ADIPOSITY IN ZOO GORILLAS (*GORILLA GORILLA GORILLA*): THE EFFECTS
OF DIET AND BEHAVIOR

by

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Dedicated to

Bryan Less

Bud, Eileen and Jon Hoellein

Bebac and Mokolo

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Adiposity in Zoo Gorillas (*Gorilla gorilla gorilla*): The Effects of Diet and Behavior

Abstract

by

ELENA HOELLEIN LESS

All animals have adaptations for manipulating and digesting the food available in their natural habitats. In captive environments, however, it can be difficult to provide diets that are both nutritionally and functionally analogous to natural diets. In the wild, western lowland gorillas travel long distances while foraging and consume a diet composed primarily of large quantities of green plant material. This diet is high in fiber and low in caloric density. In contrast, gorillas in North American zoos consume a diet that contains a high percentage of commercially prepared biscuits and domesticated fruit. This biscuit-based diet is low in volume, and fiber, and is calorically dense, substantially reducing the need for gorillas to spend large amounts of time finding and processing food. Typical captive gorilla diets, along with low levels of activity, may lead to obesity, other medical problems and the performance of undesirable behaviors. This study presents the results of two lines of research related to these issues. First, adiposity in gorillas was explored by creating a primate mass index (PMI) and analyzing how diet and activity might contribute to increased adiposity. Second, the impact of a low-starch, high-fiber, high-volume diet which does not contain biscuits was investigated on a smaller subset of gorillas to examine both behavioral and health effects. Results

suggested that both weight and a new index, the primate mass index (PMI) were useful measures of obesity. Results of the biscuit-free diet change revealed a reduction, and in some cases elimination, of regurgitation and reingestion behavior (R/R) and a significant reduction of hair-plucking behavior. An increase in feeding/foraging was observed, although overall gorilla activity levels were not consistently increased with this diet change. Health analyses from serum collected before and after the diet change indicated that this diet reduced insulin and cholesterol, but increased triglycerides at one institution and decreased triglycerides at another institution. Future research will identify if there is a link between obesity and the development of heart disease in zoo gorillas.

Chapter 1: Introduction: Adiposity in primates

'Adiposity' refers to body composition or, more specifically, the amount of fat an organism possesses (Frankenfield et al., 2001). Importantly, fat provides a means for energy storage. The amount of fat an organism possesses fluctuates throughout its lifetime, increasing early in life, during pregnancy and lactation and with aging (Zafon, 2007). However, at any point in an organism's life, it can possess too much fat or be obese. 'Obesity' is defined as having an excess amount of adipose tissue (i.e., body fat) in relation to lean body mass (Richardson et al., 2000). Obesity is considered to be more severe than merely being 'overweight' (i.e., an increase in body weight relative to height) (Lopaschuk et al., 2007). In fact, in humans, it is possible to be of normal weight, but to still exhibit signs of metabolic obesity if adiposity is high or concentrated in the abdominal area (Ruderman et al., 1998).

Several factors contribute to an increased amount of fat in all organisms. Two of the most commonly studied are diet and activity levels. It is well-known that consuming more calories than are being burned in a given day leads to weight gain. Diets that are high in saturated fat, simple sugars, and starch and lower in fiber are typically also associated with being calorically dense and can cause a rapid accumulation of fat tissue (Astrup, 2008). Additionally, when an individual is inactive, the number of calories burned per day is limited, making it easy to over-consume. Therefore, a combination of these two factors (a calorically dense diet and inactivity) can readily lead to obesity (Stein and Colditz, 2004).

In humans, obesity is associated with inflammation and insulin resistance, which are in turn risk factors for the development of cardiac disease, hypertension and type II diabetes, among other health complications (Stein and Colditz, 2004). Therefore, there is

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a need to reduce obesity to help prevent the development of these types of diseases. Not

surprisingly, there is a focus on, among other areas, diet and activity. In terms of diet,

health experts encourage the consumption of diets which are high in fiber and low in fat

in humans (Astrup, 2008). In terms of activity, it is currently recommended that adults

spend 20-60 minutes a day, 3 to 5 days a week engaged in exercise at a level of 40-85%

of their heart rate to promote weight loss, improve cardiovascular risk factors and

decrease mortality (Bensimhon et al., 2006). Determining whether obesity is an issue for

captive gorillas is an important first step in understanding and improving their health.

The evolution of obesity in humans and the consequences for zoo-housed primates

Wells (2006) discussed the development of obesity in humans in the context of

both proximate and ultimate causes. Specifically, it is important to consider the

evolutionary history and fitness benefit of fat deposition before attempting to understand

the proximate causes. Wells hypothesized that periods of food scarcity during the course

of human evolution, often changing along with seasonality, led to a need to store energy

more efficiently and to quickly allocate energy to competing tissues (Wells, 2006). This

idea has become known as the "thrifty genotype" hypothesis or the idea that natural

selection favored a gene which codes for physiological adaptations to promote energy

efficiency (Bellisari, 2008; Neel, 1962). Neel (1962) hypothesized that these

physiological adaptations center around the ability of the pancreas to more efficiently

lower blood glucose levels after a feeding session. This lowered glucose would more

rapidly trigger the next feeding session which would lead to more food consumed over

the course of an entire feeding event. This extra food intake would be converted to fat

and would over time lead to an increase in fat deposition which could be utilized in future

times of food scarcity. In other words, individuals with this thrifty genotype would have

an advantage because they would accumulate fat stores more proficiently during times of

food abundance to prepare them for periods of famine (Neel, 1962). Other benefits to the

accumulation of fat stores included: (1) buffering from malnutrition; a higher fat store

can keep nutrition constant during periods of energy fluctuation, (2) regulating

reproduction; women cannot conceive unless they have a specific amount of fat and

higher fat stores allowed women to conceive even during periods of famine, (3) assisting

with offspring growth; the highest need for fat stores is during brain development in the

infant and increased fat stores in the mother allowed for maximum brain development in

the infant even during times of famine, (4) improved immune function; an immune

response requires large amounts of energy, higher fat stores sustain immune function

during famine and (5) sexual selection; women with higher fat content were more readily

able to conceive regardless of seasonal energy fluctuations which means that men may

have begun to select females with more adipose tissue as mates to preserve their own

fitness—thus allowing this thrifty gene to be evolutionarily selected (Wells, 2006).

However, with the advent of industrialization, food became more abundant and easier to

obtain, coupled with an increasingly sedentary lifestyle. Currently, the thrifty genotype

adaptation may contribute to the current obesity epidemic (Bellisari, 2008).

Animals living near humans are also showing trends of increasing obesity

(Klimentidis et al., 2010). It is unclear at this point why this relationship exists. One

idea is that just as easily as humans can access food for themselves they can provide

energy dense foods to animals without them having to engage in the prolonged bouts of

energy-expending foraging behavior that may be associated with feeding. This sets the

stage for animals to deposit excess adipose tissue and perhaps become obese. In particular, captive zoo environments share many qualities of western culture and can affect animals' adiposity. Specifically, captive animals such as primates in many cases are not as active as their wild counterparts and may ingest the same amount or a greater number of calories per day.

Comparison of wild vs. zoo primate feeding practices

In nature, primates have evolved in areas with a diverse array of available food and as a result of this, may exhibit a wide variety of feeding styles and have evolved varying gut morphologies. As Richard (1985) discusses, the order Primates comprises animals that feed on a variety of things including, insects, plant gums, fruit, seeds, leaves, herbs and combinations of all of these. The method by which a primate chooses to forage is referred to as 'optimal foraging theory' (Richard, 1985). This theory describes how natural selection and the environment shape the evolution of feeding behavior and then subsequently morphology (and some interplay between these two), but also predicts adaptive learning. An extension of this theory is the 'optimal diet theory'. This theory considers the energy content of the food and the time it takes to acquire a food item, assigning a value to specific food items (Richard, 1985). Primates have evolved some flexibility within this framework to switch between food groups during times of food scarcity or specific weather/seasonal changes to the extent that any physiological specialization will allow. As an example, gorillas take most of their diet from plants and fruits and have developed an extensive colon to process this low quality, high fiber diet (Lambert, 1998; Milton, 1999). However, within this diet strategy, they can switch to more frugivorous vs. folivorous diets seasonally depending on availability (Remis, 1997)

and some species have specialized to be more folivorous in general based on habitat elevation and decreased availability of fruit [e.g. mountain gorillas] (Harcourt and Stewart, 2007).

Beyond food consumption, foraging for food is a key aspect of primate life and varies according to the type of food consumed. Since the cultivated vegetables and fruits grown in developed cultures vary significantly from what animals consume in the wild (Milton, 1999), it has been difficult for zoos to mimic the type of plans and fruits consumed by wild primate species. It is also difficult to create an environment where primates must forage to the extent that they do in the wild.

Historically, zoos tended to vary significantly in the food provided to captive animals and often the type of diets offered were ultimately inadequate (Ratcliffe, 1963). For this reason, Ratcliffe (1963) formulated a series of "cakes" that could be fed to many zoo animals, including the great apes, which he felt would ensure proper nutrition and allow for some consistency in diet among institutions. Animals fed these diets exhibited improved nutrition. Some agreed that these "cakes" could serve as a nutritional supplement to a diet of fruits and vegetables, but disagreed that they should be fed as a main constituent in the diet (Cousins, 1976). Cousins (1976) also argued specifically for gorillas that fruit should only be fed as a supplementary food source because wild gorilla diets are focused mainly on folivorous plants (but see more recent articles supporting frugivory as common in wild western lowland gorillas such as Remis, 1997). There are currently several brands of commercial primate biscuits that serve as one component or in some cases as the staple food item of primate diets which also include fruits and vegetables. These biscuits are an improved form of these original "cakes" and do help to

serve as a multi-vitamin and source of fiber for primates. One characteristic of these biscuits is that they are high in carbohydrates in the forms of starch and sugar (with some containing as much as 30% starch) and are also high in calories. A diet high in simple carbohydrates is associated with significant health concerns (Kopp, 2006), which may especially be the case for gorillas that eat low amounts of sugar and starch in the wild. Furthermore, because primates can quickly consume most of their day's calories by ingesting primate biscuits, feeding these types of biscuits eliminates a key element of primate feeding ecology—the necessity to forage for long periods of time. Additionally, similar to the Western human diet, the diets of many captive primates not only consist of energy dense foods and high amounts of carbohydrates, they are also high in fat (zoo gorilla diets: 6.6% fat v. wild gorilla diets: 0.5% fat) and low in dietary fiber (Popovich and Dierenfeld, 1997). Such diets may be significantly different from natural diets for primate species that evolved to consume high fiber, low carbohydrate and fat diets, as is the case for western lowland gorillas (Popovich et al., 1997).

Are zoo gorillas obese?

The western and eastern species of gorillas vary in their degree of frugivory with the western species consuming the highest number of fruit species in the diet (65-120 species) and the eastern subspecies mountain gorilla consuming the least (0-35 species) (Harcourt and Stewart, 2007). One of the reasons for this variation is the altitude-related differences in their environment. Diversity of edible fruits decreases with increasing altitude, thus the mountain gorillas do not have access to the variety of fruit species available to western gorillas and depend on a more folivorous diet (Harcourt and Stewart, 2007). Even for western lowland gorillas (WLGs), the only subspecies currently

maintained in North American zoos, diet is influenced by seasonal fruit availability
(Remis, 1997). During the non-fruiting season, a large component of gorilla diet consists
of leaves, stems and pith (Popovich et al., 1997). Because these foods are not calorically
dense, wild gorillas must eat a bulky diet (large amounts of high fiber food) to gain
proper nutrition. The fiber content of the wild gorilla diet fluctuates throughout the year
and ranges from 52-96.5% (Popovich et al., 1997). Gorillas have evolved an extensive
hindgut fermentation system to exploit this type of diet (Stevens and Hume, 1995).

In North America, there are ~350 gorillas maintained across 52 zoos (Lukas et al.,
2010b). In contrast to their diet in the wild and the associated physiological adaptations,
zoo gorilla diets provide on average only 14% dietary fiber (Ogden and Wharton, 1997).
The National Research Council recommends 10-15% of the diet as fiber for all primates,
but this is much lower than what gorillas eat in the wild. Recommended zoo gorilla diets
also contain significant amounts of commercial primate biscuits (15% of the diet) and
fruit (7%) (Schmidt, in press). The domesticated fruit consumed by zoo gorillas contains
considerably more sugar than the fibrous fruit available in African forests (Milton, 1999;
Popovich et al., 1997). Available evidence suggests that diets containing high levels of
rapidly-digested starch do not encourage good gastrointestinal health (as measured by
gut flora biodiversity or intestinal epithelial cell cytotoxicity in pigs) (Martinez-Puig et
al., 2007).

Currently, heart disease is the leading cause of death in captive male gorillas
(Meehan and Lowenstine, 1994) which is a disease associated with obesity in humans. It
is unknown what precursors and risk factors are associated with the development of heart
disease in male gorillas. It is known that the observed heart disease is fibrosing

cardiomyopathy, or hardening of the heart muscle fibers, as opposed to atherosclerosis, or clogging of the arteries, which is the most common form of heart disease in humans (Varki et al., 2009). Despite the difference in types of heart disease observed, the first step being taken by researchers is to investigate risk factors of heart disease in humans as potential risk factors for the development of heart disease in gorillas. Obesity is one risk factor for the development of heart disease in humans (Stein and Colditz, 2004), but has not been defined or studied in gorillas. Results of the research presented here can be used in the future by researchers to examine if obesity is indeed a risk factor for the development of heart disease in gorillas.

The effect of gorilla diet on activity and undesirable behaviors

Wild gorillas spend a significant amount of time eating and manipulating food. To date, three studies reported activity budgets for wild western lowland gorillas (Magliocca and Gautier-Hion, 2002; Masi et al., 2009; Remis, 1994). Although these studies primarily utilize data collected at forest clearings (and thus only a portion of gorillas' overall time budget) they suggest that time spent resting ranges from 0-28% and time spent locomoting ranges from 11.7-16.5%. The majority of time (54.5-72%) is spent feeding and foraging. Although the proportion of time spent feeding may be inflated by the fact that gorillas come to these clearings to feed, additional data suggests that gorillas are active at other times. Western lowland gorillas in the wild need to travel each day in order to obtain a sufficient quantity of food (Remis, 1997), and while foraging, they walk more than 1 km per day (Bellisari, 2008). Wild gorillas also spend time climbing when feeding and when building night nests (Remis, 1997). Climbing is classified as vigorous activity in macaques (Major, 1998). In captivity, gorillas do not

need to forage over long distances or climb to obtain food. This likely contributes to greater amounts of time spent inactive (32.8-75.8%) and lower amounts of time feeding/foraging (9.3-35.8%) (Lukas et al., in prep).

Nonhuman primates occasionally exhibit behaviors thought to occur only in captivity and are considered undesirable. In gorillas, an undesirable behavior has been characterized as a behavior that occupies a disproportionate amount of time in the zoo environment as compared to the activity budgets of wild populations (Lukas, 1999a). These can include behaviors that are related to feeding such as regurgitation and reingestion (R/R) (Gould and Bres, 1986; Lukas, 1999a) and coprophagy (Akers and Schildkraut, 1985; Gould and Bres, 1986). Other self-directed behaviors, including hair-plucking and self-injurious behaviors, were suggested to be related to stress (Lutz et al., 2003), serve a stimulatory function (Christenson and Mansueto, 1999) or are socially transmitted during development (Less et al., in press). The causes of these behaviors are difficult to determine and, once established, the behaviors may be difficult to extinguish. Rumination behavior is observed in humans (Johnston, 1993) and is similar to R/R in gorillas (Lukas, 1999a). The behavior can be reduced by behavioral interventions such as providing aversive stimuli when the behavior is being performed (Sanders-Dewey and Larson, 2006) or combining several techniques such as rewarding other behaviors, interrupting the behavior, and exercise (Wrigley et al., 2010). Also successful, and in some cases more so than behavior modifications, dietary modifications such as feeding the individual to satiation (Johnston, 1993) or feeding them to satiation with starchy items in particular has reduced this behavior almost to zero (Dudley et al., 2002). Other dietary manipulations such as increasing caloric intake, changing food consistency and

increasing food quantity have had mixed results on regurgitation behavior in humans (Dudley et al., 2002). The contrast between the low-volume, calorically-dense diets that captive gorillas typically receive and the high-volume, low-calorie diets consumed by their wild counterparts suggests that diet composition may have a role in this behavior. Though quantitative data are limited, available research suggests that increasing both opportunities for foraging and fiber content and volume of the diet greatly reduces regurgitation and reingestion (Gould and Bres, 1986; Hill, 2004; Ruempler, 1992) and hair-plucking behavior in gorillas (Hill, 2004).

The specific components of gorilla diets can also have a significant impact on rates of undesirable behaviors and on feeding activity. The removal of milk from gorilla diets greatly reduced regurgitation and reingestion (R/R) behaviors (Lukas, 1999b). Another diet manipulation has been to increase fiber in gorilla diets. Cleveland Metroparks Zoo added a fiber supplement to the gorilla diets and found that gorillas had improved stool consistency, but that the increased fiber had no real measurable effect on the gorillas' behavior and, in fact, even slightly increased the rate of R/R (Lukas et al., 2010a). Other researchers increased fiber by providing larger amounts of plant material and reducing fruit (but still feeding commercial biscuits) and found that gorillas spent more time exhibiting natural foraging behavior, spent less time inactive, and showed improved stool consistency (Savini et al., 2000). Researchers at a German zoo conducted a diet change consisting of larger amounts of browse (or twigs, shoots and leaves) and reducing simple carbohydrates, milk and meat products, but did not report if commercial biscuits were ever part of the diet. In response, they found that R/R was eliminated (Ruempler, 1992).

As demonstrated, feeding greater quantities of vegetables and browse can increase feeding activity and reduce undesirable behaviors while the increase in fiber associated with large amounts of vegetables and browse can improve stool consistency. However, no one has tested the effect of different types of non-browse diets and different levels of activity on adiposity in gorillas. It was hypothesized that calorically dense diets that are high in starch and low in fiber are correlated with adiposity in gorillas. Additionally, it was hypothesized that a lack of physical activity in gorillas contributes to adiposity. This study also tested a new form of diet which includes removing biscuits and replacing them with a greater volume of produce. It was hypothesized that increasing produce in the diet should increase time spent feeding and also decrease undesirable behavior. Therefore, this study identified a practical way, using diet, for zoos to improve the activity levels, undesirable behavior, nutrition and decrease the adiposity of their gorillas.

Methods for Measuring Adiposity

This research focuses on two different indirect ways of measuring body fat mass (i.e., adiposity). The first involves measuring serum biomarkers (discussed below). This approach is specific, but problematic because it involves the use of serum which can be difficult to obtain from zoo gorillas. Many gorillas are not trained for blood sample collection while awake, so they must be anesthetized to obtain serum and for those that are trained it is still difficult or currently impossible to collect sufficient blood samples from them. Many zoos do not regularly anesthetize their animals which makes serum collection particularly difficult. It is also problematic because animals are fasted before exams, which can affect circulating biomarker levels, and because serum collection is

typically a one-time occurrence, it only provides a one-time point sample which reflects fat at that moment, not over a longer time course. The second approach is a measure of adiposity and involves creating a body mass index (BMI) for gorillas. This is the first attempt to create a BMI for gorillas and hopefully will provide an easier method for caretakers to monitor adiposity in their animals.

Serum Biomarkers

Insulin resistance

After a meal in a healthy human, plasma glucose increases which leads to an increase in plasma insulin. This insulin acts upon receptors located on certain cell types such as fat, muscle and liver to increase glucose uptake returning plasma glucose levels to normal. Insulin resistance is a condition in which cells become less sensitive to insulin so insulin does not have the effects on receptors mediating glucose uptake that it does in normal humans (Reaven, 1988). This leads to elevated serum levels of insulin and eventually to elevated levels of serum glucose which characterize a diabetic state (Defronzo et al., 1992). In humans, both insulin resistance and type II diabetes are associated with heart disease and obesity (Kahn et al., 2001; McFarlane et al., 2001). Type II diabetes has not yet been defined in gorillas and very few gorillas have been reported to exhibit this disease (Dennis et al., in prep). Insulin and glucose levels were measured in gorillas to gain more information on insulin resistance in this population. The relationship of glucose to insulin (G/I index) was also calculated which is a marker of insulin resistance in humans (Quon, 2001).

Adiposity biomarkers

    a. Leptin

First described in 1994, leptin is a peptide hormone secreted by adipose tissue (Zhang et al., 1994). Leptin plays an important role in energy balance regulation (Houseknecht et al., 1998). Studies of humans and rodents in a steady-state (not food deprived, i.e., 'zero energy balance'), found that leptin concentrations accurately reflected body mass (Maffei et al., 1995). When organisms are food deprived, leptin levels decline (Kolaczynski et al., 1996). This decline in leptin provides feedback to neural circuits to promote feeding behaviors. Conversely, when an organism has overfed, leptin levels remain high, signaling the brain that satiety has been achieved. Leptin essentially functions as an "adipostat" to promote energy balance (Houseknecht et al., 1998). Obese individuals in particular are characterized by extremely elevated leptin levels, a state referred to as 'hyperleptinemia' (Spiegelman and Flier, 1996).

Hyperleptinemia combined with 'hyperphagia', or overeating, results in 'leptin resistance' and in most cases obesity (Morrison, 2008). Three conditions have been identified that may result in leptin resistance: (1) a defect in the leptin receptor, (2) limitations of the leptin system and/or (3) circulatory defects (Houseknecht et al., 1998). The first and second conditions refer to genetic and evolutionary hypotheses regarding leptin resistance. The third condition is particularly interesting in that the environment can play a strong role. Leptin circulates bound to transport proteins. In obese individuals with high leptin levels, these proteins become saturated and leptin begins circulating in the free form. Unbound leptin was reported to correlate with increasing obesity and BMI in humans (Sinha et al., 1996).

Most research on the relationship between body composition and leptin has been conducted with humans, laboratory mice, domestic dogs (Ishioka et al., 2007), and

animals used in agriculture such as pigs, sheep and cows (Houseknecht et al., 1998). Non-human primate studies have mainly focused on monkeys, including macaques (Muehlenbein et al., 2005), baboons (Banks et al., 2001; Muehlenbein et al., 2003) and vervet monkeys (Whitten and Turner, 2008). Only one study has examined leptin in apes and found that it increased with weight in female chimpanzees, but not in males (Bribiescas and Anestis, 2010). No studies of leptin in any of the other great ape species have been published.

Leptin is also significant with regard to its role in insulin resistance and inflammation (see next section for more information on inflammation). Leptin administration reduces insulin sensitivity in rats suggesting that leptin resistance may perpetuate an insulin resistant state (Perez et al., 2004). Leptin also has pro-inflammatory properties and has been shown to increase the production of inflammatory cytokines, which in turn increase production of acute phase proteins (Otero et al., 2006). Leptin correlates with increasing BMI in healthy humans (van Dielen et al., 2001), but this relationship is not evident once a person has developed type II diabetes (Tatti et al., 2001). Therefore, it is important to assess in any organism whether type II diabetes is evident before using leptin as an indicator of fat mass.

b. Adiponectin

Adiponectin is a peptide hormone also secreted by adipose tissue. The release of adiponectin from fat cell in vitro was increased by insulin and by the inhibition of TNF-α (Fain et al., 2007). However, unlike leptin, adiponectin concentrations are lower in obese humans (Weyer et al., 2001). Specifically, adiponectin is negatively correlated with increasing BMI in humans (Arita et al., 1999). Adiponectin acts upon cells in liver and

muscle to make them more sensitive to the effects of insulin thereby increasing glucose

uptake (Berg et al., 2002; Fantuzzi, 2005). Furthermore, adiponectin interferes with

macrophage recruitment during inflammation (Yokota et al., 2000). Therefore, it is

thought that unlike leptin, adiponectin has anti-inflammatory properties and helps to

improve insulin sensitivity (Berg et al., 2002; Tilg and Moschen, 2006).

Other biomarkers of obesity:

Two other biomarkers will be measured based on their association with obesity in

humans: triglycerides and cholesterol.

a. *Triglycerides*

Triglycerides are fatty acids linked to glycerol and comprise the fat that is stored in

adipocytes. These triglycerides function to provide energy to the body. High levels of

triglycerides are associated with obesity, type II diabetes and heart disease in humans

(Tzagournis, 1978) . Serum triglycerides positively correlate with increasing BMI in

female chimpanzees (Videan et al., 2007). Triglycerides will be measured as a marker of

adiposity in gorillas and also in conjunction with body condition measurements.

b. *Cholesterol*

Cholesterol is a lipid that is not a source of energy but rather is a precursor for cell

membranes, bile salts, and steroid hormones. Cholesterol levels can be associated with

fat mass in humans (Choi et al., 2003). Cholesterol levels can also be separately

associated with diet. In particular, it can be lower in humans (Brown et al., 1999) and

gorillas (Dennis et al., unpublished data) on higher fiber diets. In humans, high

cholesterol levels are associated with the development of heart disease in the form of

atherosclerosis (Kritchevsky, 1962). Although atherosclerosis does not appear to be

associated with heart disease in apes (Varki et al., 2009), zoo gorillas do have higher cholesterol levels than wild gorillas (Schmidt et al., 2006) potentially indicating that zoo gorillas are not being fed diets with enough fiber or that they may be obese.   Total serum cholesterol was measured in conjunction with gorilla diets and also as a potential correlate of obesity.

