testosterone concentrations [15] or plasma hemodilution [3,16]. Another possible but less studied factor that might affect PSA is insulin resistance and diabetes. Diabetes has been reported to be associated with decreased serum PSA levels and two studies have reported that diabetic humans have 10–20% lower PSA than non diabetics [17–19]. Presently there is an urgent need to understand the link between PSA and obesity/diabetes. Lack of a good animal model that can be used to study how metabolic conditions affect PSA has contributed to the slow progress in this area. In this study we report on the preliminary results on the use of the cynomolgus monkey as an animal model that can be used to study the effect of obesity and related comorbidities on serum PSA. Cynomolgus monkeys are native to Southeast Asia; although fruits and seeds make up a large proportion of their dietary intake, they are also known to eat birds, lizards, frogs, and fish. Therefore, this species is best considered an opportunistic omnivore. Cynomolgus monkeys also develop diabetes naturally with changes in plasma lipids and lipoprotein and pancreatic islet lesions similar to those that occur in human diabetics [20–22].

Using cynomolgus monkeys as animal models we have shown that serum PSA is correlated with prostate weight. Similar results have been reported in men and it is now widely accepted that serum PSA has a good predictive value for assessing prostate volume [23–24].

The present cross-sectional studies of PSA with age indicate that PSA concentration increases with advancing age ($r=0.548$; $p=0.000072$). These results are similar to data from human subjects. Osterling et al. [25] reported that PSA is directly correlated with patient age ($r=0.43$; $p<0.0001$). The similarity of the amount of PSA in the serum of cynomolgus monkeys to humans has been reported before by several others. Neal et al. [9] reported that baseline PSA of the experimental animals as well as control animals ranged from 1.18–4.16 ng/ml. Williams et al. [26] reported average blood PSA levels of 0.86ng/ml in cynomolgus monkeys.

In this study we have shown that cynomolgus monkeys increase in BMI as a result of a dietary change and that change results in serum PSA changes similar to those in obese humans. The association between insulin and serum PSA did not reach a significant level ($p=0.058$); however, we observed significant negative association with HOMA-IR (a measure for insulin resistance).

The finding of hepatic steatosis and increases in the liver enzymes AST and ALT suggest that the HSHF diet perturbs a variety of metabolic processes in the mammalian body and deserves further investigation.

The principal limitation of our feeding study is the small number of animals used, the short duration of the feeding, the relatively young age and that some of the animals did not increase their BMI. The animals used in this study were of a relatively young age (5–7 years) considering that the lifespan of cynomolgus monkeys is ~ 30 years. However, the conclusions are still valid as prostatic diseases take time to develop and are a result of instructions given to cells early in life.

We did not measure plasma volume in the animals used in this study and therefore we cannot discuss if the PSA changes seen were due to hemodilution as has been reported in humans. However the significant correlation of PSA with HOMA-IR highlights the need to investigate the role of insulin signaling pathways in regulation of PSA in obese/diabetic subjects.

In summary this preliminary study has shown that cynomolgus monkeys can be used as an animal model to investigate the link between obesity and diabetes on one hand and PSA changes resulting from prostate gland pathology on the other. The potential impact of diabetes on PSA warrants further investigation in future studies to understand whether there is any potential correlation between diabetes and prostate cancer.

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Figure 1.
Relationship between serum PSA, body weight, prostate weight and age in the cynomolgus monkey colony.

Figure 2. Relationship between change in PSA and change in body weight for animals fed a high sugar high fat diet for 49 weeks.

Figure 3. Relationship of PSA and body weight and body mass index at baseline and at 49 weeks after feeding a high sugar high fat diet.

Figure 4.

Relationship between serum PSA, insulin and insulin resistance in animals fed a high sugar high fat diet at baseline and at 49 weeks.

Figure 5.
Hepatic steatosis. Note lipid vacuoles hepatocytes (H-E staining).

Table 1

Composition of HSHF diet compared to normal low sugar low fat monkey diet

	Energy density (kcal/g)	kcal as fat (%)	kcal as carbohydrate (%)	kcal as simple sugar (%)	kcal as protein (%)
Low sugar low fat baseline diet ¹	3.26	13.81	67.16	2.95	19.03
High sugar high fat ²	4.33	38.46	51.00	10.72	10.53

¹ From chemical analysis of micronutrient composition provided by manufacturer

² From chemical analysis of nutrient composition from Covance Laboratories (Madison, WI)

Table II

DEXA, serum profile measurements and other physiological parameters (mean±SD) in cynomolgus monkeys fed a high sugar high fat diet

	Baseline	49 weeks	<i>p</i> -Value 0–49 weeks
Body weight (kg)	5.48±1.09	7.09±2.20	0.001
Lean mass (g)	4572±968	5309±1363	0.002
Fat mass (g)	289±264	1059±1002	0.002
BMI (kg/m ²)	11.13±1.85	13.53±4.02	0.0035
PSA (ng/ml)	0.818±0.40	1.07±0.43	0.063
Serum Insulin µU/ml	22.47±17.3	70.98±88.7	0.038
	6	8	
Fasting Glucose mg/dl	65.67±9.41	74.8±23.18	0.14
Insulin resistance (HOMA-IR)	3.80±3.59	12.87±16.2	0.034
		7	
Serum aspartate aminotransferase U/L	39.8±8.31	48.8±11.40	0.021
Serum alanine aminotransaminase U/L	20.13±6.2	31±11.42	0.0013