

## The Effects of a Low-Starch Diet on Behavior and Insulin Resistance Syndrome in Several Primate Species

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In humans, type II diabetes is often preceded by a state of insulin resistance (Martin et al., 1992). Insulin resistance is a condition in which the body produces insulin but tissues, specifically muscle, fat and liver cells, do not appropriately respond to the presence of insulin with the uptake and utilization of glucose. In humans, insulin resistance syndrome (also known as metabolic syndrome) is defined in a patient as having three of the following criteria: increased waist circumference, increased serum triglycerides, reduced HDL, increased blood pressure, and increased fasting blood glucose (Grundy et al., 2004). The World Health Organization adds increased serum insulin levels as a criterion to this syndrome (Beilby, 2004).

In humans and laboratory rats, the presence of insulin resistance is linked to high glycemic index foods or foods that contain high levels of dietary starch or simple sugars (Holt et al., 1997; Wiseman et al., 1996). Many diet items we feed in zoos such as root vegetables and commercial diet can contain 30-70% starch (Less, 2012). For this reason, we propose switching several primate species to a low-starch commercial diet, Mazuri L/S Primate Diet ®. This diet will be implemented on several select primate species including orangutans, Hamadryas baboons, red ruffed lemurs and ring tailed lemurs.

**Hypothesis:** Some zoo primates may have diet-induced insulin resistance syndrome.

**Specific aims:** To examine the effect of a low-starch diet on serum parameters associated with insulin resistance syndrome.

### **Methods and procedures:**

A serum sample will be obtained for each primate prior to and after the implementation of a low-starch diet. Anesthetic used should be the same for the before and after exams (and if possible, we ask that zoos avoid the use of medetomidine which can lower insulin levels). A commercially available enzyme-linked immunosorbent assay (ELISA) kit will be used to analyze serum insulin (Mercodia, 10-1132-01). This assay was previously validated for use with the above species in the CMZ Endocrinology Laboratory. Serum glucose, cholesterol, and triglycerides will be measured using an IDEXX vettest autoanalyzer.

For orangutans, we are specifically contacting institutions that are able to collect awake blood samples. For this species, we request 2 serum samples prior to the diet change and one monthly sample per individual for a year following the diet change.

All nutrition analyses will be calculated using Zootrition ® software. The PI has modified this database to include simple carbohydrates from USDA's nutrient database. The diet change will involve exchanging the current commercial pellet for the Mazuri L/S pellet (as well as reducing root vegetables and fruit) in such a way as to keep the total kilocalories of the overall diet constant and aim for a diet that is < 9% starch and in most

cases, < 10% sugar. (See the attached report of what the diet may look like for select species). Diets will be constructed and analyzed for nutritional balance by the PI or can be constructed/analyzed by the participating zoo's nutritionist (we ask that the nutritionist communicate with the PI during this process). All diets constructed by the PI will need to be approved by the chief veterinarian, relevant curator, relevant associate curator and keepers. The diet transition will occur in agreement with standard operating procedures for diet transitions at the zoo (typically transitioned over a 9 day period, with 25% of new diet and 75% of original diet offered over the first three days, then three days of 50% each of both diets, then the final three days 75% new diet and 25% original diet until diet is completely switched over on the tenth day). Rough consumption of the current diet and the low starch diet will be assessed by keeper staff (the PI can provide forms for assessment if the institution does not regularly do this). Additionally, keepers will be asked to report daily fecal consistency prior to and after implementation of the diet change. Regular (weekly or bi-weekly) weights will need to be obtained and recorded prior to, during and after implementation of the new diet. Any increases or decreases in body weight will be discussed with the curators and veterinarians to determine whether such weight change is beneficial for the animal or needs to be addressed by a change in husbandry or a health exam. Any additional concerns identified by keeper staff relating to this diet change will be discussed and dealt with by all relevant keeper, curator and veterinarian staff.