Body Mass Index

The serum paramters previously described are important tools for measuring adiposity in an individual. However, measuring these serum biomarkers can be invasive and labor intensive as it requires the collection and analysis of blood samples. Weight alone is not a very accurate measure of obesity as it does not take into account size of the organism or body composition. One commonly used method for determining whether an individual is overweight is a body mass index (BMI), calculated as weight/height^2 in humans. BMI can be measured non-invasively and increasing BMI correlates with obesity in humans (Dietz and Bellizzi, 1999). Thus, BMI may be a useful tool for noninvasively assessing gorilla body composition if validated with hormone measures of adiposity.

BMI was used to calculate whether an individual was overweight in studies using humans (World Health Organization), baboons (Muehlenbein et al., 2003), chimpanzees (Videan et al., 2007) and macaques (Muehlenbein et al., 2005). In male macaques, researchers calculated a modified BMI: weight (kg)/divided by crown-rump length squared (km) (Muehlenbein et al., 2005). They found that leptin levels and abdominal fat deposits were positively correlated with BMI in male macaques, supporting the contention that a revised body mass index for gorillas should correlate with adiposity and

circulating hormones. However, in a study of adult male baboons, BMI did not correlate with leptin levels (Muehlenbein et al., 2003). Based on the differences between male macaques and baboons, the researchers concluded that baboons may just have a lower body fat percentage than other primates (Muehlenbein et al., 2003) and other observers noted that male baboons have a low percentage of body fat (approximately 6%) (Rutenberg et al., 1987) compared to what is normal for macaques (12%) (NRC, 2003). The modified BMI along with measurements of skinfolds was found to correlate with serum triglycerides and glucose in female chimpanzees (Videan et al., 2007). The male chimpanzee BMIs, although high, did not correlate with these serum measurements, which the authors hypothesize is because they contain large amounts of lean body mass as opposed to fat mass (Videan et al., 2007). Typical body fat varies between primate species and likely, also varies based on environment.

In gorillas, four adult (2 males, 2 females) wild-born captive individuals had body fat percentages ranging from 19.4-26.6% as measured via post-mortem dissection (Zihlman and McFarland, 2000). Furthermore, Zihlman and McFarland (2000) found that bone constituted 10.2-12.7% and muscle constituted 36.1-37.9% of body mass. There have been no reports on body mass composition in wild gorillas. There is also a need for a larger sample size studying body mass in gorillas and there have been no studies aimed at determining any associations between BMI measurements and leptin and/or adiponectin.

Body composition and hormone levels

It is expected that BMI, leptin, and adiponectin will be normally distributed within the captive gorilla population. Furthermore, it is well known that there are gender

and age differences in body composition among all mammals and this is expected to hold true for gorillas. In fact, in humans, adipose tissue (along with leptin) is increased during three specific stages: early in life, during pregnancy and lactation, and with aging (Zafon, 2007). For this reason, data in this study will be controlled for gender, age, and reproductive status. Also, human females have higher adiposity and leptin than males (Ahmed et al., 1999). Female gorillas have been shown to have a higher range of percent body fat than males (Zihlman and McFarland, 2000), and thus are likely to have higher leptin levels than male gorillas if they are similar to humans. Leptin concentrations also vary with reproductive state in human and other mammalian females. Leptin is increased in pregnant (Henson and Castracane, 2000) and lactating humans (Mukherjea et al., 1999) and baboon females [(Henson et al., 1999), but see (Butte et al., 1997)] therefore we will also need to account for reproductive state. In male macaques, leptin increased with age from adolescence to adulthood in conjunction with a parallel increase in adiposity (Muehlenbein et al., 2005). One study found that juvenile captive baboons had higher leptin to weight ratios than their wild counterparts, but that leptin concentrations were more similar between the two groups as they aged (Banks et al., 2001). Therefore, it is important to control for age, gender and reproductive state when attributing differing leptin levels to different states of body composition.

Measuring adiposity as a husbandry tool

It is currently unknown what a healthy weight is for gorillas. Weight alone may not be a good sole measurement for evaluation, but should be considered in relation to size of the animal and body composition. It is also unknown how the weights of captive gorillas compare to wild gorillas (or what this means in terms of body composition). One

study comparing the weights of wild and captive gorillas found that there was not a significant difference (Leigh, 1994). However, this study did not consider size or body composition and was based on a relatively small number of wild gorillas. Considering the current weights of captive gorillas in relation to leptin, adiponectin and BMI will allow for a better understanding of what may be considered obese in the captive gorilla population. In their study of BMI in chimpanzees, Videan et al. (2007) classified obese females as those with BMIs over 20% of the mean. They also defined overweight females as having a BMI 10-20% above the mean. By these calculations, they determined that approximately 10% of females are obese and 10% are overweight (Videan et al., 2007). Because leptin, adiponectin and triglycerides (see next section) indirectly reflect body fat mass, there may be a better chance of correlating body measurements with these measurements of obesity as opposed to glucose which was used in the chimpanzee study. An additional issue with Videan's study is using the mean as the basis for defining obesity in that the mean itself could already be high, i.e. the entire population is obese on average. In humans, body composition measurements are used to assess obesity and these measurements exhibit positive relationships with the same serum biomarkers in this study. Other primate species define obesity by measuring body fat directly using calipers or doubly labeled water and comparing these measures with human ranges of serum biomarkers (Hotta et al., 2001). In this study, human healthy ranges of leptin, adiponectin and triglycerides will be used to attempt to assess obesity in gorillas.

Thesis objective

In this thesis, I conducted five studies with captive gorillas that will examine adiposity and the factors that predict it. I considered how diet, behavior and health are all integrated and essential for the successful management of gorillas in zoos.

In the first study (chapter 2), I aimed to describe the serum biomarkers used throughout the rest of the study. This chapter assessed for correlation between serum biomarkers and examine the effects of age.

In chapter 3, I determine the association between non-invasively collected gorilla body measurements and the serum markers of adiposity and insulin resistance. These measurements can serve as a tool for zoo keepers and managers to monitor body condition of their animals.

In Chapter 5, I aimed to demonstrate that diet and activity can be correlated with adiposity and insulin resistance in gorillas. More specifically, I predicted that diets higher in fiber while also being lower in starch and caloric density will lead to lower amounts of adiposity and insulin resistance than the alternative. Additionally, I predicted that gorillas that spend more time engaged in active behavior will also have lower amounts of these variables. As a side study, in Chapter 4, I also explored zoo keeper ratings of activity and how this compares to collected behavioral data. This was a novel study that examined how reliable subjective opinions of activity level in gorillas were compared to objectively collected data.

Chapter 6 of this dissertation focused on a smaller experimental study. Here I put a subset of gorillas on a higher fiber diet that was subsequently lower in caloric density and starch. This diet was created by removing primate biscuits, reducing fruit and root vegetables and increasing alfalfa and leafy greens . Body condition/health data was

collected before and after this diet change to identify a role of diet in the development of adiposity. Behavioral data were collected to evaluate the influence of diet on the presence of undesirable behaviors.

I finished the thesis by developing an outlook of future research that provides more insights on unanswered questions of adiposity and health in gorillas and factors influencing them. I also provided recommendations for zoos to implement based on this research to better define and manage adiposity in their gorillas.

Chapter 2: Correlations of serum biomarkers of obesity, insulin resistance and inflammation in zoo gorillas

In humans, obesity is associated with inflammation and insulin resistance which are in turn risk factors for the development of cardiac disease, hypertension and type II diabetes, among other health complications (Stein and Colditz, 2004). There are several serum biomarkers that represent the progression of obesity, insulin resistance and inflammation.

Insulin resistance is a condition in which cells become less sensitive to insulin so insulin does not have the effects on receptors mediating glucose uptake that it does in normal humans (Reaven, 1988). This leads to elevated serum levels of insulin and eventually to elevated levels of serum glucose which characterize a diabetic state (Defronzo et al., 1992). In humans, both insulin resistance and type II diabetes are associated with heart disease and obesity (Kahn et al., 2001; McFarlane et al., 2001).

Leptin is also significant with regard to its role in insulin resistance and inflammation. Leptin administration reduces insulin sensitivity in rats suggesting that leptin resistance may perpetuate an insulin resistant state (Perez et al., 2004). Leptin also has pro-inflammatory properties (Otero et al., 2006). Leptin has been shown to increase the production of inflammatory cytokines, which in turn increase production of acute phase proteins, or a class of proteins that increase during inflammatory events (Otero et al., 2006). Leptin positively correlates with increasing BMI and fat mass in healthy humans (van Dielen et al., 2001), but this relationship is not evident once a person has developed type II diabetes (Tatti et al., 2001). Therefore, it is important to assess in any

organism whether type II diabetes is evident before using leptin as an indicator of fat mass.

Adiponectin is a peptide hormone also secreted by adipose tissue. However, unlike leptin, adiponectin concentrations are lower in obese humans (Weyer et al., 2001). Specifically, adiponectin is negatively correlated with increasing BMI in humans (Arita et al., 1999). Adiponectin acts upon cells in liver and muscle to make them more sensitive to the effects of insulin thereby increasing glucose uptake (Berg et al., 2002; Fantuzzi, 2005). Furthermore, adiponectin interferes with macrophage recruitment during inflammation (Yokota et al., 2000). Therefore, it is thought that unlike leptin, adiponectin has anti-inflammatory properties and helps to improve insulin sensitivity (Berg et al., 2002; Tilg and Moschen, 2006).

Triglycerides are fatty acids linked to glycerol and comprise the fat that is stored in adipocytes. These triglycerides function to provide energy to the body. High levels of triglycerides in the body are associated with obesity, type II diabetes and heart disease in humans (Tzagournis, 1978) and serum triglycerides correlate with increasing BMI in female chimpanzees (Videan et al., 2007).

In many obese humans, there may be elevated levels of leptin, insulin, glucose, and triglycerides with subsequent low levels of adiponectin and glucose to insulin ratio (Mojiminiyi et al., 2006). In some instances where obese individuals develop type 2 diabetes, leptin levels can begin to decline (Abdelgadir et al., 2002). If type 2 diabetes progresses to the point where beta cell function in the pancreas is diminished, insulin levels may also begin to decline (Johnson et al., 1986; Kahn, 2003).

The purpose of this chapter is to describe the relationships between these serum biomarkers in zoo gorillas. These relationships, once understood, will be used to define obesity, examine body composition measurements such as BMI, and examine effects of diet. In this study, leptin, adiponectin and triglycerides were measured as markers of obesity. Insulin, glucose, and glucose to insulin ratio were measured as markers of insulin resistance.

Methods

Subjects

The current study included 101 adult gorillas housed at 32 institutions accredited by the Association of Zoos and Aquariums with a total of 58 males and 43 females. The gorillas ranged in age from 8-49 years of age.

Hormone Collection and Analysis

Each participating institution sent a 4 ml serum sample collected at the individual gorilla's routine veterinary exam. Previously banked samples were accepted if they were collected no earlier than 2008. Commercially available kits were used to analyze leptin (10-1199-01; Mercodia), adiponectin (K1002-1, B-Bridge International, INC) and insulin (10-1132-01; Mercodia). Each of the assays was previously validated for use with gorilla serum in the Cleveland Metroparks Zoo Endocrinology Laboratory. Glucose and triglycerides were measured using an IDEXX Vettest 8008 Chemistry Autoanalyzer.

Statistics

For both males and females separately, each variable was assessed for normality using a Kolmogorov-Smirnov Goodness-of-Fit test and log transformed if not normally distributed. Specifically, for females, adiponectin and insulin measurements were log

transformed and for males, the leptin measurements were log transformed. All body composition measures were compared between males and females with t-tests. They were all different ($p < 0.05$), thus data for males and females will be reported separately throughout this paper. All statistics were calculated using SPSS®.

Serum biomarker correlations and statistics

All serum biomarkers were assessed for correlation with each other using Pearson correlations for males (Table 3) and females (Table 6). To assess biomarkers in various glucose and insulin concentrations, males and females were split into four groups. The cut-off for high glucose was based on the fasting glucose levels in the human literature (Genuth et al., 2001) and set at 100 mg/dl. For insulin, the mean was used for males and females as the cut-off for high insulin levels (males: 5.24 mU/l, females: 11.7 mU/l). The four groups consisted of: (1) low glucose (glucose < 100 mg/dl), low insulin (insulin < mean), (2) low glucose (glucose < 100 mg/dl), high insulin (insulin > mean), (3) high glucose (glucose > 100 mg/dl), high insulin (insulin > mean), and (4) high glucose (glucose > 100 mg/dl), low insulin (insulin < mean). Serum biomarkers were graphed according to these different groups. Univariate ANOVAs were used to compare the means of the serum biomarkers between these 4 groups and differences in the groups. When significant differences were discovered, the different means were determined using LSD post-hoc tests. Alpha was set at 0.05 for all statistical tests.

Results

a. *Males*

The relationship between each serum biomarker and age is reported (Table 1). Leptin ($r = 0.423$, $p=0.001$), triglycerides ($r =0.348$, $p=0.010$) and insulin ($r=0.304$,

p= 0.022) increased with age while adiponectin (r = -0.206, p=0.124) and glucose (r= -0.116, p=0.391) did not show a relationship with age.

Table 1. Serum biomarkers and their relationship with age in males.

Serum biomarker	Increase with age?
Leptin	Yes, r=0.4, p=0.000
Adiponectin	No, r= -0.2, p=0.124
Triglycerides	Yes, r= 0.3, p=0.010
Insulin	Yes, r=0.3, p=0.022
Glucose	No, r= 0.1, p=0.661

The ranges and averages of all serum biomarkers are reported (Table 2).

Table 2. Descriptive statistics for each serum biomarker.

Biomarker	Low	High	Mean	Median	St Dev
Leptin (ng/ml) (n=58)	0.30	19.40	5.95	3.74	5.78
Adiponectin (µg/ml) (n=57)	0.16	2.13	0.69	0.59	0.43
Triglycerides (mg/dl) (n=54)	39.0	375.0	135.0	121.0	6.9
Insulin (mU/l) (n=57)	0.43	19.90	5.24	4.02	4.41
Glucose (mg/dl) (n=58)	44.0	187.0	97.0	89.5	31.6

The correlations between serum biomarkers are reported (Table 3).

Table 3. Serum biomarker correlations in males. Leptin, adiponectin, triglycerides and insulin were correlated with each other, while glucose was not correlated with other variables.

	L	A	T	I	G
L		r= -0.5 p=0.000	r= 0.5 p=0.000	r=0.4 p=0.001	r= -0.0 NS
A			r= -0.4 p=0.003	r= -0.4 p=0.005	r= -0.1 NS
T				r= 0.5 p=0.000	r= -0.0 NS
I					r= -0.1 NS
G					

The serum biomarkers of obesity exhibited correlations, in particular, leptin, insulin and triglycerides were positively correlated with each other and each negatively correlated with adiponectin (Table 1).

Non-linear relationships

Glucose did not exhibit linear relationships with the other serum biomarkers. In particular, there appeared to be distinct groups of individuals: (1) glucose < 100 mg/dl, insulin , insulin < 5.24 mU/l (mean) (n=23), (2) glucose < 100 mg/dl, 5.24 > mU/l (n=12), (3) glucose > 100 mg/dl, insulin > 5.24 mU/l (n=3) and (4) glucose > 100 mg/dl, insulin < 5.24 mU/l (n=19) (Figure 1, Table 4).
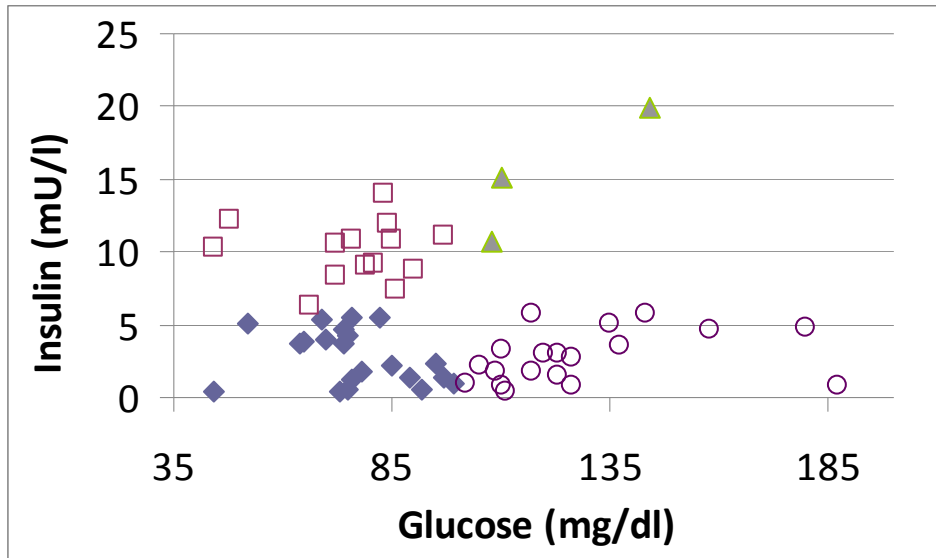
Figure 1. Varying insulin and glucose concentrations in males. Diamonds represent gorillas with a glucose < 100 mg/dl and insulin < 5.24 mU/l; squares represent gorillas with a glucose < 100 mg/dl and insulin > 5.24 mU/l; triangles represent gorillas with a glucose > 100 mg/dl and insulin > 5.24 mU/l; and circles represent gorillas with a glucose > 100 mg/dl and an insulin < 5.24 mU/l.

Furthermore, a univariate ANOVA revealed a trend towards leptin levels varying significantly among glucose and insulin concentrations (F= 2.505, p=0.069) with post-hoc tests revealing that leptin levels were significantly higher in group 3 (high glucose, high insulin) than groups 1 and 4 (group 3 vs. group 1, p= 0.015; group 3 vs. group 4, p=0.024) (Figure 2).

Figure 2. Leptin concentrations at various glucose and insulin levels in males. Leptin in group 3 was significantly higher than leptin levels in group 1 and group 4 (as denoted by the *).

Adiponectin did not vary significantly among glucose and insulin concentrations

(F=1.807, p=0.157), though it appeared to be lower in the 3rd group (Figure 3).
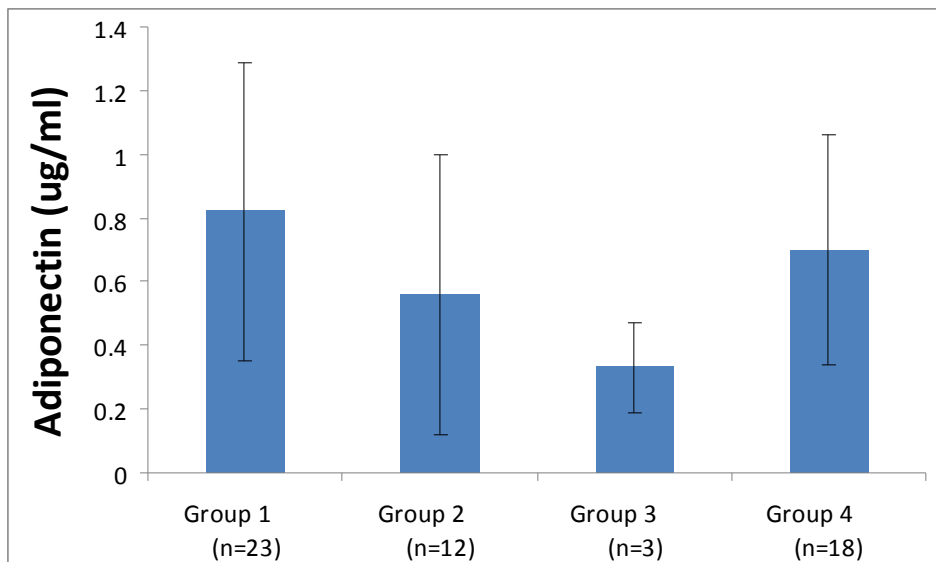
Figure 3. Adiponectin concentrations at various glucose and insulin levels. Adiponectin did not differ significantly amongst groups.

Triglycerides varied significantly across glucose and insulin concentrations (F=2.980, p=0.040) with post-hoc tests revealing that triglyceride levels were higher in group 3 than the other groups (group 3 vs. group 1, p=0.008, group 3 vs. group 2, p=0.032, group 3 vs. group 4, p=0.006) (Figure 4).
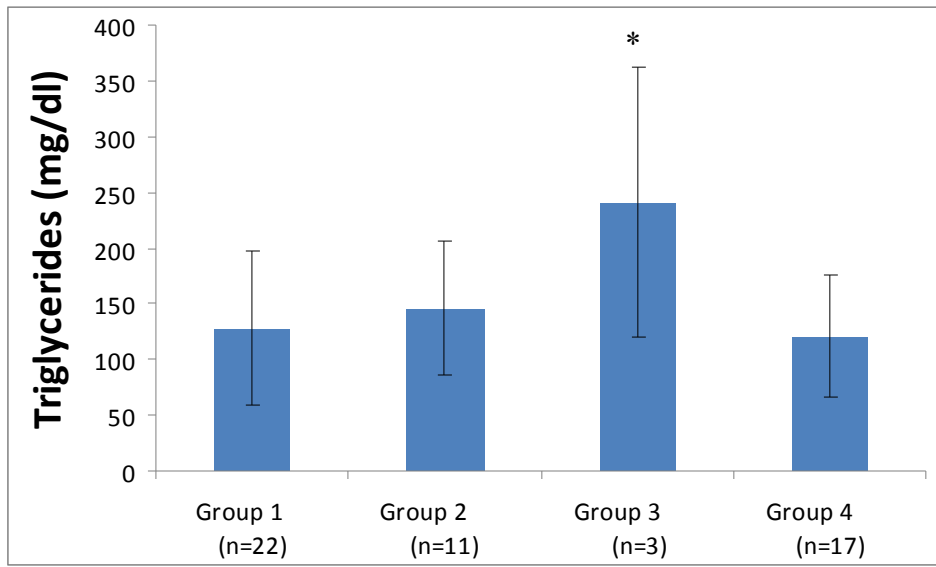
Figure 4. Triglycerides in varying glucose and insulin concentrations. Triglycerides in group 3 were significantly higher than the other groups (as denoted by the *).

Table 4. Serum biomarkers in each insulin/glucose group in males.

Serum biomarker	Age (± SD)	Group	Mean	Median	St Dev
Leptin (ng/ml)	20 ± 10	1 (n=23)	4.5	2.3	4.8
	25 ± 9	2 (n=12)	8.3	7.3	6.7
	29 ± 2	3 (n=3)	15.0	16.2	3.7
	22 ± 9	4 (n=19)	5.0	2.8	5.1
Adiponectin (ug/ml)	20 ± 10	1 (n=23)	0.8	0.8	0.5
	25 ± 9	2 (n=12)	0.6	0.4	0.4
	29 ± 2	3 (n=3)	0.3	0.3	0.1
	22 ± 9	4 (n=18)	0.7	0.6	0.4
Triglycerides (mg/dl)	20 ± 10	1 (n=22)	128.4	110.0	69.3
	25 ± 9	2 (n=11)	145.9	131.0	59.9
	29 ± 2	3 (n=3)	240.7	205.0	120.5
	22 ± 9	4 (n=17)	121.1	116.0	54.7

b. *Females*

Leptin (r = 0.423, p=0.001) and triglycerides (r =0.348, p=0.010) increased with age while adiponectin (r = -0.206, p=0.124), insulin (r= 0.023, p=0.884), and glucose (r= -0.015, p=0.25) did not show a relationship with age (Table 5).

Table 5. Serum biomarkers and their relationship with age in females.

Serum biomarker	Increase with age?
Leptin	Yes, r=0.3, p=0.045
Adiponectin	No, r= -0.0, p=0.907
Triglycerides	Yes, r=0.7, p=0.000
Insulin	No, r=0.1, p=0.502
Glucose	No, r= -0.0, p=0.925

The range and averages of biomarkers are reported in Table 6.

Table 6. Descriptive statistics for each serum biomarker in females.

Biomarker	Low	High	Mean	Median	St Dev
Leptin (ng/ml) (n=42)	0.78	29.7	9.34	7.26	7.49
Adiponectin (µg/ml) (n=41)	0.16	2.65	0.74	0.48	0.63
Triglycerides (mg/dl) (n=36)	54.0	350	162	128	94.3
Insulin (mU/l) (n=42)	0.270	74.3	11.6	3.14	18.9
Glucose (mg/dl) (n=42)	35.0	177	98.0	91.5	27.7

The correlations between serum biomarkers are reported in Table 7.

Table 7. Serum biomarker correlations in females. Leptin and triglycerides were correlated with each other and adiponectin and glucose were correlated with each other. Insulin was not correlated with any other variables.

	L	A	T	I	G
L		**r= -0.6** **p=0.000**	**r= 0.6** **p=0.000**	r= 0.2 NS	r= 0.2 NS
A			*r= -0.3* *p=0.074*	r= -0.1 NS	**r= -0.3** **p=0.037**
T				r= 0.2 NS	r= -0.2 NS
I					r= -0.1 NS
G					

Leptin, adiponectin and triglycerides were all significantly correlated with each other except for adiponectin and triglycerides which exhibited a trend (r= -0.3, p=0.074). Insulin and glucose did not exhibit linear correlations with any of the serum biomarkers except for glucose and adiponectin (r= -0.3, p=0.037).

Non-linear correlations

As in males, there appeared to be distinct groups of individuals: (1) glucose < 100 mg/dl, insulin , insulin < 11.7 mU/l (mean) (n=18), (2) glucose < 100 mg/dl, 11.7 > mU/l (n=8), (3) glucose > 100 mg/dl, insulin > 11.7 mU/l (n=2) and (4) glucose > 100 mg/dl, insulin < 11.7 mU/l (n=14) (Figure 5; Table 8).
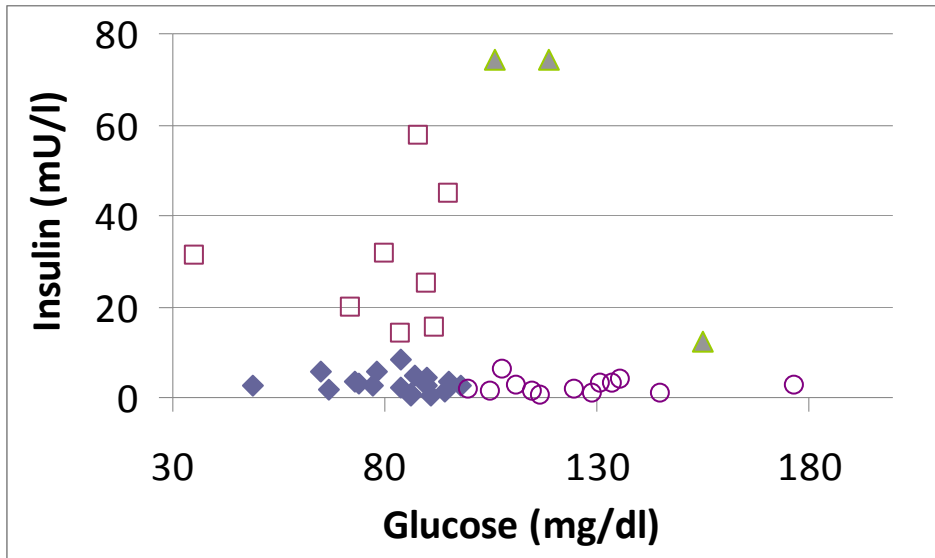
Figure 5. Varying insulin and glucose concentrations in females. Diamonds represent gorillas with a glucose < 100 mg/dl and insulin < 11.7 mU/l; squares represent gorillas with a glucose < 100 mg/dl and insulin > 11.7 mU/l; triangles represent gorillas with a glucose > 100 mg/dl and insulin > 11.7 mU/l; and circles represent gorillas with a glucose > 100 mg/dl and an insulin < 11.7 mU/l.

Furthermore, a univariate ANOVA revealed that leptin levels varied significantly among glucose and insulin concentrations (F= 3.745, p=0.019) with post-hoc tests revealing that leptin levels were significantly higher in group 3 (high glucose, high insulin) than groups 1, 2 and 4 (group 3 vs. group 1, p= 0.004; group 3 vs. group 2, p=0.002; group 3 vs. group 4, p=0.009) (Figure 6).
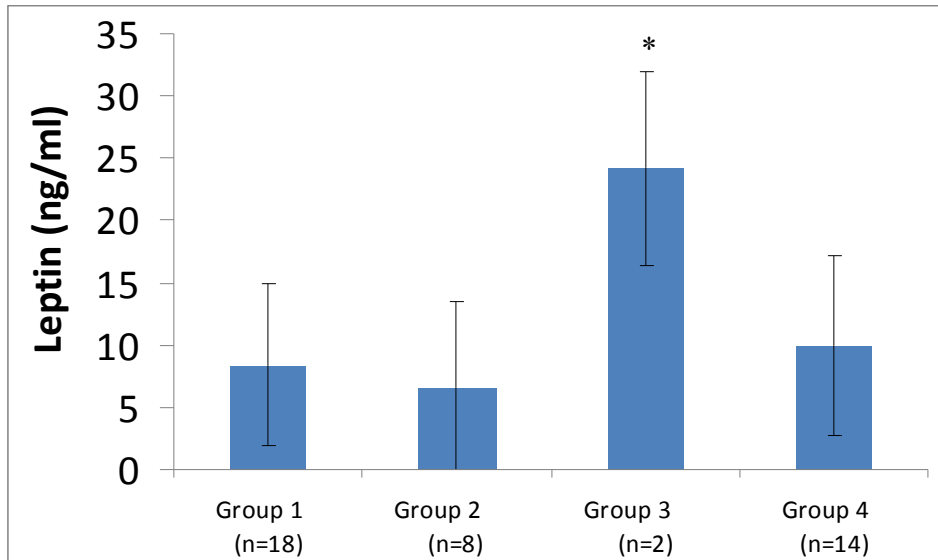
Figure 6. Leptin concentrations at various glucose and insulin levels in females. Leptin in group 3 was significantly higher than leptin levels in the other groups (as denoted by *).

A univariate ANOVA revealed that adiponectin levels varied significantly among glucose and insulin concentrations (F= 3.036, p=0.041) with post-hoc tests revealing that adiponectin levels were significantly lower in group 3 (high glucose, high insulin) than groups 1 and 2 (group 3 vs. group 1, p= 0.018; group 3 vs. group 2, p=0.018) (Figure 7).
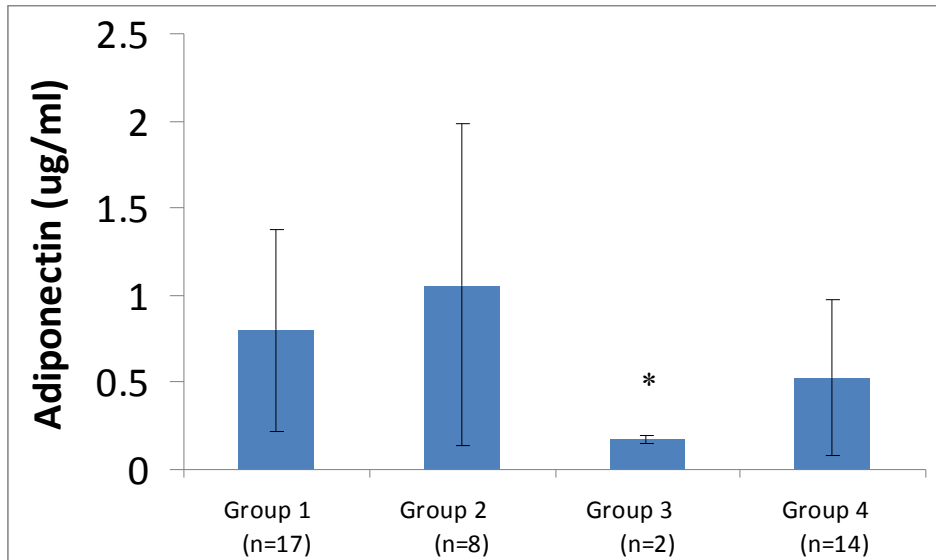
Figure 7. Adiponectin concentrations at various glucose and insulin levels in females. Adiponectin in group 3 was significantly lower than adiponectin levels in groups 1 and 2 (ad denoted by *).

A univariate ANOVA revealed that a trend towards triglyceride levels varying significantly among glucose and insulin concentrations (F= 2.858, p=0.052) with post-hoc tests revealing that triglyceride levels were significantly higher in group 3 (high glucose, high insulin) than groups 1, 2 and 4 (group 3 vs. group 1, p= 0.009; group 3 vs. group 2, p=0.008; group 3 vs. group 4, p= 0.016) (Figure 8).
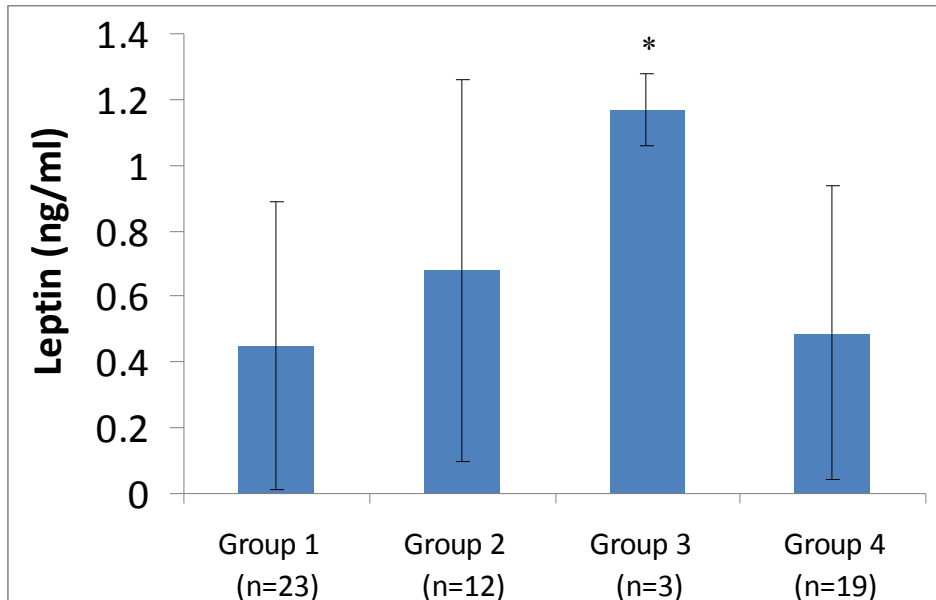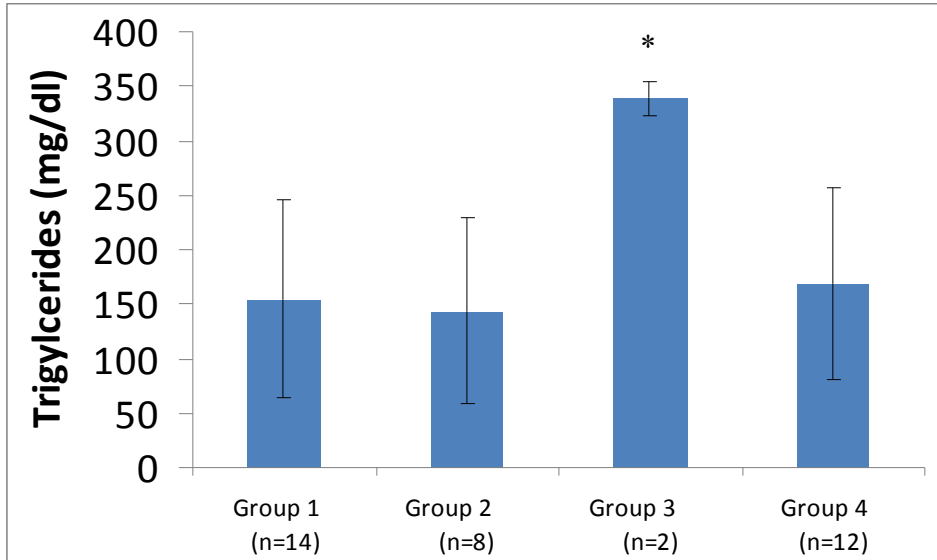
Figure 8. Triglyceride concentrations at various glucose and insulin levels in females. Triglycerides in group 3 were significantly higher than triglyceride levels in the other groups (as denoted by *).

Table 8. Serum biomarkers in each insulin/glucose group in females.

Serum biomarker	Mean age (± SD)	Group	Mean	Median	St Dev
Leptin (ng/ml)	22 ± 11	1 (n=18)	8.4	5.8	6.5
	17 ± 7	2 (n=8)	6.6	3.0	6.8
	35 ± 1	3 (n=2)	24.2	24.2	7.8
	21 ± 11	4 (n=14)	10.0	8.1	7.2
Adiponectin (ug/ml)	22 ± 11	1 (n=17)	0.8	0.5	0.6
	17 ± 7	2 (n=8)	1.1	0.9	0.9
	35 ± 1	3 (n=2)	0.2	0.2	0.0
	21 ± 11	4 (n=14)	0.5	0.4	0.5
Triglycerides (mg/dl)	22 ± 11	1 (n=14)	154.5	108.5	91.3
	17 ± 7	2 (n=8)	143.5	105.5	86.4
	35 ± 1	3 (n=2)	339.0	339.0	15.6
	21 ± 11	4 (n=12)	169.1	132.5	87.9

Discussion

Several gorillas in this study exhibited levels of leptin, triglycerides, insulin and glucose that are considered high compared to human and nonhuman primate standards. Additionally, if adiponectin is considered as a biomarker of obesity in gorillas and the human literature is used as a reference (healthy adiponectin levels > 4 ug/ml) (Im et al., 2006), 100% of our sample, including males and females, may be obese. A study on captive macaques found that adiponectin levels were lower than what was reported as healthy in humans (Hotta et al., 2001), but it is unknown whether macaques have naturally lower adiponectin levels than humans or if that entire population was also obese. Further research is needed to determine if this is indeed the case or if adiponectin

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levels in non-human primates are naturally lower than human adiponectin levels. Either way, any obesity in the gorilla population may be a problem because we know from humans that obesity is associated with the development of certain disease states, such as heart disease and type 2 diabetes (Stein and Colditz, 2004). Heart disease has been identified as the number one cause of death for male gorillas in zoos (Meehan and Lowenstine, 1994) so future research efforts should be focused on determining if there is a relationship between obesity and the development of heart disease in gorillas. Type 2 diabetes has been reported in very few gorillas compared to other species (Dennis et al., in prep), but the high glucose levels in these data suggest that it may be under reported or that glucose values may be different for gorillas compared to other species.

For both males and females, the serum biomarker correlations showed similarities to data for humans. In humans, leptin is negatively associated with adiponectin (Weyer et al., 2001) and positively associated with triglycerides (Haluzik et al., 1999) which we also found for both male and female gorillas. Additionally, leptin and triglycerides increase with age in humans (Castelli, 1986; Mann et al., 2003) and similarly increased with age in both male and female gorillas. Adiponectin has been shown to increase with age in humans (Cnop et al., 2003), but not in rhesus macaques (Hotta et al., 2001). This relationship was not observed for gorillas. It may be that this relationship holds true for humans, but not non-human primates. Another possibility for not being able to observe this relationship might be that gorillas and macaques do not live as long as humans. For this to hold true, the increase in adiponectin with age observed in humans would have to be exponential as opposed to linear. The Cnop et al. (2003) study found that this

relationship was linear so this is not likely to be the reason. Future research may be needed to determine why this relationship does not exist in gorillas.

Because research on obesity in gorillas is in its infancy, comparison to humans is currently the easiest method to make assumptions about health. However, caution must also be taken in comparing gorillas to humans. Although they are genetically very similar, they are different in many ways such as digestion strategy, diet, anatomy/morphology, among countless other traits. What might be considered a healthy range in biomarkers for humans might not be healthy for gorillas.

In both males and females, four different groups of glucose and insulin concentrations were observed: group 1: healthy levels of glucose (based on human standards), low insulin levels; group 2: healthy levels of glucose, high insulin levels; group 3: high glucose and high insulin levels; and group 4: high glucose and low insulin levels. In humans, diabetes is defined as a fasting serum glucose concentration > 126 mg/dl (Genuth et al., 2001). Insulin resistance occurs when individuals have normal to high glucose concentrations, but consistently high insulin levels (Reaven and Laws, 1994). Insulin resistance often, but not always, precedes the development of type 2 diabetes in humans (Lillioja et al., 1993). If human standards are used for when glucose levels become borderline high (100 mg/dl) (also considering that other non-human primates such as macaques report lower glucose levels than humans, (Vyas et al., 2004), gorillas in group 2 and 3 could be considered insulin resistant and gorillas in groups 3 and 4 could be considered diabetic. If this is the case, then 39% of males and 38% of females may be considered diabetic.

In some cases, as diabetes progresses in humans and domestic cats, the beta cells in the pancreas lose function and decrease production of insulin (Johnson et al., 1986; Kahn, 2003). In particular, diabetic cats are characterized by low fasting insulin levels (Johnson et al., 1986). This may be what is occurring in group 4. Leptin was lower in group 4 and leptin is lower in diabetic humans with lower insulin levels (Abdelgadir et al., 2002). Advanced diabetes can lead to hepatic lipodosis or "fatty liver", which leads to lower serum triglycerides (Lim et al., 2010). This might explain the lower triglycerides in group 4. The increased triglycerides in group 3 for both males and females suggests that this is the group where obesity is at its highest before the progression of diabetes. In future chapters, the goal is to validate body composition measures and diet/activity parameters (independent variables) with these serum biomarkers (dependent variables) by observing if there are linear relationships between the independent and dependent variables. Because of this, individuals in group 4 will be excluded from the body composition measure and serum biomarker comparisons, but included again when defining recommendations for these parameters.

Chapter 3: Validating Body Composition Measures of Adiposity in Zoo Western Lowland Gorillas (Gorilla gorilla gorilla)

'Adiposity' refers to body composition or, more specifically, the amount of fat an organism possesses (Frankenfield et al., 2001). 'Obesity' is defined as having an excess level of adipose tissue (i.e., body fat) in relation to lean body mass (Richardson et al., 2000). Obesity is considered to be more severe than merely being 'overweight' (i.e., an increase in body weight relative to height) (Lopaschuk et al., 2007). In fact, in humans, it is possible to be of normal weight, but to still exhibit signs of metabolic obesity if adiposity is high or concentrated in the abdominal area (Ruderman et al., 1998). In humans, obesity is associated with inflammation and insulin resistance which are in turn risk factors for the development of cardiac disease, hypertension and type II diabetes, among other health complications (Stein and Colditz, 2004). Therefore, there is a need to reduce obesity to help prevent the development of these types of diseases.

Animals living near humans are also showing trends of increasing obesity (Klimentidis et al., 2010). Specifically, captive animals such as primates are rarely as active as their wild counterparts, although they may ingest the same amount or more calories per day. Inactivity in humans is associated with the development of obesity and related diseases such as heart disease (Bensimhon et al., 2006). Currently, heart disease is the leading cause of death in captive male gorillas (Meehan and Lowenstine, 1994). It is anecdotally suspected that gorillas are obese and less active than wild counterparts, but the parameters for obesity in gorillas have not yet been defined.

This paper focuses on two indirect ways of measuring body fat mass (i.e., adiposity) in gorillas. The first involves measuring serum leptin and adiponectin, hormones produced by adipose cells, along with serum triglycerides, which comprise the

fat that is stored in adipocytes. In humans, it is generally accepted triglycerides should be

< 150 mg/dl (Miller et al., 2011). There was some disagreement in the literature on

healthy ranges for leptin and adiponectin because of variation observed in means in obese

and healthy individuals. For this study, the following ranges will be used: leptin (1-5

ng/ml for males; 7-13 ng/ml for females) (Couillard et al., 1997), and adiponectin (levels

should not fall below 4.0 μg/ml) (Im et al., 2006) because they appear to be

representative of the majority of the literature. For leptin, Couillard et al. (1997) reported

ranges for males and females at ~25% body fat which were used as the upper limit for the

healthy ranges in this study.

Leptin has been measured in a few species of non-human primates including

several macaque species (Muehlenbein et al., 2005) and baboons (Banks et al., 2001). In

rhesus macaques, leptin levels averaged 20 ng/ml compared to lean macaques which

averaged 4.3 ng/ml (Hotta et al., 2001). Adiponectin has only been measured in rhesus

macaques and was lower in obese macaques which averaged 1.4 ug/ml compared to 2.7

ug/ml in lean macaques (Hotta et al., 2001). Upon the development of type 2 diabetes,

leptin levels in the macaques decreased back towards lean levels, but the adiponectin

remained low (Hotta et al., 2001). Triglyceride levels have been reported in chimpanzees

(Videan et al., 2007). No male chimpanzees in that study had a triglyceride level above

150 mg/dl, while overweight females (> 10% above the mean) exhibited on average a

triglyceride level of 191 mg/dl compared to lean females which exhibited an average

triglyceride level of 125 mg/dl (Videan et al., 2007). All non-human primate studies

compared these serum values to what was reported as normal for humans. This is the first

study to report leptin and adiponectin levels in captive gorillas. Although triglycerides

are routinely measured clinically, this is the first study to report ranges of triglycerides for a large sample of gorillas.

This approach is specific, but problematic because it involves the use of serum which can be difficult to obtain from zoo gorillas. Most gorillas are not trained for blood sample collection while awake, so they must be anesthetized to obtain serum. Many zoos do not regularly anesthetize their animals which makes serum collection particularly difficult. It is also problematic because a one-time serum sample only provides one data point that reflects fat at that moment, not over a longer time course.

The second approach is a measure of adiposity and involves examining weight, creating a body mass index (BMI) for gorillas and examining other measures of gorilla size (such as head length and back length) as well as back width (measures underneath the shoulder, at the widest point of the back and right above the hips). It is currently unknown what a healthy weight is for gorillas. Weight alone may not be a good measurement of body condition, but should be considered in terms of the size of the animal and body composition. We also do not know how the weights of captive gorillas compare to those of wild gorillas (or what this means in terms of body composition). One study that compared the weights of wild and captive gorillas reported that there was not a significant difference (Leigh, 1994). Another study reported that, on average, wild male western lowland gorillas weigh 170 kg and wild females weigh 71.5 kg (Smith and Jungers, 1997). However, this study did not consider size or body composition and was based on a relatively small sample of gorillas at one study site.

BMI can be a useful tool for controlling for the size of the individual when examining body composition. BMI can be measured non-invasively and an increasing

BMI correlates with obesity in humans (Dietz and Bellizzi, 1999). BMI has been used to calculate whether an individual was overweight in studies using humans (Flegal et al., 2002), baboons (Muehlenbein et al., 2003), chimpanzees (Videan et al., 2007) and macaques (Muehlenbein et al., 2005). However, a BMI must be validated because it does not always correlate with increasing fat mass. In male macaques, researchers calculated a PMI (non-human primate mass index): weight (kg)/divided by crown-rump length squared (m) (Muehlenbein et al., 2005). They found that leptin levels and abdominal fat deposits were positively correlated with PMI in male macaques, supporting the contention that a PMI for gorillas should correlate with adiposity and circulating hormones. However, in a study of adult male baboons, PMI did not correlate with leptin levels (Muehlenbein et al., 2003). Based on the differences between male macaques and baboons, the researchers concluded that baboons may just have a lower body fat percentage than other primates (Muehlenbein et al., 2003) and other observers noted that male baboons have a low percentage of body fat (approximately 6%) (Rutenberg et al., 1987).

This PMI along with measurements of skinfolds was found to correlate with serum triglycerides and glucose in female chimpanzees (Videan et al., 2007). The male chimpanzee PMIs, although high, did not correlate with these serum measurements, which the authors hypothesized was because they contain large amounts of lean body mass as opposed to fat mass (Videan et al., 2007). Therefore, in some cases a PMI can be a useful measure to control for body size, but it must be validated for each species. Certain modifications of a PMI or additional measurements may be more useful depending on the physiology of the species.

Another measurement commonly used in humans to examine body fat is waist circumference. In humans, waist circumference is correlated with serum leptin levels (Staiger et al., 2003). Gorillas are hind gut fermenters and as such they have an extensive colon for processing fiber. Therefore, actual waist circumference may not be a useful measure. However, it is possible that gorillas may still carry body fat in their torso region. For this reason, back width measurements were collected as part of this study.

Obesity can begin to be defined in the captive gorilla population by using serum biomarkers such as leptin, adiponectin and triglycerides to characterize body composition. In their study of PMI in chimpanzees, Videan et al. (2007) were able to predict what might be considered obese for females by considering BMIs over 20% of the mean as belonging to obese females. They also defined pre-obese or overweight females as having a BMI 10-20% above the mean. By these calculations, they determined that approximately 10% of females are obese and 10% are at risk for obesity (Videan et al., 2007). This definition can be problematic because the majority of a population might be obese, which could cause an inflated mean.

This study examined adiposity in zoo gorillas by investigating correlations between body composition measures to determine if there are associations between them as observed in humans. Because serum samples for hormone analysis are not as easy to obtain, body composition measurements were validated by comparison to serum biomarkers for regular use by gorilla managers. Weight and PMI are two measurements that will be examined as these are more traditionally used measures of adiposity. However, because it is unknown whether these measurements will be useful for gorillas, several other potential measurements of size (crown to rump length, back length and

head length) and potential measurements of body fat/width of the back (shoulder width, hip width and widest point of the back) were measured. Again, the overall goal was to find any measure that can be non-invasively collected to help managers determine if their gorillas are obese. Furthermore, recommendations of healthy ranges of these body composition measures will be defined in captive gorillas by comparing these measures between gorillas with varying glucose and insulin concentration (see Ch. 2).

Methods

Subjects

The current study includes 101 adult gorillas housed at 29 institutions throughout the Association of Zoos and Aquariums with a total of 58 males and 43 females. The gorillas ranged in age from 8-49 years of age (mean = 21.75, median = 20.5). Not all zoos were able to provide every measurement for each gorilla so the sample size varies with each measurement (Table 1; Table 3), but all individuals did have a serum sample that was analyzed.

Body Measures

Measurements (Appendix 1) were taken on awake gorillas in holding or exhibit areas. A measurement could also be taken at a veterinary exam instead of on an awake gorilla. Measurements were made on 69 gorillas. Of these, 75.4% (52 out of 69) were made within six months of serum collection. For males, 57% of measurements were taken at exams vs. awake and for females, 65% of measurements were taken at exams as opposed to awake. PMI was calculated as suggested previously, weight (kg)/ (crown to rump length (m))^2.

Hormone Collection and Analysis

Each participating institution sent a 4 ml serum sample collected at the individual

gorilla's routine veterinary exam. Previously banked samples were accepted if they were

collected no earlier than 2008. Serum samples for this study were accepted no later than

2010. Serum samples were analyzed at the Cleveland Metroparks Zoo Endocrinology

Laboratory. The Leptin ELISA Kit (10-1199-01; Mercodia) was designed to measure

total serum leptin in human samples. This assay was validated for gorilla serum. The

Mouse/Rat Adiponectin ELISA Kit (K1002-1, B-Bridge International, INC) designed to

measure total serum adiponectin in mouse or rat samples was also validated for use with

gorilla serum. Triglycerides in serum were measured using IDEXX Vettest 8008

Chemistry Autoanalyzer.