A paired t-test (or Wilcoxon signed rank test depending on data normality) will be used to compare fasting concentrations of all biomarkers before and after the diet change. Significance for all tests will be set at  $p < 0.05$ .

**Timeline:** The pre- and post-diet change serum samples will be collected at routine veterinary exams (except see orangutans above). Primates will not begin the new diet until they have had an exam where a pre-diet change serum sample will be taken (or if they have a recent banked serum from the past 6 months). The post-diet change serum sample will be obtained at the next routine exam after the new diet is in place. No diet change will occur until all animals can be weighed on a regular basis.

**Proposed schedule:**

Spring/summer 2012 health exams of all animals; bank baseline serum samples for study

Diet transition (following health exams)

Spring/summer 2013 health exams of all animals, collect follow-up serum samples for study

Summer 2013 – analysis of serum samples for study

**Rough budget:** Mazuri is willing to provide Mazuri L/S biscuits for a year free of charge to institutions participating in this diet change.

**Anticipated assistance required by institution staff members:**

Vet staff: Collect serum from identified individuals before and after implementation of a diet change

Nutritionist (if available, if not, PI can serve this function): Work with PI to switch commercial diet and balance all other nutrients

Keepers: Obtain weekly or bi-weekly weights on individuals. Fill out consumption reports. Monitor daily fecal consistency and report any concerns.

**Expected outcomes:** We expect to see a decrease in serum insulin, glucose, cholesterol and triglycerides after this diet change.

**Significance:** If suspected insulin resistance syndrome can be improved by modifying diet, zoo primates may be less susceptible to the development of diseases such as type 2 diabetes.

**Additional biomaterials information:**

Samples will be collected before and after the diet change from all individuals from the following species:

Ring-tailed lemur (*Lemur catta*)

Red ruffed lemur (*Varecia rubra*)

Orangutan (*Pongo pygmaeus* or *Pongo abelii* or hybrid)

**Storage/Shipment:**

The same anesthetic should be used at both times of serum collection (we prefer if medetomidine is not used as it lowers insulin levels). If samples will be sent right after collection, we ask that they be shipped with several ice packs. If samples cannot be shipped right away, we ask that they be frozen in a -80° C freezer (or below). Frozen samples should be shipped preferably on dry ice, but many ice packs will work as well. Samples will be shipped via FedEx overnight and the PI can provide the FedEx number when the institution is ready to ship the samples. We ask that the anesthetic used at the time of serum collection be included with the shipment.

**Sample information:**

Samples are requested regardless of sex or age. Samples will be discarded after use for the biomarkers listed. At least 3 samples (and a maximum of 6) per individual are requested.

**Checklist of information needed for study:**

- 3-6 serum samples per individual, each at least 2 ml
- Medical records pertaining to the time period of the samples
- Weights corresponding to the serum samples (if not available in the records)
- Diets corresponding to time period of the serum samples

## References

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Example Low-Starch Diets

**Ring-tailed lemur (per individual):**

Low starch	Grams
Mazuri L/S	25
Endive	110
Squash, summer	10
Green beans	15
Broccoli	25

**Orangutan (per individual):**

< 15% sugar

Per Individual	Grams
Mazuri Primate L/S	300
Oranges	30
Apples	30
Grapes	40
Bananas	45
Carrots	120
Celery	281.25
Yam	75
Broccoli	250
Endive	500
Romaine	500

< 12% sugar

Per Individual	Grams
Mazuri Primate L/S	300
Oranges	30
Apples	30
Grapes	20
Bananas	30
Carrots	60

Celery	187.5
Yam	75
Broccoli	450
Endive	750
Romaine	350

< 10% sugar

Per Individual	Grams
Mazuri Primate L/S	300
Oranges	30
Apples	15
Grapes	15
Carrots	60
Celery	93
Yam	75
Broccoli	125
Romaine	500
Alfalfa hay	300

**Hamadryas baboon (per individual):**

Per individual	Grams
Mazuri L/S	350
Carrots	40
Broccoli	40
Green beans	40
Endive	180