Statistics

 For both males and females separately, each variable was assessed for normality

using a Kolmogorov-Smirnov Goodness-of-Fit test and log transformed if not normally

distributed. Specifically, for females, adiponectin was log transformed and for males,

leptin was log transformed. All body composition measures were compared between

males and females with t-tests. They were all different ($p < 0.05$), thus data for males and

females are reported separately throughout this paper. All statistics were calculated using

SPSS® software.

Body composition correlations

All body composition measures were assessed for correlation using Pearson

correlations for males (Table 2) and females (Table 4). Because PMI was not correlated

with weight or any of the width measurements as might be expected for males, a

modified PMI of weight (kg)/(back length (m))^2 (therefore, excluding head length) was calculated. Correlations for the modified PMI were calculated using Pearson correlation and added to the male correlation matrix (Table 2).

Defining body composition recommendations

Univariate ANOVAs were used to compare the body composition measures with the most significant relationship with each serum biomarker between groups as defined by glucose and insulin concentrations. These groups were defined as follows for males: group 1 (glucose < 100 mg/dl, insulin < 5.24 mU/l), group 2 (glucose < 100 mg/ dl, insulin > 5.24 mU/l), group 3 (glucose > 100 mg/dl, insulin > 5.24 mg/dl) and group 4 (glucose > 100, insulin < 5.24 mU/l). These groups were defined as follows for females: group 1 (glucose < 100 mg/dl, insulin < 11.7 mU/l), group 2 (glucose < 100 mg/ dl, insulin > 11.7 mU/l), group 3 (glucose > 100 mg/dl, insulin > 11.7 mg/dl) and group 4 (glucose > 100, insulin < 11.7 mU/l). LSD post hoc tests were used to compare differences between these groups. The mean of group 1 (low glucose, low insulin) was calculated and increased by 20% as in Videan et al. 2007 to calculate what measures might be associated with hyperglycemia.

Relationships between body composition measures and serum biomarkers

Each measurement was correlated with each dependent variable (leptin, adiponectin and triglycerides) controlling for age using partial correlation analysis. Females with serum glucose levels > 100 mg/dl and insulin levels > 11.7 mU/l (n=14) (group 4) and males with serum glucose levels > 100 mg/dl and insulin levels > 5.24 mU/l (n=19) (group 4) were removed from the analysis. The purpose of this was to control for the sharp decrease in other serum biomarkers at high glucose and low insulin

concentrations (see Ch. 2). The removal of group 4 left 38 males and 28 females for analysis. Measurements that were significantly correlated with specific biomarkers were then further analyzed to define recommendations of body measures for zoological managers. The mean of the measurement in the healthy range of the serum biomarker (based on the human literature) was increased by 20% as in Videan et al., 2007 to calculate what might be considered obese. Alpha was set at 0.05 for all statistical tests.

Results

a. *Males*

Body composition measure ranges/correlations

There were many correlations amongst various body composition measures suggesting that multiple measures can be used to assess obesity (Table 1). Widest point, shoulder width and hip width were highly positively correlated with each other (Table 1). Modified PMI was not significantly correlated with weight, but was correlated with the width measurements (Table 1).

Table 1. Correlation matrix of body composition measures for males. P = PMI, M=modified PMI, W = weight, CR = crown to rump, HL = head length, BL = back length, SW = shoulder width, HW = hip width, WP = widest point. Bold type denotes significant correlations. NS= not significant.

	M	W	CR	HL	BL	SW	HW	WP
P	**r=0.6** **p=0.000**	r = 0.2 NS	**r =-0.5** **p=0.002**	r = 0.1 NS	**r =-0.5** **p=0.002**	r = 0.1 NS	r = 0.0 NS	r = 0.0 NS
M		r=0.3 NS	**r= -0.6** **p=0.000**	**r=0.5** **0.001**	**r= -0.8** **p=0.000**	**r=0.3** **p=0.037**	**r=0.5** **p=0.005**	**r=0.4** **p=0.018**
W			**r = 0.4** **p=0.005**	**r = 0.5** **p=0.002**	r = 0.2 NS	r = 0.1 NS	r = 0.3 NS	r = 0.2 NS
CR				r = 0.2 NS	**r = 0.7** **p=0.000**	r = 0.0 NS	r = 0.2 NS	r = -0.0 NS
HL					r = -0.3 NS	**r = 0.5** **p=0.002**	**r = 0.6** **p=0.000**	**r = 0.5** **p=0.004**
BL						r = -0.2 NS	r = -0.1 NS	r = -0.3 NS
SW							**r = 0.8** **p=0.000**	**r = 0.8** **p=0.000**
HW								**r = 0.76**
WP								

The range and averages of body composition measures are reported (Table 2). None of the body composition measures increased with age.

Table 2. Body measurements and hormones (range and averages for males).

Parameter	Low	High	Mean	Median	St Dev
Weight (kg) (n=42)	136	246	177	177	52.9
BMI (kg/m^2) (n=41)	115	272	173	163	34.9
Modified BMI (kg/m$^{2)}$) (n=38)	197	617	313	298	98.0
Crown to rump (cm) (n=41)	81.5	122	103	104	10.3
Head length (cm) (n=36)	17.8	33.5	26.5	27.3	3.73
Back length (cm) (n=38)	55.9	99.1	77.1	77.8	9.60
Shoulder width (cm) (n=38)	28.0	97.0	61.4	57.2	14.8
Hip width (cm) (n=39)	20.0	71.0	45.9	42.4	10.2
Widest point (cm) (n=42)	38.5	84.0	54.8	50.8	12.2

Relationships between body composition measures and serum biomarkers

Partial correlations between body composition measures and serum biomarkers are reported in Table 3. Partial correlations revealed that none of the body composition measures were significantly related to all of the measured serum biomarkers. Additionally, several body composition measures, including PMI, crown to rump, head length, back length, shoulder width, and widest point showed no significant relationship

to any of the biomarkers. Weight exhibited a significant negative relationship with adiponectin and a significant positive relationship with leptin. Modified PMI exhibited a significant positive relationship with both triglycerides. Hip width indicated a trend towards a negative relationship with adiponectin.

Table 3. Partial correlation matrix (controlling for age) of body composition measures for males. M=modified PMI, W = weight, CR = crown to rump, HL = head length, BL = back length, SW = shoulder width, HW = hip width, WP = widest point, L=leptin, A=adiponectin, T=triglycerides. Bold type denotes significant correlations. Italics indicate a trend towards significance. NS= not significant.

	P	M	W	CR	HL	BL	SW	HW	WP
L	r = 0.1 NS	r = 0.1 NS	**r = 0.5 p=0.017**	r = 0.2 NS	r = 0.1 NS	r = 0.1 NS	r = 0.1 NS	r = 0.3 NS	r = 0.1 NS
A	r = -0.0 NS	r = -0.2 NS	**r = -0.5 p=0.008**	r = -0.3 NS	r = -0.3 NS	r = -0.2 NS	r = -0.1 NS	*r = -0.4 p=0.076*	r = -0.1 NS
T	r = 0.3 NS	**r = 0.5 p=0.015**	r = 0.2 NS	r = -0.3 NS	r = 0.3 NS	r = -0.3 NS	r = 0.3 NS	r = 0.4 NS	r = 0.1 NS

Defining recommendations of body composition measures

For males, only modified PMI and weight exhibited significant relationships with leptin, adiponectin and triglycerides. The mean of each body composition measure + 20% in the healthy ranges of these serum biomarkers (Couillard et al., 1997; Miller et al., 2011) was used as a cut-off point for when obesity may be an issue. Weights were also reported from wild gorillas to determine when obesity may be present (Smith and Jungers, 1997) based on a weight 20% above the mean weight (Videan et al., 2007).

Therefore, the following table can be used by gorilla managers as reference ranges for assessing obesity in their gorillas and can also help determine whether further analysis of serum leptin, adiponectin or triglycerides is needed (Table 6).

Table 4. Reference for gorilla managers to assess male gorilla obesity. If gorillas express measurements greater than the values in this table, managers should assess serum for obesity. Wild gorilla weights are as reported in Smith and Jungers 1997.

Body composition measure	Males
Wild gorilla weight (kg)	>204 kg
Wild gorilla weight (lbs)	> 449 lbs
Weight (kg)	>211 kg
Weight (lbs)	>464 lbs
Modified PMI (kg/m^2)	>358 kg/m^2
Modified PMI (lbs/ft^2)	>56 lbs/ft^2

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The two body composition measures that exhibited a relationship with serum biomarkers in males were analyzed according to the glucose and insulin concentrations and graphed if they were significant. The glucose and insulin groups were defined as 1 (glucose < 100, insulin < 5.24), 2 (glucose < 100, insulin > 5.24), 3 (glucose > 100, insulin > 5.24), and 4 (glucose > 100, insulin < 5.24). A univariate ANOVA revealed that weight did not vary significantly between glucose and insulin concentrations (F=0.013, p=0.998). A univariate ANOVA also revealed that modified PMI did not vary significantly between glucose and insulin concentrations (F=1.60, p=0. 28) (Figure 1). However, if group 4 is excluded from the analysis, modified PMI exhibited a trend towards varying significantly among glucose and insulin concentrations (F=2.695, p=0.091) with significantly higher modified PMIs in group 3 compared to group 2 (p=0.031) and a trend towards higher modified PMIs in group 3 compared to group 1 (p=0.071).
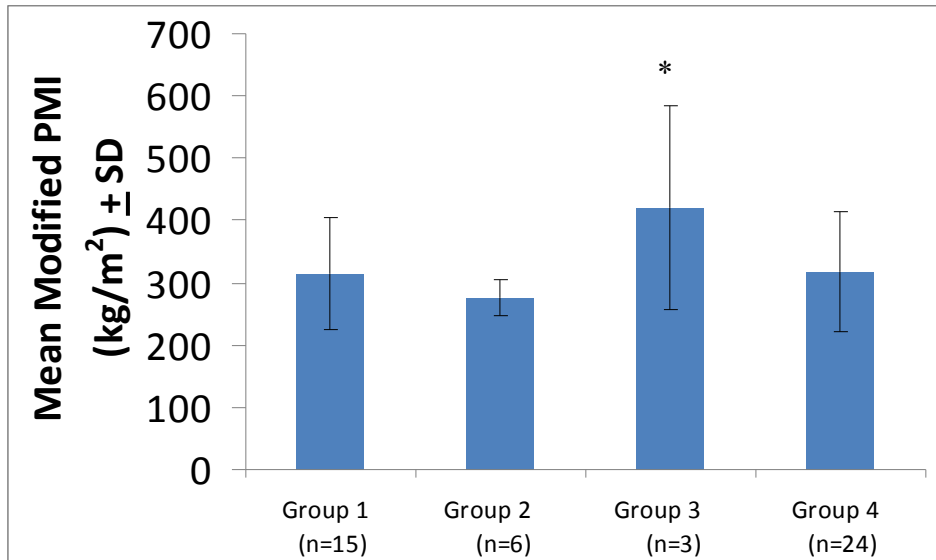
Figure 1. Modified PMI expressed via glucose and insulin concentrations. When only groups 1-3 were analyzed, modified PMI was significantly higher in group 3 than groups 1 and 2. Mean PMI in group 1 + 20% did not go above 377 kg/m^2 suggesting that PMIs above this level may be associated with hyperglycemia.

Therefore, the following table can be used by gorilla managers as reference ranges for

assessing hyperglycemia in their gorillas and can also help determine whether further

analysis of serum glucose, etc. is needed (Table 5).

Table 5. Reference for gorilla managers to assess hyperglycemia. If gorillas express PMIs greater than the values in this table, managers should assess serum for glucose and insulin concentrations.

Body composition measure	Males
PMI (kg/m^2)	>377 kg/m^2
PMI (lbs/ft^2)	> 63 lbs/ft^2

    b. *Females*

    Serum biomarker and body composition measure ranges/correlations

    There were many correlations amongst various body composition measures

suggesting that multiple measures can be used to assess obesity (Table 6). All of the

width measurements (widest point, hip width, and shoulder width) were correlated with

each other (Table 6). PMI and weight were correlated with each other and the width

measurements (Table 6).

Table 6. Correlation matrix of body composition measures for females. P = PMI, W = weight, CR = crown to rump, HL = head length, BL = back length, SW = shoulder width, HW = hip width, WP = widest point. Bold type denotes significant correlations. NS=not significant.

	P	W	CR	HL	BL	SW	HW	WP
P		**r = 0.8** **p=0.000**	r = -0.2 NS	r = 0.2 NS	**r = -0.5** **p=0.017**	**r = 0.7** **p=0.001**	**r = 0.6** **p=0.005**	**r = 0.6** **p=0.011**
W			r = 0.2 NS	r = 0.2 NS	r = -0.1 NS	**r = 0.6** **p=0.005**	**r = 0.7** **p=0.001**	r = 0.4 NS
CR				r = 0.2 NS	**r = 0.8** **p=0.000**	r = 0.2 NS	r = 0.0 NS	r = 0.1 NS
HL					r = -0.2 NS	r = 0.2 NS	r = -0.3 NS	r = 0.0 NS
BL						r = -0.1 NS	r = -0.0 NS	r = -0.1 NS
SW							**r = 0.8** **p=0.000**	**r = 0.9** **p=0.000**
HW								**r = 0.7** **p=0.002**
WP								

The range and averages of body composition measures and hormones are reported

(Table 7). None of the body composition measures increased with age except weight

which exhibited a trend towards increasing with age (r =0.329, p=0.093).

Table 7. Body measurements and hormones (range and averages for females)

Measure	Low	High	Mean	Median	St Dev
Weight (kg) (n=27)	59.1	175	95.0	205	59.2
PMI (kg/m^2) (n=26)	85.7	222	134	131	35.2
Crown to rump (cm) (n=20)	74.9	92.7	83.6	84.0	5.03
Head length (cm) (n=21)	11.8	26.0	17.9	18.0	3.13
Back length (cm) (n=20)	48.5	74.5	65.3	65.9	6.29
Shoulder width (cm) (n=22)	20.6	67.0	47.0	44.3	11.2
Hip width (cm) (n=18)	30.0	55.0	39.8	38.0	7.04
Widest point (cm) (n=19)	21.0	69.0	44.1	44.0	10.4

Relationships between body composition measures and serum biomarkers

The partial correlations between each serum biomarker and body composition measurements are reported in Table 8. Partial correlations revealed that none of the body composition measures were significantly related to all of the measured serum biomarkers. Additionally, several measures showed no significant relationship to any of the biomarkers including PMI, weight, crown to rump length, head length, and back length.

Hip width, shoulder width, and widest point all exhibited significant positive

relationships with leptin and triglycerides.

Table 8. Partial correlation matrix (controlling for age) of body composition measures for females. P = PMI, W = weight, CR = crown to rump, HL = head length, BL = back length, SW = shoulder width, HW = hip width, WP = widest point, L=leptin, A=adiponectin, T=triglycerides. Bold type denotes significant correlations. NS= not significant.

	P	W	CR	HL	BL	SW	HW	WP
L	r = 0.2 NS	r = 0.2 NS	r = 0.1 NS	r = -0.0 NS	r = 0.0 NS	**r = 0.7 p=0.003**	**r = 0.7 p=0.013**	**r = 0.6 p=0.032**
A	r = -0.3 NS	r = 0.0 NS	r = 0.4 NS	r = -0.1 NS	r = 0.4 NS	r = -0.2 NS	r = -0.4 NS	r = 0.0 NS
T	r = 0.2 NS	r = 0.4 NS	r = 0.3 NS	r = 0.5 NS	r = 0.1 NS	**r = 0.7 p=0.009**	**r = 0.7 p=0.029**	**r = 0.7 p=0.011**

Defining recommendations of body composition measures for obesity and

hyperglycemia

For females, only hip width, shoulder width and widest point exhibited significant

relationships with leptin and triglycerides. The mean of each body composition measure

+ standard deviation in the healthy ranges of these serum biomarkers (Couillard et al.,

1997; Miller et al., 2011) was used as a cut-off point for when obesity may be an issue.

Weights were used from wild gorillas to determine when obesity may be present (Smith

and Jungers, 1997) based on a weight 20% above the mean weight (Videan et al., 2007).

Therefore, the following table can be used by gorilla managers as reference

ranges for assessing obesity in their gorillas and can also help determine whether further

analysis of serum leptin or triglycerides is needed (Table 9).

Table 9. Reference for gorilla managers to assess obesity. If gorillas express measurements greater than the values in this table, managers should assess serum for obesity. Weights are from wild gorillas (Smith and Jungers, 1997).

Body composition measure	Females
Weight (kg)	>86 kg
Weight (lbs	>189 lbs
Hip width (cm)	>47 cm
Hip width (in)	>19 in
Shoulder width (cm)	>56 cm
Shoulder width (in)	>25 in
Widest point (cm)	>52 cm
Widest point (in)	>20 in

Body composition measures were analyzed according to the glucose and insulin concentrations and graphed if they were significant. The glucose and insulin groups were defined as 1 (glucose < 100, insulin < 11.7), 2 (glucose < 100, insulin > 11.7), 3 (glucose > 100, insulin >11.7), and 4 (glucose > 100, insulin < 11.7). Because there was only one female in group 3, this group was not included in statistic analyses for females. Univariate ANOVAs revealed that hip width (F=0.672, p=0.526), shoulder width (F=0.993, p=0.419, and widest point (F=0.872, p=0.477) did not vary significantly between glucose and insulin concentrations. A univariate ANOVA revealed that weight significantly varied by glucose and insulin concentrations (F=5.710, p=0.010) and that the weight of females in group 4 was significantly higher than females in group 2 (p=0.003) and there was a trend towards being higher than females in group 1 (p=0.098) (Figure 2).
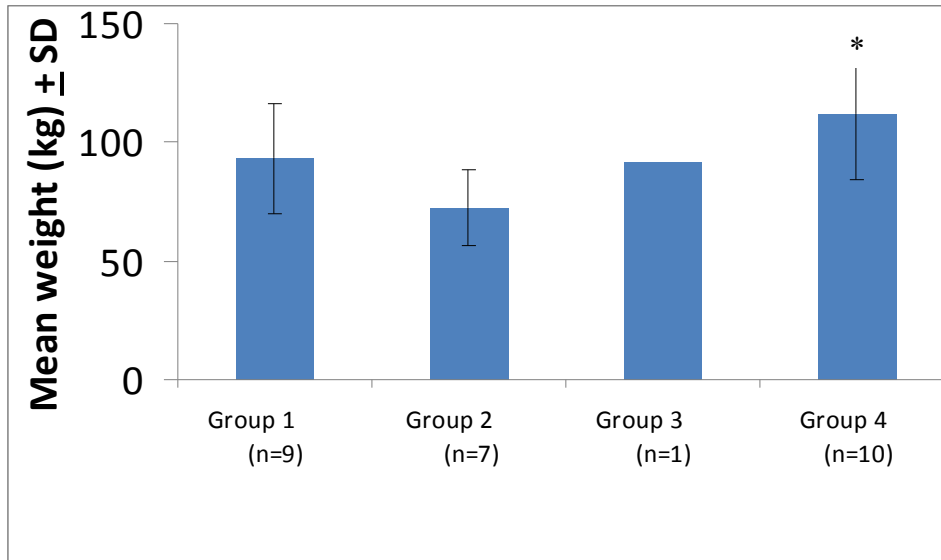
Figure 2. Weight expressed via glucose and insulin concentrations. Females in group 4 weighed significantly more than females in the first two groups (as denoted by *). The mean weight in group 1 + 20% suggests that weights above 112 kg may be associated with hyperglycemia.

A univariate ANOVA revealed that PMI significantly varied by glucose and insulin concentrations ($F=5.304$, $p=0.013$) and that the PMI of females in group 4 was significantly higher than females in group 2 ($p=0.004$) and there was a trend towards PMI being higher than females in group 1 ($p=0.051$) (Figure 3).
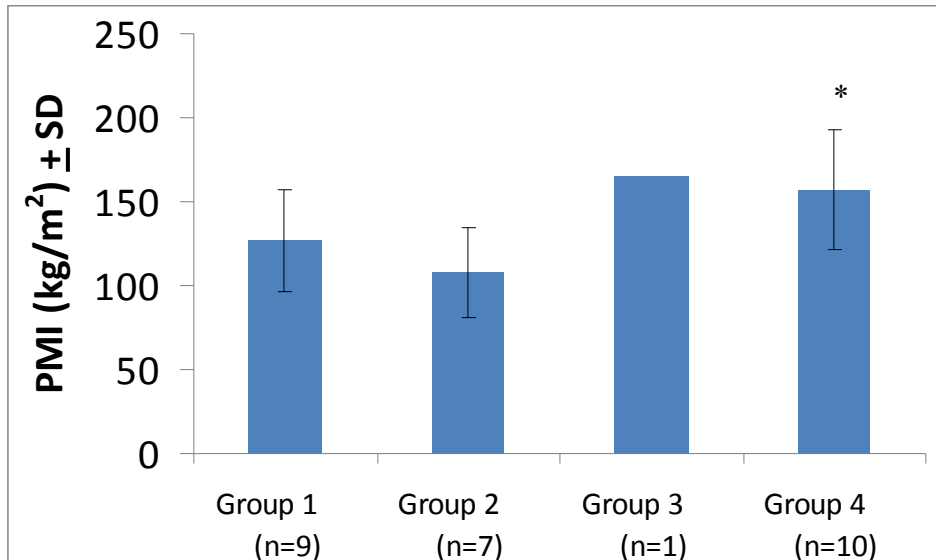
Figure 3. PMI expressed via glucose and insulin concentrations. Females in group 4 had a significantly higher PMI than females in the first two groups. Mean PMI in group 1 + 20% suggests that PMIs above 152 kg/m² may be associated with hyperglycemia.

Therefore, the following table can be used by gorilla managers as reference ranges for assessing hyperglycemia in their gorillas and can also help determine whether further analysis of serum glucose and insulin is needed (Table 10).

Table 10. Reference for gorilla managers to assess hyperglycemia. If gorillas express PMIs or weight greater than the values in this table, managers should assess serum for glucose and insulin concentrations.

Body composition measure	Females
PMI (kg/m²)	>158 kg/m²
PMI (lbs/ft²)	> 31 lbs/ft²
Weight (kg)	>112 kg
Weight (lbs)	>246 lbs

Discussion

This study aimed to identify relationships between body condition measures and serum biomarkers to determine reference ranges for obesity in zoo gorillas. Several body composition measures were significantly correlated with serum biomarkers. For males, weight emerged as the best indirect measurement of leptin and adiponectin, while

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modified PMI emerged as the best indirect measurement of triglycerides. The original PMI measure was not correlated with either weight or potential measurements of waist circumference. Historically, weight has been thought to be inaccurate as a measure of body composition in humans because it does not factor size into the equation as does a BMI calculation (Keys et al., 1972). It may be that adult male gorillas did not vary enough in size for that to play a role, simply leaving weight as the best indicator. Many arguments against the usefulness of weight and BMI as measures of obesity focus around the fact that they do not account for lean muscle mass and therefore, athletes can have a high BMI and weight, but not a high percentage of body fat (Prentice and Jebb, 2001). Gorillas do have a higher muscle mass to body mass ratio than orangutans and female gorillas have a higher muscle mass to body mass ratio than humans, but male gorillas and male humans have a similar muscle mass to body mass ratio (Zihlman and McFarland, 2000). Therefore, it did not seem likely that gorillas had such high levels of lean muscle mass that a PMI would not correlate with serum biomarkers. This is particularly true because a BMI correlates with serum biomarkers in male humans, and these data on gorillas demonstrated that weight alone exhibited a significant relationship with body fat as measured by serum leptin and adiponectin. Conversely, the relationship between triglycerides and back length suggested that gorillas with shorter torsos were more likely to have higher levels of triglycerides. One potential explanation for this might be that all male gorillas were fed similarly. Zoo diet appropriations are made without consideration for variations in body size. Therefore, a given zoo prescribed the same diet for all adult males within the zoo and thus smaller-bodied males may be receiving proportionally more food than larger bodied males, in which case shorter males may be accumulating

more adipose tissue. Head length was inversely correlated with back length suggesting that gorillas with shorter torsos also had taller heads. Feeding shorter gorillas the same as taller gorillas could lead to more adipose tissue accumulating in the sagittal crest of the head and also the increased level of serum triglycerides. Sagittal crests in gorillas contain a large pad of fatty tissue (Breuer et al., 2007) which further supports this hypothesis. This would also explain why PMI calculated with crown to rump length may not be a good measure for male gorillas. There really were no "taller" male gorillas because shorter backed gorillas had taller heads and longer backed gorillas had shorter heads eliminating any usefulness of controlling for a height measurement. In lieu of this finding, PMI was modified to exclude head length. This modified PMI was then correlated with other measures of back weight and exhibited a stronger correlation (though not statistically significant) with weight. Weight was not significantly correlated with modified PMI because there was likely more variation/error in getting the height measurements which was emphasized by squaring the height in the modified PMI calculation. A reduction in error by standardizing the measurements better probably would yield a significant correlation here. Regardless, the results indicated that the modified PMI measurement may be better for controlling for head size in gorillas than the original PMI measure. This modified PMI was also positively correlated with triglycerides and glucose indicating that shorter torso gorillas may indeed be fatter and hyperglycemic.

For females, hip width, shoulder width, and widest point were the only useful indirect measurements of body fat. In particular, female gorillas like female humans seemed to carry the majority of their fat around their hips. Interestingly, both male and

female gorillas seemed to accumulate fat in areas that were potential sexual signals. The

hips are a sexual signal for human females (Low et al., 1987) and may also be for female

gorillas. Male sagittal crests of gorillas are sexual signals for female gorillas (Breuer et

al., 2007). It makes sense from an evolutionary perspective that a member of the

opposite sex would prefer these areas that can accumulate fat as it demonstrates that

individual's ability to find food. However, in zoos, these sexual signals may be inflated

and actually misleading as individuals with more pronounced sexual signals could be at

risk for obesity and the development of associated disease states. This could provide

evidence that the thrifty genotype hypothesis is occurring in zoo gorillas, but future

research would need to focus on selection and heritability of traits in this population.

In males, weight was significantly correlated with leptin and adiponectin but did

not vary significantly when compared across various glucose and insulin concentrations.

This could mean that although weight reflected body fat, weight was not significant from

a health perspective as defined by glucose and insulin concentrations. Modified PMI

exhibited a relationship with glucose and varied significantly across glucose and insulin

concentrations when group 4 was removed from the analysis. Based on the mean plus

20%, no modified PMIs measured above 377 kg/m^2 in group 1. Although modified PMIs

in group 3 ranged from 285-586 kg/m^2 (mean $\pm$ st dev), a modified PMI > 377 kg/m^2

could put a male gorilla at risk for the development of hyperglycemia. Modified PMI

exhibited a lower mean in group 4. Weight decreases in advanced type II diabetes in

other herbivores such as horses (Durham et al., 2009). This could explain what is

occurring in group 4.

In females, hip width, shoulder width and widest point were correlated with leptin and triglycerides, but did not vary across glucose and insulin concentrations. Weight and PMI were significantly higher in group 4 than other groups. Using the mean + 20%, the highest weight for group 1 was 112 kg, 88 kg for group 2, and 112 kg for group 3. The range in weights for group 4 (mean $\pm$ st dev) was 85-139 kg. Therefore, a weight > 112 kg may be associated with hyperglycemia. However, the low weights in group 4 indicate that there may be other factors like diet that can affect hyperglycemia. Similarly, using mean PMI + 20%, the highest PMI for females in group 1 was 152 kg/m^2, 130 kg/m^2 for group 2, and 198 kg/m^2 for group 3. The range in PMIs for group 4 was 122-192 kg/m^2. Therefore, a PMI greater than 152 kg/m^2 may be associated with hyperglycemia. As with weight, the low PMIs could indicate that there are other factors, like diet, affecting hyperglycemia. The lower weights and PMIs in group 4 may also be associated with advanced disease.

Moving forward, managers should assess these non-invasively collected body composition measures in their gorillas to assess if further evaluation of serum insulin or glucose levels is needed. Previous data suggest that the average weight of wild gorillas is 170 kg for males and 71.5 kg for females (Smith and Jungers, 1997). These weights should still be used as the guidelines for gorilla weights. For females, the hip widths, shoulder widths and widest points mentioned in this chapter can be used by managers to assess if their animals may be at risk for obesity. The weights and PMIs mentioned in this chapter help to assess if a gorilla may be at risk for hyperglycemia. In other words, gorillas with high weights and PMIs may be at risk for the development of obesity or diabetes. Additionally, caution should be exercised when using the modified PMI and

PMI measurements. Anecdotally, some of the vertical measurements (crown to rump length and back length) seemed to underestimate the actual size of the gorilla. This could be due to variance in measurements; for example, one data collector may have started the measuring tape just above the rump while another started at the bottom. There could also be differences in gorilla posture with some individuals sitting up straighter than others. It is recommended that if managers find that their gorillas have high weights or modified PMIs that they further explore their blood work before officially implementing a weight loss plan. Again, future research is needed to link obesity with disease states.

Additionally, it may be important to standardize recommendations for food consumption across all males. To reduce risk of obesity, it may be better to feed gorillas with shorter torsos a reduced diet compared to that recommended for gorillas with longer torsos. For example, consider a situation where two male gorillas both weigh 205 kg, but have different torso lengths of 70.0 and 99.0 cm respectively. The former male gorilla has a modified PMI over 377 and the latter has a modified PMI under 377. It might be advisable for the former male gorilla to lose at least 21 kg to be just under the modified PMI threshold. The modified PMI measurement can therefore serve as a guide in helping managers decide if they may be feeding their gorillas to excess. However, to reiterate, if managers find that their gorillas exceed the body composition measurement guidelines they should first collect the relevant biological data i.e., glucose, triglycerides, etc., before implementing a weight loss program. The next chapter of this thesis examines dietary practices, nutrition and activity levels and their potential contribution to obesity in zoo gorillas.

Chapter 4: Assessing Inactivity in Zoo Gorillas Using Keeper Ratings and Behavior Data

Introduction

Zoo behaviorists commonly struggle with issues of small sample size (Kuhar, 2006). One recommended way to address this problem is to conduct a study involving multiple institutions (Swaisgood and Shepherdson, 2005). However, conducting direct observational behavior research at multiple institutions can be time consuming and costly (Carlstead et al., 1999). Alternatively, it can be difficult for zoo keepers and managers to make time in their already busy schedules to collect behavior data for an investigator.

Systematic data collection is traditionally preferred over keeper surveys when assessing animal behavior because it is more objective, it can be collected across different times, seasons and environments and is a quantitative form of measurement (Crockett, 1996). However, qualitative assessments are commonly used to interpret not just what an animal does, but what its subjective experience might be like (Wemelsfelder, 2007). Subjective assessment can be made objective through behavioral observations and examining if observers tend to define the subjective state of an animal in a statistically similar way (Wemelsfelder, 1997; Wemelsfelder, 2007) . Subjective assessments should be included in integrated studies of animal welfare to supplement more detailed and rigorous behavioral observation methods (Wemelsfelder, 2001). In particular, there have been instances where behavior data has either confirmed (Lukas and Thompson, 2002) or disputed (Less et al., in prep) keepers' understanding of specific animal behavior problems, but the truth about the behavior problems would not have been clarified without systematic behavior data collection. Conversely, keeper assessments are much

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quicker and easier to obtain. In personality and animal welfare research, several studies have validated keeper assessments of behavior by correlating them with systematically collected behavior data (Carlstead et al., 1999; Gosling, 2001; Kuhar et al., 2006). Therefore, in some instances keepers can serve as proxies for the animals they care for and have demonstrated an ability to provide reliable and valid assessments of these animals' well-being and behavior (Whitham and Wielebnowski, 2009). Specifically, validating keeper assessments can be helpful in the context of a multi-institutional study where large amounts of data need to be collected from many locations.

The goal of this study was to validate subjective keeper assessments of captive western lowland gorilla (*Gorilla gorilla gorilla*) activity levels against systematically collected behavior data. These data were used to examine if high levels of inactivity in gorillas are correlated with increased adipose tissue (Less et al., in prep). We propose two disparate but complementary ways to characterize inactivity in gorillas: being "idle", or at rest, performing no otherwise defined active behaviors, or being "immobile", physically stationary or not locomoting, (see definitions of idle and immobile; Appendix 1). To date, research on gorillas typically refers to the former when discussing inactivity. However, both of these ways of looking at inactivity can be useful for managing gorillas. From a behavioral standpoint, the manager's goal is to see a wide variety of dynamic behaviors occurring in zoo gorillas to indicate they are enriched and able to display a wide range of species-typical behaviors. Locomotion is also a species-typical behavior in gorillas but from a health standpoint the goal may be to see a gorilla physically mobile for a specific amount of time throughout the day not just as natural behavior but also as a

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form of physical exercise to stay fit. This study employs both as it is unclear what keepers may refer to when characterizing an animal as inactive.

An additional goal of this study is to determine if there is a relationship between these two classifications of behavior. Furthermore, the purpose of validating a keeper rating of inactivity is to increase the sample size of activity information on gorillas as only a subset of zoos may be willing or able to use staff or volunteer time to collect behavior data on their animals.

Methods

  Survey question

A survey questionnaire was sent to 15 Association of Zoos and Aquariums (AZA) institutions housing western lowland gorillas (Table 1). One or two people (typically a lead keeper or manager) completed the survey for each adult male (>12 yrs old), adult female (>8 yrs old), and subadult/juvenile gorilla that was housed at their institution and for which they also had collected behavior data. In cases where more than one person responded, ratings were averaged together.

In 66% of cases, the data collector was different than the person answering the survey question. The survey question was distributed via email and collected using SurveyMonkey® (SurveyMonkey, Palo Alto, CA) software.

Table 1. Survey question assessing keeper ratings of gorilla activity

How would you characterize this individual's overall activity level?
1. Very high-individual runs/climbs frequently and walks around exhibit often.
2. High-individual walks around exhibit frequently and occasionally runs and climbs.
3.Moderate-individual occasionally walks around exhibit, spends some time inactive.
4.Sedentary-Individual is highly inactive, walks only when necessary (i.e. to shift, etc.)

In cases where more than one person responded, ratings were averaged together.

Behavioral data collection

Behavioral data for the project were collected using group scan sampling (Altmann, 1974). To generate activity budgets for the gorillas, scan samples of each gorilla group were conducted at 3 min intervals during 30 minute observation sessions. Each day was divided into four time slots: 9:00 h-11:00 h, 11:00 h AM-13:00 h, 13:00 h-15:00 h, 15:00 h-17:00 h. Each institution needed to collect two 30-min observations per time period (providing eight 30-min observations total on each gorilla group). Observations did not have to be collected on the same day. If institutions took a month to collect data, for example, they may have chosen to only collect two observations per week for that month. Every data collector was reliability tested by video until they gained > 90% agreement with the PI. This video can be accessed at: http://www.clemetzoo.com/gorillassp/Research.html. No more than two people collected behavioral data at each institution.

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Scoring behavioral activity vs. physical activity

On each scan, the observer had to score whether each gorilla was idle or engaged in a defined active behavior and also whether the gorilla was immobile or mobile (see Chapter 5). Behavioral activity was scored to assess the percentage of time gorillas spent idle versus engaged in various other active behaviors, but physical activity was scored to examine how much time gorillas spent locomoting within the exhibit to assess activity level from a health perspective. A study in macaques determined that heart rate increased the greatest amount when an individual moved from being immobile to mobile, although it could increase if an individual was immobile and also engaged in active behaviors such as feeding (Major, 1998). Hence we used this behavioral category to examine activity in terms of health/exercise. Both behavioral activity and physical activity were scored at each scan. For example, during one observation, a gorilla might spend a majority of the time immobile but much less time both immobile and idle (Figure 1).

Physical Movement	Immobile				Mobile
Behavioral Activity	Idle	Feed/forage	Scratch	Object directed	Locomote

Figure 1. Theoretical schematic of a gorilla time budget. During each observation, both physical movement and behavioral activity were scored. All of the behaviors in each category were mutually exclusive from each other, but the categories were not.

Additionally, while immobile, several more dynamic behaviors such as feeding and scratching may have been occurring. Therefore, it might be stated that this gorilla did not spend much time behaviorally inactive or "idle" but they did spend quite a bit of time not physically moving or "immobile."

Data analysis

Mean percentage of time spent in a behavior was calculated from the scan data. Because some individuals were occasionally not visible, percentage of time in each behavior was calculated by dividing number of scans spent performing the behavior by the total number of visible scans, providing an estimate of proportion of visible time engaged in a behavior (Stoinski et al., 2004). In this case, we were only interested in the time spent immobile or idle.

For all continuous data, each variable was assessed and found to be normally distributed using a Kolmogorov-Smirnov Goodness-of-Fit test. Using Spearman rank correlations, the correlation between the survey rating and time spent immobile as calculated from the collected behavior data was assessed. The correlation between time spent immobile and time spent idle was also examined. Chi-square analyses were used to compare survey ratings across age/sex groups and one-way analysis of variance was used to compare time spent immobile/idle across age/sex groups. Alpha was set at 0.05 for all statistical tests.

Results

Demography

All 15 zoos (i.e. 100%) responded, providing a sample of 30 adult males, 29 adult females plus 14 subadult/juvenile gorillas. Subjects ranged in age from 1 to 52 years. The mean age for males was 23.5, for females 26.4 and subadult/juveniles 5.9. Adult males and females were most likely to be rated at moderate activity while juveniles were most often rated at very high activity (Table 2). The difference in survey rating proportion is significant χ^2, (10, N=73) = 36.5, p = 0.000).

Table 2. Survey ratings by number per age/gender classification

Survey Rating	# of Males	# of Females	# of Juvenile
Very high activity	2	3	11
High activity	7	8	2
Moderate activity	13	14	1
Sedentary	4	3	0

The average time spent immobile for all gorillas was 87.2%. The average time spent immobile for males was 90.0%, for females 87.2% and for juveniles 81.8%. An analysis of variance showed that the difference in time spent immobile was significant, F (2,72) = 3.3, p = 0.042. Post hoc analyses using the Tukey post hoc correction for significance indicated the average time spent immobile was significantly higher for males than juveniles (p = 0.032). The average time spent idle for all gorillas was 54.0%. The average time spent both immobile and idle for males was 56.8%, for females 57.9% and for juveniles 39.8%. An analysis of variance showed that the difference in time spent idle was significant, F (2,72) = 13.4, p = 0.000. Post hoc analyses using the Tukey post hoc correction for significance indicated that the average time spent idle for juveniles was significantly lower than the average time spent idle for both adult males and adult females (p= 0.000).

Survey correlation

Time spent immobile was significantly correlated with an increasingly inactive ranking by staff (Spearman rank, r = 0.338, p = 0.003); Figure 2)
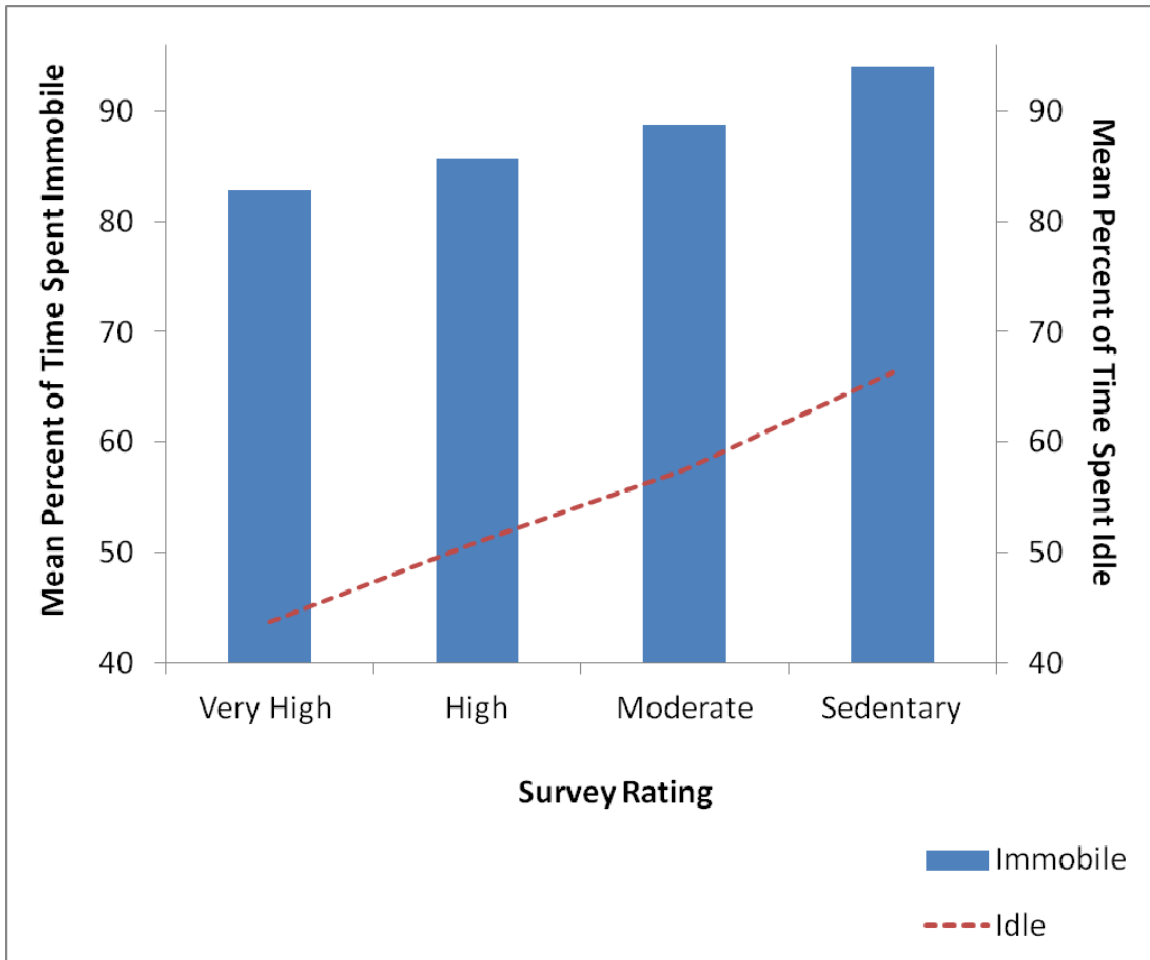
Figure 2. Mean time spent immobile and idle per activity rating.

Time spent idle was significantly correlated with time spent immobile (Spearman rank, r = .393, p = 0.001) and time spent idle was also significantly correlated with an increasingly inactive ranking by staff (Spearman rank, r = 0.474, p = 0.000; Figure 2).

Discussion

Interestingly, gorillas in general spend quite a bit of time immobile and male gorillas were the most immobile of the three experimental groups, but time spent immobile and idle did not differ significantly between males and females demonstrating that the sexes are equally inactive. Additionally, it is not surprising that juveniles spent less time immobile and idle than adult gorillas as juvenile primates are known to be more

active and playful than adults (Pereira and Fairbanks, 2002), a finding that has also been reported for gorillas in zoos (Lukas et al., 2008; Stoinski et al., 2004). Wild gorillas spend 0-28% of time idle (Magliocca and Gautier-Hion, 2002; Masi et al., 2009; Remis, 1994). To our knowledge, no research to date has reported time spent immobile in wild gorillas. Regardless, these data are consistent with previous reports indicating zoo gorillas are more sedentary than their wild counterparts; for example, zoo gorillas spend 20-25% of time consuming and foraging for food (Gould and Bres, 1986; Lukas, 1999b) compared to 54.5-72% in wild gorillas (Magliocca and Gautier-Hion, 2002; Masi et al., 2009; Remis, 1994). The difference in activity levels between wild and captive gorillas may contribute to health problems thought to occur only in the zoo population, such as heart disease (Meehan and Lowenstine, 1994). Therefore, further research needs to focus on health and welfare implications of inactivity in zoo gorillas and interventions that might mediate the negative health effects of a sedentary lifestyle.

This study adds to the growing evidence that crude staff ratings of behavior can, in some cases, be a proxy for systematically collected behavior data. This is significant because it can allow for a larger sample size without the high time and monetary costs of collecting behavior data at each institution. However, it is still recommended that ratings be validated for each new species and each new behavior, as specific behaviors may be harder to accurately rate (Gosling, 2001). Specifically, while the entire ethogram for the larger study was quite complex, this study only validates the measures of behavioral activity and physical activity. It likely would have been much more difficult to find ways to validate all of the scored behaviors with keeper assessments, so this approach might have only been successful because we did limit the behaviors that were rated. Care

should be taken to assess the situation as there may be instances where behavior data

collection alone is a preferred method over keeper assessments in addressing a behavior

problem. A combination of the two may be useful in monitoring health, behavior, and

welfare in zoo animals over long periods of time (Watters et al., 2009; Whitham and

Wielebnowski, 2009). Furthermore, future assessments of gorilla health and behavior in

zoos should incorporate the two distinct measures of inactivity developed here. It may be

that physical activity has greater implications for health than behavioral activity. Further

research is needed to determine if this is indeed true.

Chapter 5: The Relationship between Diet, Activity, and Adiposity in Captive Gorillas

Introduction

In nature, primates have evolved in areas with a diverse array of available food and as a result of this, may exhibit a wide variety of feeding styles and evolved varying gut morphologies. As Richard (1985) discusses, the order Primates comprises animals that feed on a variety of things including, insects, plant gums, fruit, seeds, leaves, herbs and combinations of all of these. Primates have evolved some flexibility within this framework to switch between food groups during times of food scarcity or specific weather/seasonal changes to the extent that any physiological specialization will allow. As an example, gorillas take most of their diet from plants and fruits and have developed an extensive colon to process this low quality, high fiber diet (Lambert, 1998; Milton, 1999). However, within this diet strategy, they can switch to more frugivorous vs. folivorous diets depending on seasonal availability (Remis, 1997) and some gorilla species have specialized to be more folivorous in general based on foods available at higher altitudes (Harcourt and Stewart, 2007).

Beyond food consumption, foraging for food is a key aspect of primate life and varies according to the type of food consumed. It has been difficult for zoos to mimic not both the type of plants and fruits consumed by wild primate species, as the cultivated vegetables and fruits grown in the country of captivity can vary significantly from what animals consume in the wild (Milton, 1999), but also to create an environment where primates can spend time foraging at the level that they do in the wild.

In North America, there are approximately 350 gorillas maintained across 52 zoos (Lukas et al., 2010b). The diets zoos feed are quite different from the diets that gorillas evolved to consume in the wild. Gorillas have evolved to process a very high fiber diet. In contrast to their diet in the wild which provides on average 74% dietary fiber (Popovich et al., 1997), zoo gorilla diets provide on average only 14% dietary fiber (Ogden and Wharton, 1997). Recommended zoo gorilla diets also contain significant amounts of commercial primate biscuits (15% of the diet), and fruit (7%; Schmidt, in press). The domesticated fruit consumed by zoo gorillas contains considerably more sugar than the fibrous fruit available in African forests (Milton, 1999; Popovich et al., 1997). Available evidence suggests that diets containing high levels of rapidly-digested starch, as are found in commercial primate biscuits, do not encourage good gastrointestinal health (as measured by gut flora biodiversity or intestinal epithelial cell cytotoxicity) in other hind gut fermenters, such as pigs (Martinez-Puig et al., 2007).

Diets are inexorably linked to activity in all species. To date, three studies reported activity budgets for wild western lowland gorillas (Magliocca and Gautier-Hion, 2002; Masi et al., 2009; Remis, 1994). Although these studies primarily utilize data collected at forest clearings (and thus represent only a portion of gorillas' overall time budget) they found that time spent resting ranges from 0-28%. Western lowland gorillas in the wild need to travel each day in order to obtain a sufficient quantity of food (Remis, 1997), and while foraging, they walk more than 1 km per day (Bellisari, 2008). In captivity, gorillas do not need to forage over long distances or climb to obtain food. This likely contributes to greater amounts of time spent inactive (32.8-75.8%) (Lukas et al., in prep).

Inactivity is related to weight gain in many organisms, including humans (Cecchini et al., 2010; Stein and Colditz, 2004). Several factors contribute to obesity in different species. Two of the most commonly studied variables are diet and activity levels. It is well-known that consuming more calories than are being burned in a given day leads to weight gain. Diets that are high in saturated fat, simple sugars, and starch and lower in fiber are typically considered calorically dense and can cause a rapid accumulation of fat tissue (Astrup, 2008). Additionally, when an individual is inactive, the number of calories that are metabolized per day is limited, making it easy to over-consume. Therefore, a combination of these two factors (a calorically dense diet and inactivity) can readily lead to obesity (Astrup, 2008; Cecchini et al., 2010; Stein and Colditz, 2004).

In humans, obesity is associated with inflammation and insulin resistance which are in turn risk factors for the development of cardiac disease (Manson et al., 1990), hypertension (Rahmouni et al., 2004) and type II diabetes (Venables and Jeukendrup, 2009). Accordingly, there is a call to reduce obesity in the human population and not surprisingly, there has been a focus on diet and activity levels. In terms of diet, health experts encourage the public to adopt diets which are high in fiber, low in fat, and high in low-energy density food items such as fruits, vegetables and whole grains (Astrup, 2008). In terms of activity, it is currently recommended that adults engage in a 20-60 minute bout of exercise 3 to 5 days a week at a level of 40-85% of their maximum heart rate to promote weight loss, improve cardiovascular risk factors and decrease mortality (Bensimhon et al., 2006).

The focus of this study was to demonstrate that diet and activity can be correlated with adiposity and insulin resistance in zoo-housed gorillas (see Chapter 1 for greater detail on these biomarkers). Leptin, adiponectin and triglycerides served as biomarkers of adiposity. Insulin and glucose served as biomarkers of insulin resistance. Cholesterol levels were increased on low fiber diets in humans (Anderson et al., 1994; Jenkins et al., 2000), so were also measured as biomarkers of low fiber diets in this study. Specifically, it was predicted that diets higher in fiber while also being lower in starch and caloric density would be associated with lower adiposity and a reduced incidence of insulin resistance. Additionally, it was predicted that gorillas that spend more time engaged in active behavior would also have lower levels of these biomarkers.

Methods

Subjects

The current study included 101 adult gorillas housed at 32 institutions accredited by the Association of Zoos and Aquariums with a total of 58 males and 43 females. The gorillas ranged in age from 8-49 years of age. Not all zoos were able to provide every measurement for each gorilla so the sample size varies with each measurement (Table 1; Table 4), but a serum sample was analyzed for all individuals.

Body Measures

Body measurements were made on 69 gorillas (see previous chapter), either while awake or during veterinary exams. Of these, 75.4% (52 out of 69) were made within six mos. of serum collection, while the other 24.6% were made greater than six mos. from the time of serum collection. For males, 57% of measurements were taken at exams vs. awake and for females, 65% of measurements were taken at exams as opposed to awake.

PMI was calculated as suggested previously for females, weight (kg)/ (crown to rump length (m))^2. Modified PMI was calculated as suggested previously for males, weight (kg)/back length (m))^2.

Diets

A report of each gorilla's daily diet was submitted by the home institution with items reported on an 'as fed' basis (g). Dietary starch, neutral detergent fiber (NDF), total dietary fiber (TDF), and kilocalories were calculated using Zootrition® software. The total amount (g) of commercial biscuits and fruit was also calculated. These dietary parameters were used as predictors of biomarkers (Table 3 & 6).

Activity

A survey was sent to 15 Association of Zoos and Aquariums (AZA) institutions housing western lowland gorillas. One or two people (typically a lead keeper or manager) completed the survey for each adult male (>12 yrs old), adult female (>8 yrs old), and subadult/juvenile gorilla that was housed at their institution and for which they also had collected behavior data. In cases where more than one person responded, ratings were averaged together. In 66% of cases, the data collector was different from the person answering the survey question. The survey was distributed via email and collected using SurveyMonkey® (SurveyMonkey, Palo Alto, CA) software. This survey was validated against behavioral data collected by the PI and keeper staff (see previous chapter).

Hormone Collection and Analysis

Each participating institution sent a 4 ml serum sample collected at the individual gorilla's routine veterinary exam. Previously banked samples were accepted if they were collected no earlier than 2008. In addition to leptin, adiponectin and triglycerides (see

chapter 2), commercially available kits were used to analyze insulin (10-1132-01; Mercodia). Each of the assays were previously validated for use with gorilla serum in the Cleveland Metroparks Zoo Endocrinology Laboratory. Glucose and cholesterol were measured using an IDEXX Vettest 8008 Chemistry Autoanalyzer.

Statistics

For males and females separately, each variable, and diet parameters were assessed for normality using a Kolmogorov-Smirnov Goodness-of-Fit test. For males, all diet parameters were normally distributed. For females, insulin, TDF and NDF were not normally distributed and were log transformed. All activity and diet parameters were compared between males and females using t-tests. Activity did not differ significantly between males and females (see previous chapter). However, all diet parameters differed between males and females, augmented by the fact that males are fed greater quantities of food. For these reasons, results will be reported separately for males and females. All statistics were calculated using SPSS® software.

Diet parameter correlations

All biomarkers were assessed for correlation amongst one another using Pearson correlations for males (Table 2 and 3) and females (Table 5 and 6).

Relationships between diet parameters and serum biomarkers

Each diet measurement was partially correlated with each serum biomarker while controlling for age. Alpha was set at 0.05 for all statistical tests.

Defining diet and activity recommendations

Univariate ANOVAs were used to compare the body diet parameters with the most significant relationship with each serum biomarker between groups as defined by glucose and insulin concentrations. These groups were defined as follows for males: group 1 (glucose < 100 mg/dl, insulin < 5.24 mU/l), group 2 (glucose < 100 mg/ dl, insulin > 5.24 mU/l), group 3 (glucose > 100 mg/dl, insulin > 5.24 mg/dl) and group 4 (glucose > 100, insulin < 5.24 mU/l). These groups were defined as follows for females: group 1 (glucose < 100 mg/dl, insulin < 11.7 mU/l), group 2 (glucose < 100 mg/ dl, insulin > 11.7 mU/l), group 3 (glucose > 100 mg/dl, insulin > 11.7 mg/dl) and group 4 (glucose > 100, insulin < 11.7 mU/l). LSD post hoc tests were used to compare differences between these groups.

Because the survey rating of activity was significantly correlated with age, with juveniles being more active than adults, the age range was limited to only adult individuals. Once gorillas reached 20 years of age, the survey rating of activity no longer demonstrated a significant correlation with age so these individuals were used in analysis. However, this led to a distribution of gorillas mainly in the moderate activity category which provided a limited range for statistical analysis.

Results

    a. *Males*

       Diet parameter correlations

The range and average cholesterol and diet parameters are reported in Table 1.

Table 1. Cholesterol and diet parameters (range and averages for males).

Measure	Low	High	Mean	Median	St Dev
Cholesterol (mg/dl) (n=58)	131	377	243	246	60.8
Amount of biscuits (g as fed) (n=56)	100	2380	1140	1100	674
Amount of fruit (g as fed) (n=48)	250	1580	816	847	364
TDF (g of dry matter) (n=50)	128	1320	483	413	230
NDF (g of dry matter) (n=49)	105	1130	401	364	209
Starch (g of dry matter) (n=44)	36.0	798	373	393	188
Kilocalories (n=51)	1690	8010	5008	4650	1777

Table 2 shows the correlations between diet parameters. Leptin was significantly negatively correlated with cholesterol (r= -0.3, p=0.022) and adiponectin was significantly positively correlated with cholesterol (r= 0.4, p= 0.007). Trigylcerides were significantly positively correlated with cholesterol (r= -0.3, p=0.039). Only the amount of biscuits fed (r= -0.3, p=0.047) was significantly negatively correlated with age.

Table 2. Correlation matrix of diet parameters for males. TDF=total dietary fiber, NDF=neutral detergent fiber, KC=kilocalories. Bold type denotes significant correlations.

	Biscuits	Fruit	TDF	NDF	Starch	KC
Biscuits		r=0.0 NS	**r= 0.3 p=0.048**	**r= 0.3 p=0.024**	**r = 0.9 p=0.000**	**r = 0.6 p=0.000**
Fruit			r=0.2 NS	r= 0.1 NS	r=0.1 NS	r=0.1 NS
TDF				**r = 0.9 p=0.000**	r = 0.3 NS	**r = 0.8 p=0.000**
NDF					r = 0.2 NS	**r = 0.8 p=0.000**
Starch						**r = 0.6 p=0.000**
KC						

Relationships between diet parameters and serum biomarkers

Partial correlations were used to examine relationship between diet parameters and serum biomarkers (Table 3). None of the diet parameters demonstrated a significant relationship with glucose or triglycerides. Biscuits, dietary NDF, dietary starch and kilocalories emerged as the diet parameters exhibiting a significant relationship with the most serum biomarkers. The amount of biscuits and NDF exhibited a significant negative relationship with leptin, while exhibiting a significant positive relationship with adiponectin and cholesterol. Biscuits exhibited a significant negative relationship with insulin. Dietary starch exhibited a significant positive relationship with cholesterol and glucose. Kilocalories exhibited a significant positive relationship with cholesterol. There was a trend towards a significant negative relationship between kilocalories and insulin.

Table 3. Partial correlation matrix (controlling for age) of diet parameters for males. TDF = total dietary fiber, NDF=neutral detergent fiber, KC=kilocalories, L=leptin, A=adiponectin, T=triglycerides, G=glucose, I=insulin, C=cholesterol. Bold type denotes significant correlations. NS= not significant.

	Biscuits	Fruit	TDF	NDF	Starch	KC
L	**r = -0.3** **p=0.045**	r = 0.0 NS	r = -0.1 NS	**r = -0.5** **p=0.012**	r = -0.1 NS	r = -0.2 NS
A	**r = 0.4** **p=0.012**	r = 0.2 NS	r = 0.2 NS	**r = 0.4** **p=0.035**	r = 0.1 NS	r = 0.3 NS
T	r = 0.0 NS	r = -0.1 NS	r = 0.0 NS	r = 0.1 NS	r = -0.0 NS	r = 0.1 NS
G	r = 0.0 NS	r = -0.2 NS	r = 0.1 NS	r = -0.0 NS	**r = 0.5** **p=0.023**	r = 0.1 NS
I	**r = -0.3** **p=0.026**	r = -0.1 NS	r = -0.3 NS	r = -0.2 NS	r = -0.2 NS	*r = -0.3* *p=0.069*
C	**r = 0.6** **p=0.000**	r = -0.2 NS	**r = 0.5** **p=0.009**	**r = 0.6** **p=0.000**	**r = 0.4** **p=0.034**	**r = 0.5** **p=0.004**

Defining diet recommendations

The two diet parameters that exhibited a relationship with serum biomarkers were analyzed according to the glucose and insulin concentrations and graphed if they were significant. The glucose and insulin groups were defined as 1 (glucose < 100, insulin < 13.1), 2 (glucose < 100, insulin > 13.1), 3 (glucose > 100, insulin >13.1), and 4 (glucose > 100, insulin < 13.1). A univariate ANOVA revealed that NDF did not differ significantly between glucose and insulin concentrations (t=1.612, p=0.200). A univariate ANOVA revealed that biscuits did vary significantly between glucose and insulin concentrations (F=3.262, p=0.030), with males in group 1 consuming more biscuits than males in group 2 (p=0.010) and group 3 (p=0.036) (Figure 1).
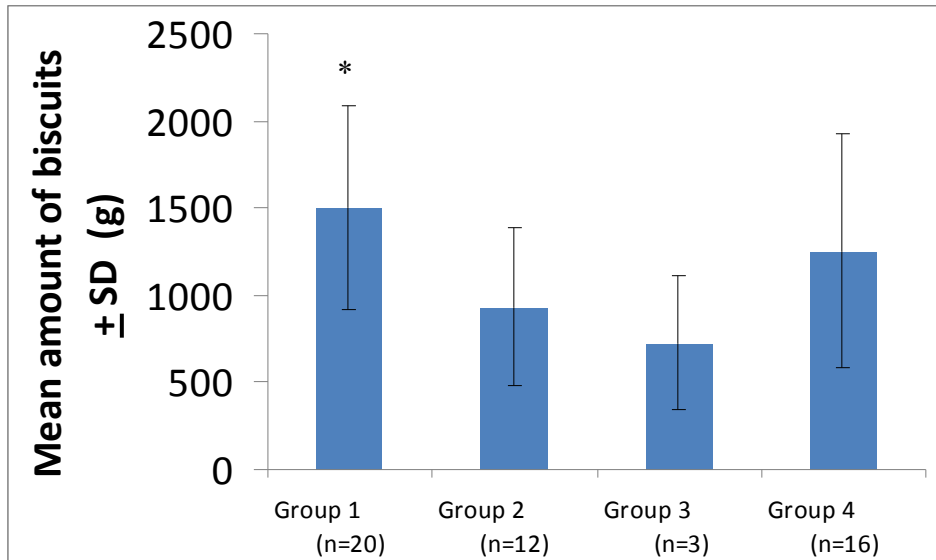
Figure 1. Amount of biscuits fed expressed via glucose and insulin concentrations. Males in group 1 consumed significantly more biscuits than males in groups 2 and 3.

b. *Females*

The range and averages of cholesterol and diet parameters are reported in Table 4.

Table 4. Cholesterol and diet parameters (range and averages for females).

Measure	Low	High	Mean	Median	St Dev
Cholesterol (mg/dl) (n=42)	128	344	196	191	48.4
Amount of biscuits (g as fed) (n=37)	100	1400	560	530	334
Amount of fruit (g as fed) (n=37)	200	1870	614	527	352
TDF (g of dry matter) (n=33)	128	750	272	228	163
NDF (g of dry matter) (n=32)	105	713	220	176	158
Starch (g of dry matter) (n=24)	41.2	495	161	152	82.2
Kilocalories (n=33)	1630	5790	2790	2510	1080

The correlations between all of the diet parameters are reported in Table 5. Cholesterol was not correlated with any of the other serum biomarkers. Only cholesterol (r= 0.5, p=0.001) increased with age.

Table 5. Correlation matrix of diet parameters for females. TDF=total dietary fiber, NDF=neutral detergent fiber, KC=kilocalories. Bold type denotes significant correlations.

	Biscuits	Fruit	TDF	NDF	Starch	KC
Biscuits		r= -0.2	r= 0.3	r= 0.3	**r = 0.8**	**r = 0.4**
Fruit			**r=0.4**	**r= 0.4**	r= -0.0	**r=0.5**
TDF				**r = 1.0**	r = 0.2	**r = 0.8**
NDF					r = 0.0	**r = 0.8**
Starch						r = 0.3
KC						

Diet parameters were compared to serum biomarkers using partial correlations and controlling for age (Table 6). No diet parameters demonstrated a significant relationship with insulin or cholesterol. The amount of fruit emerged as the parameter that was significantly correlated with the most biomarkers. The amount of fruit had a significant positive relationship with leptin, triglycerides and glucose, while also exhibiting a significant negative relationship with adiponectin. There was a significant negative relationship between calories and inuslin. There was also a trend towards a significant negative relationship between starch and adiponectin (t=1.957, p=0.069). There was a trend towards a significant positive relationship between calories and leptin (t=1.957, r= 0.7, p=0.064).

Table 6. Partial correlation matrix (controlling for age) of diet parameters for females. TDF = total dietary fiber, NDF=neutral detergent fiber, KC=kilocalories, L=leptin, A=adiponectin, T=triglycerides, G=glucose, I=insulin, C=cholesterol. Bold type denotes significant correlations. Italics denote trends toward significance.NS= not significant.

	Biscuits	Fruit	TDF	NDF	Starch	KC
L	r = -0.1 NS	**r = 0.7 p=0.000**	r = 0.1 NS	r = 0.2 NS	r = -0.1 NS	*r = 0.4 p=0.086*
A	r = 0.1 NS	**r = -0.6 p=0.002**	r = -0.1 NS	r = -0.1 NS	r = 0.3 NS	*r = -0.4 p=0.061*
T	r = 0.1 NS	**r = 0.5 p=0.028**	r = 0.4 NS	r = 0.2 NS	r = 0.2 NS	r = 0.3 NS
G	r = -0.2 NS	**r = 0.6 p=0.004**	r = -0.1 NS	r = -0.1 NS	r = 0.2 NS	r = 0.1 NS
I	r = -0.2 NS	r = 0.3 NS	r = -0.3 NS	r = -0.3 NS	r = -0.1 NS	**r = -0.4 p=0.038**
C	r = 0.1 NS	r = -0.1 NS	r = 0.1 NS	r = 0.1 NS	r = 0.2 NS	r = 0.1 NS

Defining diet recommendations

The two diet parameters that exhibited a relationship with serum biomarkers were analyzed according to the glucose and insulin concentrations and graphed if they were significant. The glucose and insulin groups were defined as 1 (glucose < 100, insulin < 13.1), 2 (glucose < 100, insulin > 13.1), 3 (glucose > 100, insulin >13.1), and 4 (glucose > 100, insulin < 13.1). A univariate ANOVA revealed that calories did not vary significantly between glucose and insulin concentrations (F=1.487, p=0.239). A univariate ANOVA revealed that fruit varied significantly between glucose and insulin concentrations (F=6.038, p=0.002) and that the fruit consumed by females in group 3 was significantly higher than group 1 (p=0.000, group 2 (p=0.002) and group 4 (p=0.000) with a trend towards the amount of fruit being higher in group 1 than in group 2 (p=0.077) (Figure 2). However, if females from group 3 were removed from the analysis, the ANOVA was not significant (F=2.125, p=0.136).
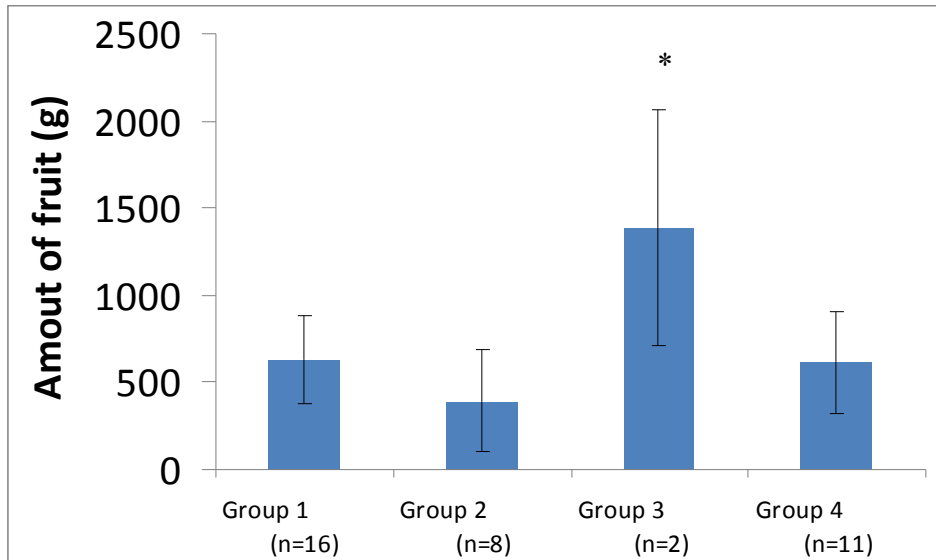
Figure 2. Amount of fruit expressed via glucose and insulin concentrations. Females in group 3 consumed significantly more fruit than females in the other groups.

Activity levels were not significantly correlated with any biomarkers.

Discussion

The results of this study demonstrate that there are significant correlations between diet and serum biomarkers of obesity and insulin resistance in the captive gorilla population. The amount of biscuits fed was negatively correlated with age in males indicating that as males get older they are fed fewer biscuits. Managers may cut down on biscuits fed as older gorillas may start to appear more obese or as they advance in age. Furthermore, males were fed about twice as much food as females which was not surprising because they are approximately twice the body size of females. However, the diets provided here were what zoos feed the entire group of gorillas based on what they estimate each individual eats. Many females may eat more than what is estimated on paper so to say that males consume twice as much food as females in zoo could be erroneous.

For males, biscuits (and the other highly correlated diet parameters NDF, starch and kilocalories) exhibited negative relationships with leptin and insulin, while exhibiting a positive relationship with adiponectin and cholesterol. Commercial diets or "biscuits" were the main source of fiber in gorilla diets, but also the main source of starch and calories. It may be that the fiber provided by the biscuits improved insulin sensitivity, but the paradoxical increase in cholesterol may be explained by saturated fat present in the biscuits (Medallion Labs analysis of Marion Leafeater® biscuits). The high fiber content of the biscuits likely led to lower obesity as well. Because biscuits were associated with higher levels of cholesterol and cholesterol is associated with the development of heart disease in humans (Assmann and Gotto, 2004), it may be good to drastically reduce the amount of biscuits and find other sources of NDF.

In females, fruit appeared to be associated with obesity. All obesity biomarkers were correlated with the amount of fruit in the diet as was glucose. Females in group 3 consumed significantly more fruit than females in the other groups. Using the st dev, the mean + st dev for group 1 was 882 g of fruit, 689 g of fruit for group 2, and 905 g of fruit for group 4. The range of fruit fed in group 3 was 707-2067 g of fruit. Diets containing amounts of fruit > 905 g may be associated with hyperglycemia. The hyperglycemia seen in animals that are receiving fruit in lower amounts suggest that there may be other sources of hyperglycemia other than fruit. The lower amount of fruit fed in group 4 deserves further research. One possibility may be that because group 4 was associated with increased weight and PMI, managers may decrease fruit in the diets in an attempt to decrease weight.

There were sex differences in which diet parameters associated with the serum biomarkers with biscuits exhibiting a relationship with more serum biomarkers in males and fruit exhibiting a relationship with more biomarkers in females. One reason for this may be that male gorillas get twice as many biscuits as females. Biscuits are a diet item that is often hand fed by keepers so it could be likely that they consume twice as much of this diet item as females. Therefore, they may be getting twice the fiber, but also twice the cholesterol as females. Fruit is a diet item that is often fed scattered throughout the exhibit so it is unknown how much fruit females are actually consuming. They could be eating the same amount or more fruit than males which may be why this diet parameter was significant for them. Additionally, there was a trend towards calories correlating with serum biomarkers in females. On paper, females eat only half of what males eat, but again what females are actually consuming on exhibit may be the same amount of calories as males.

Activity was not significantly correlated with any of the biomarkers. This could mean that diet has greater implications for obesity than activity levels. Another possibility is that treating inactivity as a categorical variable limited the range of data to lumping gorillas all within one survey rating. However, if the physical inactivity of all adult gorillas placed them in either group 3 (moderate activity) or group 4 (sedentary) (see Chapter 4), then most gorillas spent between 90-98% of time physically inactive. This indicates that all of the gorillas in the population may be so equally inactive that there was not a wide enough range in activity levels to assess correlations. The best way to understand activity in gorillas may be to experimentally increase activity and observe if this improves health parameters. Several studies have demonstrated ways to increase

activity in gorillas. One study found that rotating gorilla groups between different enclosures led to greater activity levels generalized in both enclosures (Lukas et al., 2003). Another study on chimpanzees found that providing variable feeding times led to greater activity levels throughout the day (Bloomsmith and Lambeth, 1995). Care should be taken to ensure that increasing activity levels does not also increase stress levels. These types of methods can be compared with serum biomarkers to determine if increasing activity has an effect on health.

One limitation of this study was that the history of the gorillas was not examined. For example, a gorilla may have been fed a very unhealthy diet for several years and then transferred to a zoo with a healthier diet, but the health status would reflect the previous diet. Therefore, longitudinal studies of gorillas at the same institution are needed to examine the effect of specific diets on the development of obesity and associated health issues. Future research is needed to examine the relationship between biomarkers and disease states such as heart disease and diabetes in gorillas to determine if obesity (and potentially diet) contributes to disease development. Furthermore, experimental approaches are needed to determine the role that fiber, starch and sugar have in the development of obesity. Biscuits may be a healthy diet item as the only source of fiber, but the high levels of sugar, starch and saturated fat they contain could have deleterious health effects. Finally, there needs to be an experimental approach to increasing activity levels in gorillas in order to determine if activity is playing a role in the development of obesity.

Chapter 6: Removal of Primate Biscuits from Gorilla Diets: The Impact on

Behavior, Adiposity and Health

Introduction

Wild gorillas spend a significant amount of time eating/manipulating food. To

date, three studies reported activity budgets for western lowland gorillas (Magliocca and

Gautier-Hion, 2002; Masi et al., 2009; Remis, 1994). Although these studies primarily

utilize data collected at forest clearings (and thus only a portion of gorillas' overall time

budget) they suggest that time spent resting ranges from 0-28% and time spent

locomoting ranges from 11.7-16.5%. The majority of time (54.5-72%) is spent

feeding/foraging. Although the proportion of time spent feeding may be inflated by the

fact that gorillas come to these clearings to feed, wild gorillas undoubtedly spend a

considerable portion of their day engaged in active behaviors. Western lowland gorillas

in the wild need to travel each day in order to obtain a sufficient quantity of food (Remis,

1997), and while foraging, they walk more than 1 km per day (Bellisari, 2008). Wild

gorillas also spend time climbing when feeding and when building night nests (Remis,

1997). Climbing is classified as vigorous activity in macaques (Major, 1998). In

captivity, gorillas do not need to forage over long distances or climb to obtain food. This

likely contributes to greater amounts of time spent inactive (32.8-75.8%) and lower

amounts of time feeding/foraging (9.3-35.8%) (Lukas et al., in prep).

Nonhuman primates occasionally exhibit behaviors thought to occur only in

captivity and are considered undesirable. In gorillas, an undesirable behavior is defined

as a behavior that occupies a disproportionate amount of time in the zoo environment as

compared to the activity budgets of wild populations (Lukas, 1999a). These can include

behaviors that are related to feeding such as regurgitation and reingestion (Gould and

Bres, 1986; Lukas, 1999a) and coprophagy (Akers and Schildkraut, 1985; Gould and

Bres, 1986). Other self-directed behaviors, including hair-plucking and self-injurious

behaviors, were suggested to be related to stress (Lutz et al., 2003), serve a stimulatory

function (Christenson and Mansueto, 1999) or are socially transmitted during

development (Less et al., in press). The causes of these behaviors are difficult to

determine and, once established, the behaviors may be difficult to extinguish. However,

at least in the case of regurgitation and reingestion in humans, the behavior can be

reduced by behavioral interventions such as providing aversive stimuli when the behavior

is being performed (Sanders-Dewey and Larson, 2006) or combining several techniques

such as rewarding other behaviors, interrupting the behavior, and exercise (Wrigley et al.,

2010). Also successful, and in some cases more so than behavior modifications, dietary

modifications such as feeding the individual to satiation (Johnston, 1993) or feeding

them to satiation with starchy items in particular has reduced this behavior almost to zero

(Dudley et al., 2002). Other dietary manipulations such as increasing caloric intake,

changing food consistency and increasing food quantity have had mixed results on

regurgitation behavior in humans (Dudley et al., 2002). The contrast between the low-

volume, calorically-dense diets that captive gorillas typically receive and the high-

volume, low-calorie diets consumed by their wild counterparts suggests that diet

composition likely has a role in this behavior. Though quantitative data are limited,

available research suggests that increasing both opportunities for foraging and fiber

content/volume of the diet greatly reduces regurgitation and reingestion (Gould and Bres,

1986; Hill, 2004; Ruempler, 1992) and hair-plucking behavior in captive gorillas (Hill, 2004).

Historically, zoos tended to vary significantly in the food provided to captive animals and often the type of diets offered were ultimately inadequate (Ratcliffe, 1963). For this reason, Ratcliffe (1963) formulated a series of "cakes" that could be fed to many zoo animals, including the great apes, which he felt would ensure proper nutrition and would allow for some consistency in diet among institutions. He argued that animals fed these diets exhibited improved health. Some agreed that these "cakes" could serve as a nutritional supplement to a diet of fruits and vegetables, but disagreed that they should be fed as a main constituent in the diet (Cousins, 1976). Cousins (1976) also argued specifically for gorillas that fruit should only be fed as a supplementary food source because wild gorilla diets are focused mainly on folivorous plants (but see more recent articles supporting frugivory as common in wild western lowland gorillas such as (Remis, 1997). There are currently several brands of commercial primate biscuits that serve as one component, or in some cases, as the staple food item of primate diets which also include fruits and vegetables. These biscuits are an improved form of these original "cakes" and do help to serve as a multi-vitamin for primates. One characteristic of these biscuits that remains a concern is that they are high in carbohydrates in the forms of starch and sugar (with some containing as much as 30% starch) and are also high in calories. A diet high in carbohydrates is associated with significant health concerns, especially for folivorous and frugivorous primates (Kopp, 2006). Furthermore, because primates can quickly consume most of their day's calories by ingesting primate biscuits, feeding these types of biscuits eliminates a key factor of primate feeding ecology—the

ability to forage for long periods of time. Additionally, similar to the Western human diet, the diets of many captive primates not only consist of energy dense foods and high amounts of carbohydrates, they are also high in fat and low in dietary fiber (Popovich and Dierenfeld, 1997). Such diets may be significantly different from the natural diets of primate species that evolved to consume high fiber, low carbohydrate, low fat diets, as is the case for western lowland gorillas (Popovich et al., 1997).

The specific components of gorilla diets can also have a significant impact on rates of undesirable behaviors and on feeding activity. The removal of milk from gorilla diets greatly reduced regurgitation and reingestion (R/R) behaviors (Lukas, 1999b). Another dietary manipulation has been to increase fiber in gorilla diets. Cleveland Metroparks Zoo added a fiber supplement to the gorilla diets and found that gorillas had improved stool consistency, but that the increased fiber had no real measurable effect on the gorillas' behavior and, in fact, even slightly increased the rate of R/R (Lukas et al., 2010a). Other researchers increased fiber by providing larger amounts of plant material and reducing fruit (but still feeding commercial biscuits) and found that gorillas spent more time exhibiting natural foraging behavior and spent less time inactive along with also showing improved stool consistency (Savini et al., 2000). Researchers at a German zoo conducted a diet change which consisted of providing larger amounts of browse and reducing simple carbohydrates, milk and meat products; they found that R/R was eliminated (Ruempler, 1992). Ruempler (1992) did not report if commercial biscuits were ever part of the diet.

High fiber diets can additionally impact serum biomarkers associated with obesity, insulin resistance and inflammation in humans. Specifically, high fiber diets

alone have decreased serum levels of insulin (Weickert et al., 2006), leptin (Murakami et al., 2007) as well as glucose, triglycerides and serum cholesterol (Anderson et al., 1994). High fiber diets plus an increase in aerobic exercise decreased levels of serum amyloid A (SAA) in humans (Wegge et al., 2004).

As demonstrated, feeding greater quantities of vegetables and browse can increase feeding activity and reduce undesirable behaviors while the increase in fiber associated with large amounts of vegetables and browse can improve stool consistency. However, no one has tested the effect of different types of non-browse diets and different levels of activity on adiposity in gorillas. In this study, a new form of diet was tested which included removing biscuits and replacing them with a greater volume of produce. It was hypothesized that increasing the amount of produce in the diet would increase time spent feeding and also decrease undesirable behavior. It was also hypothesized that a high fiber, low starch diet would decrease serum leptin, insulin, triglyceride, cholesterol, and glucose levels, while increasing adiponectin levels. The goal was to identify a practical way for zoos to improve the activity levels, undesirable behavior, nutrition and adiposity of their gorillas by manipulating the diet.

Methods

Subjects

The current study included 32 gorillas housed at select institutions (Tables 1 and 2) throughout the Association of Zoos and Aquariums with a total of 12 males and 20 females. The gorillas ranged in age from 1-47 years of age. At some institutions, several gorillas did not have their diet changed and served as controls (Table 2).

Table 1. Subject information for the experimental group. All individuals in this group underwent a diet change. CMZ=Cleveland Metroparks Zoo, CZA=Columbus Zoo and Aquarium, NCZ=North Carolina Zoological Garden, TZ=Toronto Zoo, WPZ=Woodland Park Zoo and SB=studbook.

Individual	Institution	Sex	Age (study period)
Bebac SB# 872	CMZ	Male	25
Mokolo SB# 948	CMZ	Male	22
Macombo II SB# 836	CZA	Male	27
Oliver SB# 1040	CZA	Male	22
Nia SB# 1306	CZA	Female	16
Kambera Dupe SB# 1543	CZA	Female	11
Moana SB# 1678	CZA	Female	8
N'kosi SB# 1195	NCZ	Male	17
Donna SB# 336	NCZ	Female	40
Katie SB# 498	NCZ	Female	35
Hope SB# 559	NCZ	Female	33
Charles SB# 551	TZ	Male	38
Josephine SB# 524	TZ	Female	39
Johari SB# 1679	TZ	Female	8
Shalia SB# 1698	TZ	Female	8
Sadiki SB# T1203	TZ	Male	5
Vip SB# 687	WPZ	Male	31
Amanda SB# 549	WPZ	Female	40
Jumoke SB# 889	WPZ	Female	25
Calaya SB# 1699	WPZ	Female	8

Table 2. Subject information for the control group. This group did not undergo a diet change and served as a control. CZA=Columbus Zoo and Aquarium, TZ=Toronto Zoo, WPZ=Woodland Park Zoo and SB=studbook.

Individual	Institution	Sex	Age (study period)
Mumbah SB# 379	CZA	Male	45
Pongi SB# 269	CZA	Female	47
Lulu SB# 262	CZA	Female	46
Dotty SB# T1182	CZA	Female	6
Umande SB# T1217	CZA	Male	4
Ngozi SB# 1503	TZ	Female	12
Nassir SB# 1998	TZ	Male	< 1
Pete SB# 384	WPZ	Male	42
Nina SB# 330	WPZ	Female	42
Nadiri SB# 1424	WPZ	Female	14
Naku SB# 1578	WPZ	Female	10
Akenji SB# 1680	WPZ	Female	9
Uzumma SB# T1324	WPZ	Female	2

Diet Change

The proposed study necessitated a process of successive biscuit reduction, from the current level to 0% of the troop's daily diet (by weight), with each decrease occurring at three-seven day intervals over the course of 2-4 weeks. The transition time for the diet was modified per the discretion of the animal care/veterinary staff at each institution. The purpose of this gradual decrease was to allow the gorillas to adjust behaviorally to the diet change while also providing sufficient time for gut flora to respond. Associated

with the reduction in biscuits was a transition to the proposed, or similar, biscuit-free diet.

While study participation did not require precise adherence to the proposed diet, alternate

diets did not contain any commercial biscuits and had reduced amounts of fruit. All

participating institutions were also required to feed a lower overall amount of dietary

starch and an equivalent or higher level of dietary fiber. Diets adhered to the National

Research Council (NRC) guidelines for non-human primates as closely as possible.

Dietary starch, neutral detergent fiber, total dietary fiber, simple sugars and kilocalories

were all calculated using Zootrition® software (Table 3). Nutrients were calculated as

both percentage of dry matter and grams of dry matter. Table 3 reports nutrition

information for each institution before and after the diet change. An example diet for an

adult male and adult female is included except in the case of CZA where two adult males

were fed differently so both are included. To replace the neutral detergent fiber found in

biscuits, gorillas were fed alfalfa hay.

Browse and consumption

Browse was not included in the diet manipulation, but institutions were

encouraged to keep browse provision constant between the two conditions so that the diet

change was the only variable. Staff was also encouraged to monitor consumption of all

diet items. At CMZ, all diet items were weighed in and weighed out daily for a rough

measure of consumption.

Resistant Starch

There was an additional diet manipulation occurring at both CMZ and CZA as

part of another study (Dennis et al., in prep). This study involved the feeding of resistant

starch, or a type of starch that mimics the function of fiber, in particular neutral detergent

fiber. The product used was Hi-Maize Resistant Starch® made by Honeyville Food Products (www.honeyvillegrain.com). This diet manipulation had no effect on behavior (Lukas et al., 2010). However, this diet manipulation confounded the interpretation of health data and therefore the effect of this diet change on health is reported here. The timeline for the diet changes was as follows: baseline biscuit-based diet, addition of resistant starch (gorillas were on this supplement for about 3 yrs) and then the biscuit-free diet (the resistant starch supplement was kept on as part of this diet in addition to the other changes previously mentioned).

Body Measures

Measurements (see chapter 1) were taken on awake gorillas in holding or exhibit areas. A measurement could also be taken at a veterinary exam instead of on an awake gorilla. Measurements were made on 14 of the sampled gorillas.

Hormone Collection and Analysis

Only CMZ, CZA and NCZ provided serum samples for this study. A 4 ml serum sample collected at the individual gorilla's routine veterinary exam. Previously banked samples were accepted if they were collected no earlier than 2008. Serum samples were validated for use in gorillas and analyzed by enzyme immunoassay as discussed in previous chapters.

Behavioral data collection

Behavioral data for the project were collected using group scan sampling (Altmann, 1974). To generate time budgets for the gorillas, scan samples of each gorilla group were conducted at 3 min intervals during 30 minute observation sessions according to the ethogram (Table 3). In addition, all occurrences of undesirable behaviors were

counted as defined in the ethogram (Appendix 1). Each day was divided into four time slots: 9-11 AM, 11 AM-1 PM, 1-3 PM, 3-5 PM. Each institution needed to collect two 30-min observations per time period (providing us with eight 30-min observations total on each gorilla group). Observations did not have to be collected on the same day. If institutions took a month to collect data, for example, they may have chosen to only collect two observations per week for that month. Every data collector was reliability tested by video until they gained > 90% agreement with the PI. Because NCZ utilized keeper staff to collect data, they conducted scan samples at 1 minute intervals during 10 minute observations to fit around their work schedule. Tempeartures in the pre- and post-time slots were controlled for such that the mean temperatures did not vary more than 5° F (except Toronto: pre-diet change: 70°F, post-diet change: 60° F). The number of observations in the outdoor/indoor exhibits were controlled for at each institution that had both types of exhibits. At CMZ, a third condition of behavioral data was collected after weights had stabilized.

Table 3. Ethogram

Behavioral Activity	Operational Definition
Solitary Behaviors (collected by scans)—includes both static and dynamic behaviors	
Idle	Inactive, at rest, not engaged in any obvious activity or otherwise defined behavior.
Locomote	(not related to foraging) Moving actively on ground or climbing.*
Feed/forage	Actively eating, carrying, searching for or processing food, includes drinking water.**
Object manipulation	Actively handling a non-natural object that is not food. If hand is resting on object and not manipulating it, please score as inactive.
Self-directed	Includes grooming (touching, licking, inspecting hair), scratching, self-mouthing (chewing on hair or skin for >5 secs). ***
Other	Any solitary behavior that is not described by the ethogram.
Not visible	Animal is not within view of the observer.
Undesirable Behaviors (collected by scans and all-occurrence)—all are examples of dynamic behavior	
Regurgitation, reingestion	Voluntary retrograde movement of fluid into mouth, hands ,or substrate followed by subsequent consumption (one bout consists of pre-R/R behavior (stomach drumming, hand shaking, etc.), regurgitation and reingestion).
Coprophagy	Ingesting feces (one bout refers to transfer to mouth and consumption).
Hair plucking	Pulling out hair, inspecting it and potentially ingesting it (one bout consists of subsequent plucking, inspecting, ingesting or discarding).
Picking	Repeatedly disturbing an existing wound or creating a new wound (one bout ends when behavior has stopped for five seconds).
Other	Includes ear covering, tongue wagging, head or hand shaking, etc. (one bout ends when behavior has stopped for five seconds).
Social Behaviors (collected by scans)—all are examples of dynamic behaivor	
Affiliative	Animal is receiving or initiating allogrooming, social play, or sexual behavior.
Agonistic	Animal is tight-lipped, charging, chest beating, ground slapping, throwing straw, etc. at another individual or strikes another individual in an aggressive manner.
Displace	Animal approaches another, the other moves away (animal can be initiating or receiving a displace behavior).
Physical Activity (collected by scans)	
Immobile	Animal is not moving more than one body length and assumed to be at a resting heart rate.
Mobile	Animal is moving more than one body length and is assumed to have a higher than resting heart rate.

Not visible	Animal is out of sight such that the movement cannot be observed.

* Once selecting locomote, the observer will be able to select ground or climb.
** Once selecting feed/forage, the observer will be able to select the type of food.
***One selecting self-directed behavior, the observer will be able to select self-groom,
    scratch, or self-mouth.

Statistics

Each variable was assessed for normality using a Kolmogorov-Smirnov

Goodness-of-Fit test and log transformed if not normally distributed. A repeated

measures ANOVA was used to analyze pre- and post- diet change behavioral data and

institution was the between-subjects factor. A multivariate ANOVA was used to

examine differences in post-diet change behaviors across institutions. Alpha was set at

0.05 for all statistical tests. At CMZ only, a third condition of behavioral data was

compared to the biscuit-free condition to determine if behavior differed once weights had

stabilized. Only standard error bar comparisons, instead of statistics, were used because

there was a small n of only two individuals at CMZ.

Health data from the biscuit-free diet manipulation and addition of resistant starch

were similarly assessed for normality and all were normally distributed. Because health

data was only available from three institutions, individuals were divided into groups

based on whether the institution reported that they consumed the diet, exhibited poor

consumption or were the control group. A repeated measures ANOVA controlling for

consumption was used to examine differences among these groups. Alpha was set at 0.05

for all statistical tests. All data were calculated using SPSS® software.

Results

Nutrition

The biscuit-free diets varied by institution but all involved the elimination of biscuits, reduction of fruit, reduction of dietary starch, and maintenance or increase of dietary fiber and neutral detergent fiber (Table 4).

Table 4. Nutrition information for diets before and after the diet change. Nutrition information is listed as both a percent of total diet dry matter / grams of dry matter. OD=original diet, BF=biscuit-free diet, NDF=neutral detergent fiber, and TDF=total dietary fiber.

Subject	Diet Phase	TDF	NDF	Starch	Sugars	Kilocalories
CMZ male	OD	39%/454	10%/115	15%/171	11%/128	2303
CMZ male	BF	41%/950	21%/488	1%/24	8%/173	5141
CZA male	OD	26%/404	9%/138	22%/347	20%/320	4113
CZA male	BF	34%/790	12%/292	4%/88	9%/215	6176
CZA female	OD	36%/263	9%/65	18%/86	12%/85	1904
CZA female	BF	43%/562	23%/304	3%/41	8%/105	2694
NCZ male	OD	25%/508*	18%/350	24%/480*	16%/314*	3713
NCZ male	BF	36%/1270	32%/1101	2%/78	17%/604	7227
NCZ female	OD	25%/188	15%/116	19%/149	24%/180*	1741
NCZ female	BF	34%/701	27%/560	2%/41	17%/337	5142
TZ individual	OD†	28%/1140	24%/977	21%/852	16%/643	10724
TZ individual	BF†	31%/757	27%/667	21%/526	14%/342	5909
WPZ male	OD	37%/798	35%/745	7%/156	15%/320	3768
WPZ male	BF	39%/1004	36%/919	1%/30	9%/244	5021
WPZ female	OD	38%/780	36%/735	6%/135	14%/287	3381
WPZ female	BF	41%/625	38%/577	1%/18	10%/150	2533

*Many commercial diets do not report values for TDF, sugar, and starch. If a commercial diet did not have a value for TDF, sugar, or starch, values for commercial diets were estimated based on analysis of CMZ's commercial diet.
†Diet was reported per individual, not by gender. Diet analysis did not include primate muffins and sofbill gelatin because of lack of nutritional information.

Data from CMZ demonstrated 100% consumption of all food items.

Behavior of Experimental Groups

A repeated measures ANOVA of all institutions (controlling for consumption) revealed a significant decrease in the rate of R/R (F=7.65, p=0.014; Figure 1) and a significant increase in feeding (Figure 2) and the rate of coprophagy (pre-diet change mean: 0.028 bouts/hr $\pm$ 0.014, post-diet change mean: 0.109 bouts/ hr $\pm$ 0.051; F=42.3, p=0.000). The rate of coprophagy was not a normally distributed variable, even when log transformed as many individuals did not perform the behavior. There was a trend towards an institution effect on R/R (F=2.93, p=0.056) and a multivariate analysis with post hoc tests revealed that this effect occurred because the diet change significantly affected R/R in zoos other than WPZ (WPZ:CMZ-p=0.036; WPZ:CZA-p=0.051, WPZ:NCZ-p =0.013; and WPZ:TZ-p=0.025). However, when Amanda from WPZ was removed from the analysis (Amanda was receiving extra fruit in her diet to take *Salmonell*a medication), there was no institution effect (F=1.87, p=0.172). R/R was completely eliminated in three gorillas post diet change. There was a significant institution effect of feeding (F=5.697, p=0.005) and post hoc tests revealed that CMZ gorillas increased feeding significantly more than NCZ (p=0.001), WPZ (p=0.001), TZ (p=0.007) and CZA (p=0.002). There was a significant institution effect on coprophagy (F=27.9, p=0.00) and post hoc tests revealed that this occurred significantly more at CMZ than other zoos (CZA-p=0.00, NCZ-p=0.00, TZ-p=0.00) and also significantly more at WPZ than CZA (p=0.018), NCZ, (p=0.047) and TZ (p=0.019). There was a significant increase in time spent immobile (pre-diet change mean: 91.3% pf time $\pm$ 1.46; post-diet change mean= 93.8% of time $\pm$ 0.997; F=7.48, p=0.019). There was not an institutional effect on time spent immobile. Hair plucking behavior only occurred at CMZ and was reduced (pre-diet

change mean: 2.24 bouts/hr $\pm$ 1.68; post-diet change mean: 0.39 bouts/hr $\pm$ 0.11), while ear covering only occurred at CZA and increased (pre-diet change mean: 0.8 % of time $\pm$ 0.47; post-diet change mean: 3.0% of time $\pm$ 1.37).
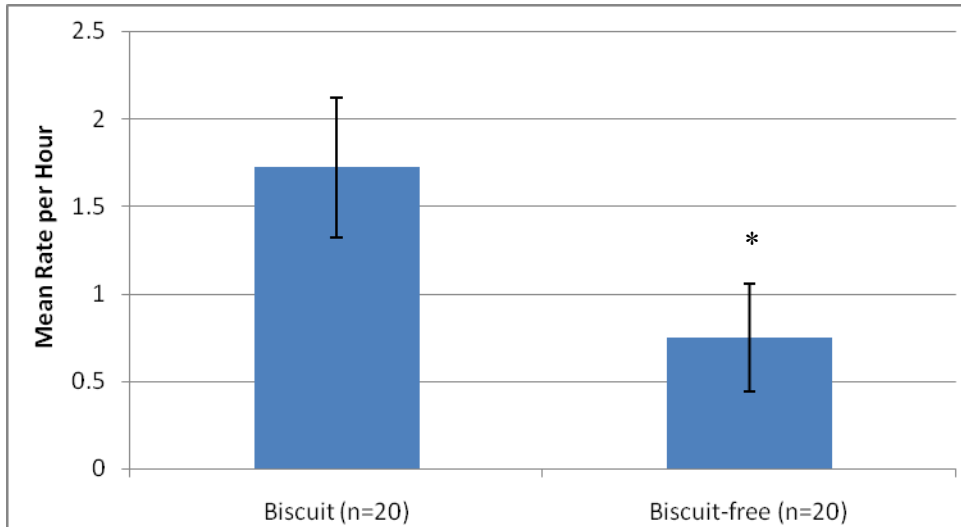

Figure 1. The rate of R/R significantly decreased after the switch to a biscuit-free diet.
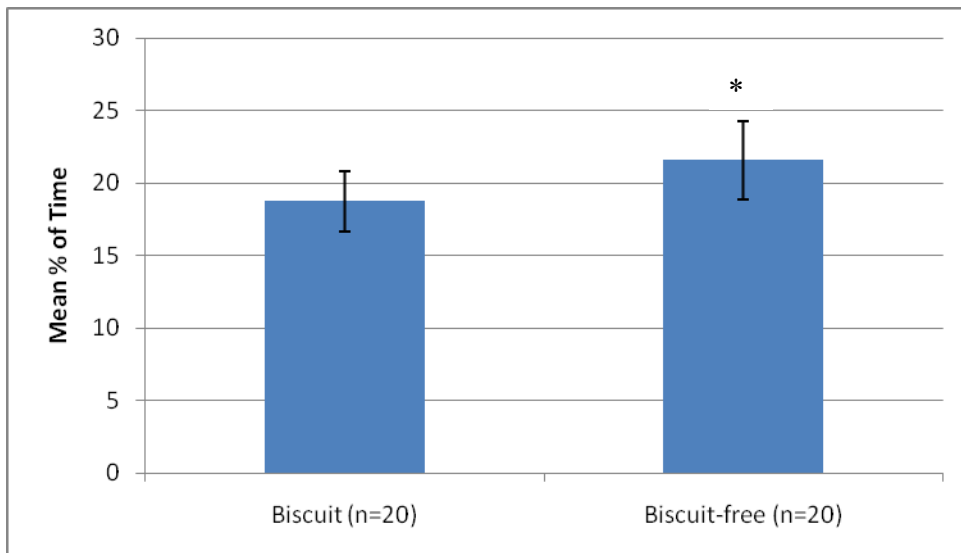

Figure 2. Feeding significantly increased after gorillas were switched to a biscuit-free diet.

At CMZ, a third condition of data collected once weights had stabilized revealed that coprophagy decreased from the initial biscuit-free data as did time spent immobile

(Figure 3, 4). All other behaviors displayed no difference between the initial biscuit-free
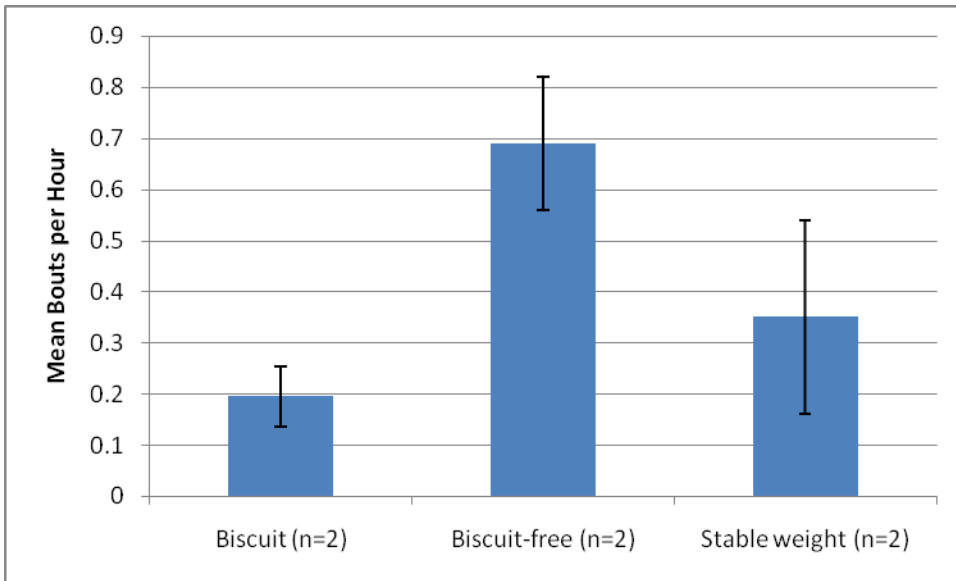
data as measured by standard error bar overlap.

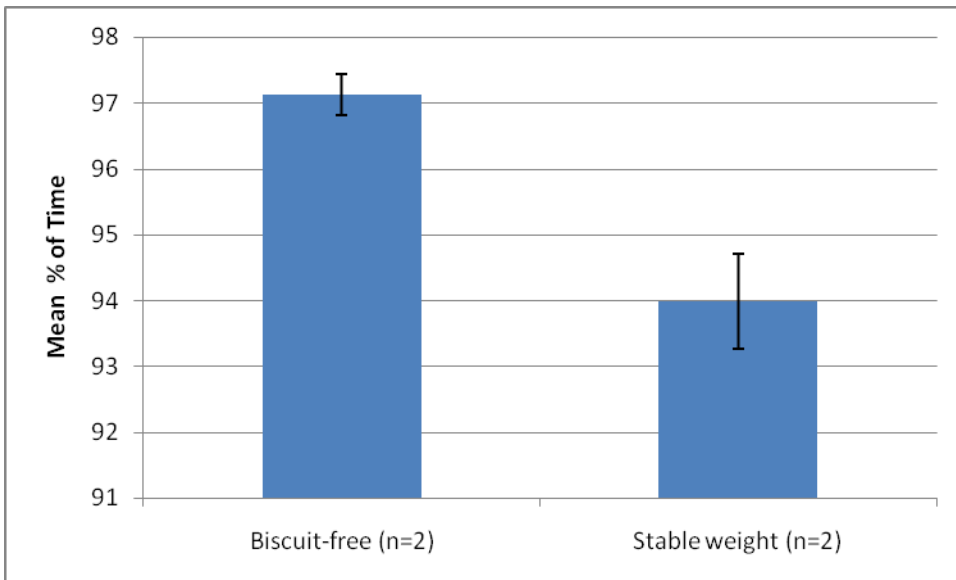Figure 3. Mean rate of coprophagy in CMZ gorillas.

Figure 4. Mean % of time spent immobile for CMZ gorillas. Time spent immobile was
not collected in the baseline biscuit-based diet for CMZ.

Behavior of Control Groups

A repeated measures ANOVA of all institutions revealed a trend towards an increase in time spent immobile (F=3.87, p=0.078). No other behaviors changed significantly for the control groups: R/R (F=0.52, p=0.488), feeding (F=2.42, p=0.147), and coprophagy (F=0.55, p=0.476).

Serum biomarkers

Serum biomarkers were compared between the individuals on the biscuit-free diet and the control group (Table 5). A repeated measures ANOVA revealed that insulin levels were significantly reduced after the diet change (F=11.5, p=0.007) with no difference between control and biscuit-free groups (F=1.72, p=0.229). Glucose to insulin ratio increased significantly after the diet change (F=35.0, p=0.000) and the control group significantly increased the glucose to insulin ratio significantly more than the biscuit-free groups (control vs. good consumption: p=0.000, control vs. poor consumption: p=0.001). The diet change significantly increased triglyceride levels (F=15.9, p=0.002), but there were no differences between control and biscuit-free groups (F=0.874, p=0.442). The diet change significantly improved cholesterol levels (F=7.12, p=0.022), but there were no differences between the control and biscuit-free groups (F=0.318, p=0.744). For glucose, there was no significant effect of the diet change on glucose (F=0.462, p=0.509), but post hoc tests revealed that glucose levels were significantly higher in the poor consumption group than the control. Leptin and adiponectin concentrations were not affected by the diet change.

Table 5. Health effects of biscuit-free diet.

Serum biomarker	Control (Change in mean after diet change)	Biscuit-free: good consumption (Change in mean after diet change)	Biscuit-free: poor consumption (Change in mean after diet change)
Leptin	+13.2	-1.2	-1.3
Adiponectin	-1.3	+1.7	-9.8
Glucose	-2.7*	-4.6*	+14.3†
Insulin	-7.8	-18.2	-24.8
Glucose:insulin ratio	+61.7*	+17.2†	+18.4†
Triglycerides	+69	+31	+56

*denotes a significant difference vs. †

Because the resistant starch supplement was present along with the biscuit-free diet change, the various diet phases were compared with one male at NCZ that was on a biscuit-free diet with no resistant starch supplement. Insulin concentrations decreased equally on both the biscuit-free diet and resistant starch supplement (Figure 5). Trigylcerides increased with the resistant starch diet, but not the biscuit-free diet (Figure 6.). Cholesterol concentrations were decreased on all diet manipulations equally (Figure 7.).
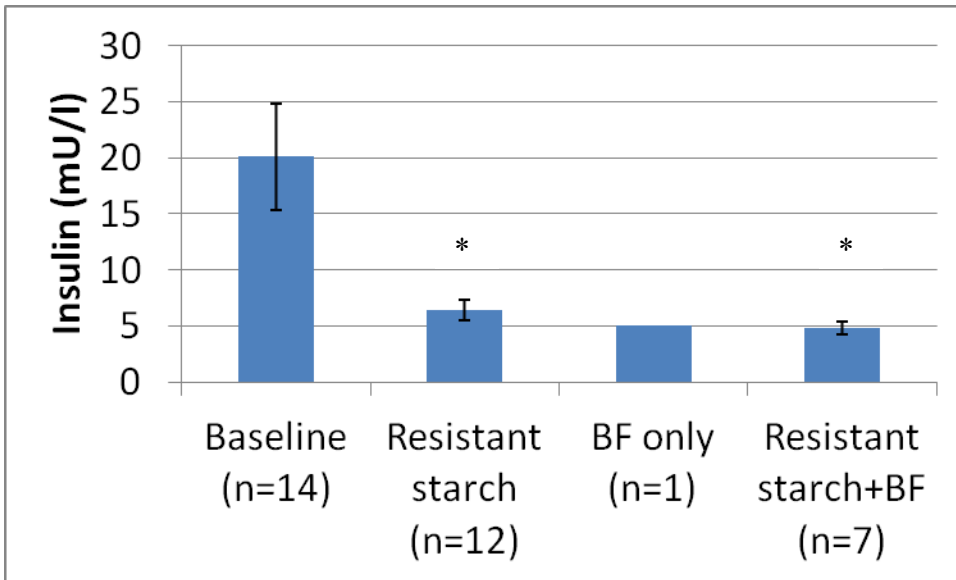
Figure 5. Insulin levels at various phases of the diet. Insulin levels did not show a difference between resistant starch and biscuit-free conditions.
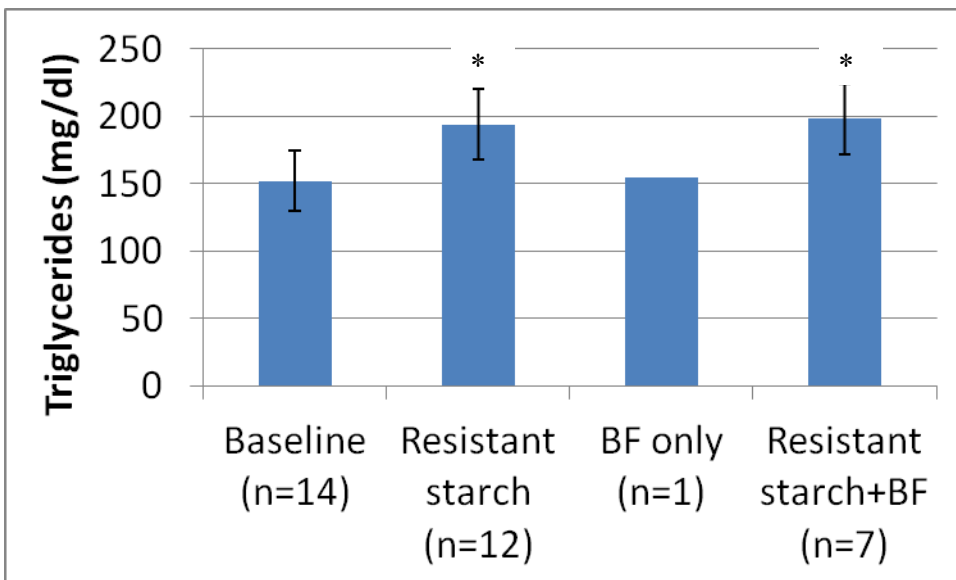

Figure 6. Triglyceride concentrations at various phases of the diet. Triglycerides increased on the diet phases including resistant starch.
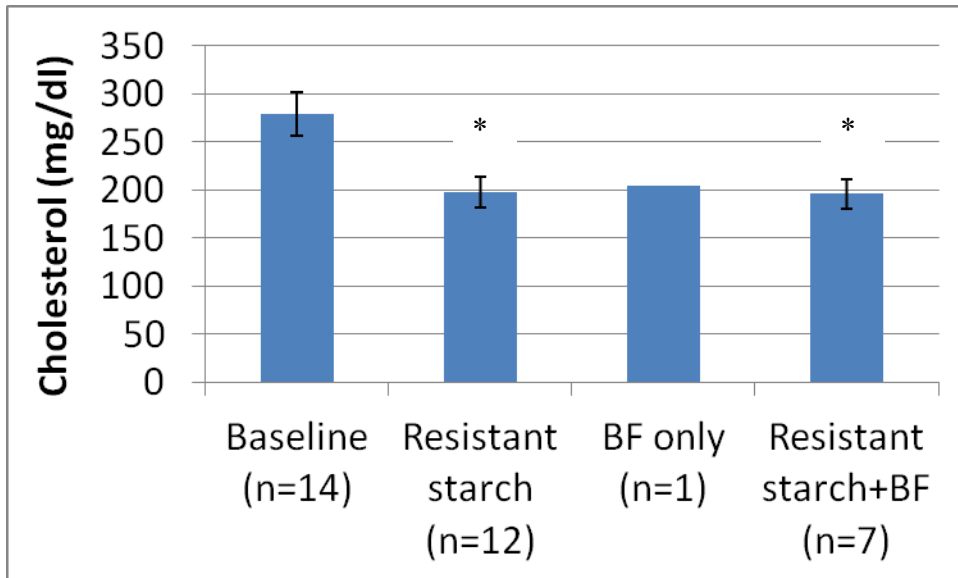
Figure 7. Cholesterol levels at various phases of the diet. Cholesterol was decreased on all diet changes but exhibited no difference between the different diet phases.

Body measurements

Body measurements were collected as described previously (Chapter 2) on two individuals. All measurements were smaller after the biscuit-free diet change except back length and hip width (for Bebac only) (Table 5).

Table 6. Body measurements before and after the diet change.

Measure	Bebac (Pre- diet)	Bebac (post-diet)	Mokolo (pre-diet)	Mokolo (post-diet)
Weight (kg)	181	164	209	177
Modified PMI (kg/m^2)	245	213	252	209
Back length (cm)	86	87	91	92
Head length (cm)	30	24	28	24
Shoulder width (cm)	42	38	53	47
Hip width (cm)	38	38	56	39
Widest point (cm)	49	43	58	50

Discussion

      This diet significantly decreased and in some cases eliminated a prevalent undesirable behavior, R/R. R/R occurs in approximately 60% of the zoo gorilla population, but has not been reported in the wild to date (Lukas, 1999a). However, the trend towards a significant institution effect revealed that WPZ was significantly different from the other zoos and did not exhibit as drastic a decrease in this behavior. One reason for this is that even though all individuals at this zoo decreased R/R behavior, one individual increased this behavior. This individual ("Amanda") was diagnosed with *Salmonella* and had to be medicated, which required her to be fed extra fruit and root vegetables to administer the medication. These "extras" likely contributed to her increase in R/R. Further supporting this finding was that the institution effect of R/R reduction was not significant when Amanda was removed from the analysis.

      Overall, this diet was effective in increasing time spent feeding. However, there was a significant institution effect demonstrating that CMZ had a much higher increase in time spent feeding than the other institutions. It is unclear why this diet had such a large effect on feeding at CMZ. One possibility is that CMZ's gorilla group was the only all-male group in the study. It may be that competition between males drove consumption of all of the food items leading to increased time spent feeding, whereas other institutions had some leftover food. Also, CMZ gorillas spent quite a bit of time consuming alfalfa hay, whereas this diet item did not appear to be consumed in entirety at other institutions. This may reflect a difference in food preference between institutions or might again be the result of having an all-male group with higher rates of competition. Another possibility is that other zoos may have been hand fed other calorically dense diet items

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which required gorillas to feel full and spend less time feeding on exhibit. For example, CZA hand fed oatmeal, beans and rice, TZ hand fed almonds and WPZ hand fed yogurt.

There was also a trend towards time spent immobile increasing after the diet change, but this also occurred in the control groups. Data from CMZ gorillas revealed that time spent immobile decreased after weights had stabilized indicating the increase in inactivity may have been related to weight loss. It is unclear why the control groups increased time spent immobile as temperature was controlled for in each condition and gorilla behavior does not change in spring vs. fall because of similar temperatures (Vreeman and Ross, 2007). It may be that some other factor, like humidity, may have influenced behavior. Another possibility is that the increase in age, although slight, may have led to an increase in inactivity. Further research is needed to elucidate this finding.

Additional undesirable behaviors were affected by this diet change. Coprophagy increased at all zoos, but more so in CMZ and WPZ. These zoos had a higher rate of coprophagy in the baseline phase suggesting that this behavior needs to be a significant part of the behavioral repertoire to see a drastic increase after the diet change. One reason for the increase may be that the higher fiber, higher volume diet leads to an increase in fecal excretion and hence consumption if that behavior already persists. However, coprophagy in wild bonobos was hypothesized to be a response to periods of food scarcity (Sakamaki, 2010). Because all gorillas lost weight during the initial implementation of the diet, they may have treated the diet change as a period of food scarcity and resorted to coprophagy. This hypothesis is further supported by the observed decrease in coprophagy that occurred after gorillas' weights stabilized at CMZ. Ear covering only occurred at CZA and increased after the diet change. Anecdotally, ear

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covering seemed to occur most often before the group was shifted to their outdoor exhibit to feed suggesting that there may have been some anticipatory stress associated with the diet change (Woods, 2000). Hair plucking decreased at CMZ and there was no decrease in this behavior in a control group at WPZ suggesting that the diet itself may have influenced this behavior. The increased foraging time may have led to less hair-plucking behavior (Hill, 2004). However, studies on whisker barbering in mice found that this behavior was associated with oxidative stress (Vieira et al., 2011). This diet change may have decreased oxidative stress in these gorillas and then subsequently hair plucking.

From a health perspective, this diet was effective at reducing insulin and cholesterol levels. However, the data were confounded by a previous study which included the addition of resistant starch to the gorilla diets. Both the biscuit-free diet and the resistant starch were equally effective at reducing insulin and cholesterol levels, which is not surprising because the biscuit-free diet was higher in fiber and resistant starch mimics the action of fiber. High fiber diets have led to a reduction in insulin (Anderson et al., 1994; Weickert et al., 2006) and cholesterol levels (Brown et al., 1999) in humans. This diet change did not have a significant effect on adiponectin or on SAA, the inflammatory marker. Adiponectin was not affected by fiber intake in humans (Weickert et al., 2006), so this result was supported. Furthermore, all body composition measurements were decreased in two individuals placed on this diet indicating that gorillas not only lost weight on this diet, but lost weight in areas suspected of housing fat deposits on gorillas.

There was an observed increase of triglycerides after moving to both a biscuit-free diet and feeding resistant starch. This result is the opposite of what might be expected

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when moving to a higher fiber diet. However, triglycerides were associated with increased obesity in humans (Tzagournis, 1978), so it may be that the extra calories associated with adding resistant starch to the diet caused the gorillas to gain weight and influenced obesity. This does not appear to be the situation for the biscuit-free diet because gorillas lost weight. Because triglycerides decreased with higher fiber diets in humans (Kiehm et al., 1976), it may be that the biscuit-free diet was not as high in fiber as suspected. For example, if gorillas were not eating all of their alfalfa hay as reported this could lead to an increase in triglycerides. The glucose to insulin ratio was improved by switching to a biscuit-free diet, but there was also a trend towards an increase in glucose levels which may explain this effect. The glucose to insulin ratio must be considered with caution as it only marks insulin resistance in individuals with normal glucose levels (Quon, 2001). Therefore, glucose levels that are too high could falsely inflate this ratio.

This diet had health and behavioral benefits, but it should be implemented with caution and with following several guidelines. Net kilocalories (as roughly estimated by Zootrition® software) were matched in the pre- and post-diet change condition, yet all gorillas lost weight. This is thought to have occurred because the higher fiber diet made the kilocalories less usable (Miles, 1992) and therefore, the overall metabolizable kilocalories were lower. Therefore, zoos should plan to feed double the kilocalories of their current diet if they plan to implement this diet and should be able to get regular (weekly) weights on their gorillas to adjust accordingly. Institutions need to be prepared for weight loss even if kilocalories are doubled. This can be addressed by feeding even more of the diet or by feeding a suitable calorically dense item to help maintain weight.

Almonds, beans (rinsed to reduce sodium content), avocados and rice were attempted to be used as substitutes but almonds increased R/R and rice is high in dietary starch. Beans and avocados worked well, but further research would need to be implemented to ensure that these are appropriate diet items for gorillas.

Another challenge with this diet is that not all gorillas consumed every diet item. CMZ was the only zoo to measure consumption and had 100% consumption. However, anecdotally, the other institutions varied in consumption. Not consuming the whole diet means that individuals were not getting the intended nutrition. This situation could lead to nutritional deficiencies or the diet being lower in fiber than expected. Therefore, zoos should weigh in and weigh out food items in the initial phases of implementing this diet and the nutrient content of the diet should be analyzed based on what is being consumed. This is a rough measure of consumption, but is better than no measure. It was essential that gorillas eat alfalfa hay as part of this diet to replace the NDF found in biscuits. If this did not occur, gorillas may not have been consuming the fiber needed which could have affected the results. Both CZA and CMZ fed resistant starch which mimics NDF function in the body. Future research needs to determine if the health effects of resistant starch are similar to that of NDF. If so, this may be an easier way to control fiber intake in gorillas that do not care for alfalfa hay. Other types of hay could also be used such as timothy grass.

Care must be taken in examining nutrients as a percentage of the diet, particularly when feeding a diet with a larger volume. For example, the percentage of total dietary fiber only appeared to increase slightly compared to the actual g of dry matter being fed, while the percentage of sugar went down compared to the g of dry matter which

increased (Table 3). It may be better to examine nutrients consumed by exotic animals based on body weight or some other variable besides the percentage of the total fed.

In conclusion, this diet led to improvements in some behaviors and health measures in zoo gorillas. Specifically, the biscuit-free diet reduced and in some cases eliminated R/R, reduce hair-plucking behavior while increasing time spent feeding. This diet was effective at reducing serum insulin levels. Therefore, it may be helpful for zoos to consider implementing this diet. However, zoos should only implement this diet if they are willing to measure consumption and monitor body weights. It is also recommended that zoos contact one of the investigators of this research for guidance before implementing this diet. Future research will focus on evaluating alternative forms of diets that may be easier or less costly to implement.

Chapter 7: Conclusion and outlook

Summary of findings and conclusions

The five studies presented in this thesis provide an important contribution to our understanding of factors influencing adiposity in captive gorillas. In the first study, serum biomarkers were described and assessed for correlation. Four groups emerged based on glucose to insulin relationships: (1) healthy glucose, low insulin, (2) healthy glucose, high insulin, (3) high glucose, high insulin, (4) high glucose, low insulin. The other serum biomarkers decreased in group 4 so this group was removed from further analyses.

In the second study, body composition measurements were assessed via linear regression to examine how they associated with serum biomarkers of adiposity. The results revealed that weight and modified PMI were associated with serum biomarkers of obesity for males. For males, a modified PMI needed to be calculated which did not account for head length, i.e. sagittal crest size. Hip width arose as the body composition measurement associated with the most biomarkers of obesity in females. For females, PMI was associated with adiponectin, one biomarker of obesity. For males and females respectively, both modified PMI and PMI exhibited relationships with glucose suggesting that some obese gorillas may be developing type II diabetes. Thus, this non-invasive method of collecting body measurements provides a promising tool in assessing obesity in zoo gorillas. The regression equations from these relationships combined with human healthy ranges of glucose and insulin were used to calculate at what point gorillas may be considered obese. For example, using the glucose and insulin concentration groupings to

examine PMI in both sexes, PMIs of 403 kg/m^2 for males and 158 kg/m^2 for females could be labeled as endpoints after which glucose might move out of its healthy range.

In the third study, methodology to increase the sample size in the fourth study was validated. In particular, a keeper assessment of activity in zoo gorillas was validated against collected behavioral data. Behavioral data can be time-consuming and costly to collect compared to keeper assessments which are quick and easy to obtain. Increasingly inactive rankings by keeper staff were correlated with gorillas spending more time inactive. Throughout this chapter, the distinction between behavioral and physical inactivity was made and it was found that adults were similarly inactive, while juveniles were more active.

The fourth investigation aimed to determine if components of diet or inactivity as assessed by keepers correlated with serum biomarkers of obesity, insulin resistance and inflammation. For diet, the nutrition focus was on the dietary starch, dietary fiber (both in the forms of total fiber and neutral detergent fiber) and kilocalories. The total amount of biscuits, as the main contributor of dietary starch and neutral detergent fiber, and the total amount of fruit, as the main contributor of dietary sugar, was assessed. In females, the amount of fruit was associated with all of the serum biomarkers of obesity. For males, the results were very mixed with the amount of biscuits/dietary starch/NDF being associated with some improvements in health and some worsening of serum biomarker levels. Activity levels did not exhibit any correlations with the serum biomarkers, however, there was not a wide range of activity levels across individuals which likely made it difficult to look for patterns.

The fifth investigation involved an experimental diet manipulation focused on increasing volume and fiber, while decreasing dietary starch. This diet had profound effects on behavior in that it eliminated an undesirable behavior in zoo gorillas, regurgitation and reingestion. This modified diet increased time spent feeding, but did not increase physical activity levels. There was a difference between institutions in the magnitude of these behavioral effects. Furthermore, this diet improved health parameters in both males and females by reducing serum insulin and cholesterol levels. However, the diet change increased triglyceride levels which deserves further exploration.

While these four studies have contributed to the body of knowledge on gorilla adiposity, behavior and diet, there remain many more unanswered questions that could be addressed in the future.

Outlook and future studies

Non-invasively collected body measurements can be a useful tool for determining obesity in gorillas when they are validated with other indirect measures of obesity such as serum leptin or triglyceride levels. Adiponectin levels were below what is considered to be healthy in humans. Further research is needed to determine if adiponectin levels are simply lower in gorillas than in humans or if all gorillas in the zoo population have unhealthy, low levels of adiponectin and what this means. Many gorillas had leptin and triglyceride levels in the normal range, so it is unclear at this point why only adiponectin was low in these individuals. Additionally, when validating body composition measurements with serum biomarkers, the assumption was made that these biomarkers were associated with health in gorillas based on data gathered in human studies. However, this research needs to be conducted for gorillas. There are a few disease states,

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particularly heart disease and perhaps type II diabetes that are known to occur in the zoo gorilla population. Therefore, future research should determine if there is an association between these disease states and these serum biomarkers.

There were correlations between diet parameters and several of our biomarkers. In particular, the amount of fruit fed was correlated with obesity in females. This relationship was not observed in males, which requires further investigation. Additionally, it was difficult to assess the distinct effects of dietary starch and fiber because both were correlated with each other and with the amount of commercial biscuits as biscuits were the main source of both. While some of the data suggest that larger amounts of biscuits in the diet can be associated with improved health, the experimental chapter of this dissertation demonstrates that health parameters can be improved by removing biscuits. Again, this likely has to do with biscuits being the main source of fiber in gorilla diets, but also the main source of dietary starch. This research demonstrates that the removal of primate biscuits does have a positive effect on health; therefore, it is likely that removing them may be a way to address health problems. However, because they do provide a large amount of dietary fiber, another source of fiber needs to be added to gorilla diets. Future studies should focus on one nutrient at a time and observing the effects. For example, it would be informative to reduce only dietary starch or dietary sugar separately in the diet and observe the effect.

The biscuit-free diet led to an increase in time spent feeding and eliminated regurgitation/reingestion behavior in some individuals. There was an effect of institution on these behaviors. Future research needs to focus on how certain institutional practices or individual differences can contribute to different behavioral responses. Furthermore,

future studies should focus on the social compostion of gorilla groups. There was a mix of bachelor and mixed-sex groups included in this work, therefore, the varying social contexts could affect adaptation to this diet.

As mentioned earlier, a decrease in insulin and cholesterol were observed after switching to a new diet. However, the insulin/cholesterol-lowering effects of the diet were similar to the insulin/cholesterol-lowering effects of adding a resistant starch supplement to the diet. The resistant starch supplement did not result in any behavioral changes, but can be as effective in improving health parameters. This diet was expensive and some gorillas had a harder time adapting to it. Gorillas that do not perform undesirable behaviors (and therefore do not have a need for these to be reduced) or institutions that cannot afford to feed a biscuit-free diet, may benefit from adding resistant starch to their diet. Future research efforts should focus on other ways of decreasing simple carbohydrates, such as low-starch biscuits.

As demonstrated, this study made important strides in understanding gorilla health, nutrition and behavior, but many questions remain unanswered. The work reported here emphasizes the importance of collaboration between zoological institutions in accommodating multi-institutional research requests. Both research of this nature and smaller case study experimental studies will be essential in furthering zoo gorilla research.

APPENDIX I. Gorilla information and body measures form.

General Information Questions:

1. Studbook # _____

2. Local ID # _____

3. House name _____

4. What is the sex of this gorilla?

5. What is the age of this gorilla?

6. What is the social group that this animal resides in (mixed-sex, bachelor, solitary, other)?

7. How would you define the rank of this individual within it's social group (dominant, subordinate, medium)?

8. When was this individual's last veterinary exam?

9. When is this individual's next veterinary exam (if this is unknown, please indicate)?

Measurements
(*We ask that these measurements are made on either awake gorillas in holding or exhibit areas or on anesthetized gorillas at exams. All measurements should be made in cm on the back of the gorilla. Please take the measurements twice. Please attach a photo of gorilla taken at the time measurements are made. Make sure the photograph is of the entire gorilla, not just a close-up of the back).*

"Crown to rump measure"
1. Measure from the bottom of the gorilla's back to the crown of the head as shown.
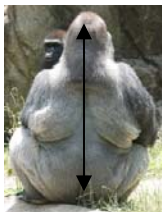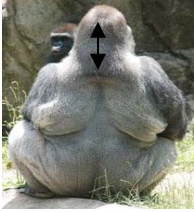
Measurement 1:   Measurement 2:

"Crown to bottom of neck measure"
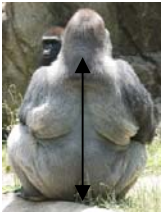2.   Measure from the bottom of the neck (even with the height of the shoulders) to the top of the crown.

          Measurement 1:   Measurement 2:

"Bottom of neck to rump measure"
3. Measure from the bottom of the gorilla's back to the bottom of the neck (even with the shoulder line).
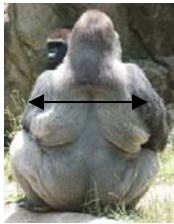
          Measurement 1:   Measurement 2:

"Width right underneath armpits"
4.   Measure from underneath the right armpit to underneath the left armpit as shown.

          Measurement 1:   Measurement 2:

"Width right above hips"
5.   Measure from right above where the right leg meets the back to where the left leg meets the back when seated in this posture.

          Measurement 1:   Measurement 2:
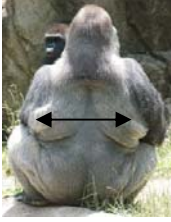
"Measure widest point of torso (roughly 2/3 down between beneath shoulder and above hips measurement"
6.   Measure 2/3 of the way down between measurement 4 and measurement 5.

 Measurement 1:   Measurement 2:

7. What is the most recent weight (lbs.) of this individual (list multiple if available and if tracked throughout course of diet change)?

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