Metabolic changes to GLUT-4 levels in urban Chacma baboons on the Cape Peninsula: raiding their way to type 2 diabetes?

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VVLJUL002

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Submitted 25 October 2013

Abstract

The Chacma baboons (Papio ursinus) *of the Cape Peninsula are established raiders of human food, leading to widespread conflict between this intelligent, adaptable species and humans. The modern Western diet that these baboons have become exposed to has many deleterious effects on health, including obesity and type 2 diabetes. The aim of this study was to investigate whether this population of baboons have lowered GLUT-4 transporter protein levels in comparison to wild-feeding baboons, as an indication of insulin abnormalities. GLUT-4 levels were analysed via Western Blot and DXA scanning was used to compare physical characteristics between these two groups. No significant difference in GLUT-4 levels was found, however the two groups differed in three physical variables, with the semi-provisioned Peninsula group having higher total weight (kg)($p<0,05$), total body lean mass (kg) ($p<0,01$) and bone mineral content (kg)($p<0,001$) than the wild-feeding controls. These results indicate that male individuals from the Peninsula population are bigger but not fatter than wild-feeding male baboons from the Eastern Cape population. Although it could not be determined whether human food is causing insulin abnormalities in the Cape Peninsula's population of Chacma baboons, this study indicates that this is a promising area of research, likely to affect the management strategies used on this population.*

Acknowledgements

I would like to thank my supervisor, Dr Cecile Reed, and my co-supervisor Dr Tertius Kohn. I would also like to thank Veeraj Goyaram and David Leith for their help with techniques.

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Introduction

Dietary and behavioral flexibility of primates

Dietary flexibility is an important reason for the success and survival of various primate species (Jaman & Huffman, 2013; Hill, 2000; El Alami, *et al.* 2012). Most primate species have the ability to take advantage of a wide range of food sources, both natural and artificial, due to their intelligence, cooperation, manual dexterity and behavioral flexibility (Kaplan, *et al*. 2011). They are able to effectively utilize human-modified landscapes and the relationship between some primate species and humans may be commensal, where primate populations take advantage of human food resources such as waste or crops, and use them to either supplement or supplant their natural diets (Gautier & Biquand, 1994). Raiding of cropland, dumps or residential areas has become a successful foraging strategy for some species of primates, especially when combined with other stress factors such as habitat-loss and subsequent restrictions to their natural ranges and available resources (Strum, 2010). Raiding may arise in situations where there is insufficient natural food, or simply when primates develop a preference for human food (Saj, *et al.* 1999).

Human food in the form of crops, waste at dumps, or residential home foodstuff is generally more spatially concentrated, easy to access and to process, is more readily digested and most importantly, more calorically concentrated, than any wild diet that primates may have (Muruthi, *et al.* 1991, Saj, *et al.* 1999). The detrimental effects of modern human foods on health are well known, and have been shown to result in the metabolic syndrome which includes disorders such as obesity and diabetes (Eckel, *et al.* 2005; Cordain, *et al.* 2005). This change in diet is therefore likely to be detrimental to the health of raiding primates. The Chacma baboons (*Papio ursinus*) of the Cape Peninsula have become established raiders of human food, resulting in widespread human-baboon conflict (Hoffman & O'Riain, 2011). The issue of the Cape Peninsula raiding baboons has attracted much concern from both the public and conservationists. Therefore a new potential health concern in the form of metabolic abnormalities due to increased exposure to human food would be of interest in order to better inform future management decisions, and encourage a less hostile interaction between humans and baboons.

Consequences of human food on primate lifestyles

In areas where humans and primates coexist, a clumped food distribution, such as is found at a waste dump, could lead to changes in behaviour and activity budgets of primates, and subsequently to changes in social structure and health (El Alami, *et al.* 2012). For primates not dependent to some degree on human food, larger distances must be covered to accumulate the same nutritional value (Altmann, *et al.* 1993), as primates naturally have to move continuously in order to forage successfully. When food resources are concentrated however, primates may demonstrate reductions in home range size and decrease the distance they travel for food each day, in spite of the fact that with concentrated resources group size and density increase (Hoffman & O'Riain, 2012). Despite the calorically rich nature of human food it is likely to have lower crude fibre content and protein levels than natural foods (Muruthi, *et al*. 1991). Refined starches specifically are lower in crude protein, and require primates to balance this high-carbohydrate diet with wild-sourced protein (Saj, *et al.* 1999). Wild food items, on the other hand, are patchily distributed and require more time and energy to access and process; meaning easily accessed human food would be more rewarding in terms of energy expenditure versus potential reward (Muruthi, *et al*. 1991). Hence, the cost to eating human foods preferentially over wild foods may be mitigated by increasing the overall feeding time and selecting higher energy food (Muruthi, *et al*. 1991).

Higher energy per unit means that human food allows the metabolic demands of primates to be satisfied sooner, and allows improved foraging efficiency (Saj, *et al.* 1999). This change has an immediate impact on activity budgets, which is possibly the most obvious change when primates are provisioned with human food (Saj, *et al.* 1999). The major differences in activity between semi-provisioned and wild groups are with time spent feeding and resting. Altmann & Muruthi (1988) found that a semi-provisioned troop of savannah baboons (*Papio cynocephalus*) in Kenya spent twice as much time resting and one-third as much time feeding, as a wild feeding troop of baboons in the same area. Also, the semi-provisioned baboons travelled significantly less than wild-feeding troops, and had ten times smaller home ranges. Semi-provisioned baboons showed a more predictable daily activity pattern, with a regular routine and home base, while wild-feeding troops showed greater variation in sleeping and foraging

patterns. In semi-provisioned troops there were significant increases in socialization and resting resulting from the reduction in time spent feeding and obtaining food (Altmann & Muruthi, 1988). Similarly, a study on Rhesus macaques (*Macaca mulatta*) in urban and rural Bangladesh found reduced feeding times, and increased socializing and resting in urban troops (Jaman & Huffman, 2013). Urban macaques spent more time feeding on provisioned foods, while rural troops spent more time on crop or wild food resources, demonstrating great behavioural flexibility to resource and habitat variability (Jaman & Huffman, 2013). Studies on Barbary macaques (El Alami, *et al.* 2012) and Vervet monkeys (*Cercopithecus aethiops*) (Saj, *et al.* 1999) found the same trend in activity budgets for semi-provisioned troops.

Consequences of human food on primate health

Modifications to health and physiology can accompany changes to activity budgets and behaviour. The close contact between humans and primates in urban environments mean there is a possibility that primates are becoming more susceptible to transmission of infectious diseases, either in the form of zoonoses (primate to human transmission) or anthroponoses (human to primate transmission) (Drewe, *et al.* 2012). Olive baboons in Kenya have been found to contract tuberculosis from human refuse (Tarara, *et al.* 1985), and a study on Chacma baboons (*Papio ursinus*) on the Cape peninsula found that 30% of baboons had antibodies reactive or cross-reactive with hepatitis A virus. This virus has a faecal-oral transmission, and close contact (such as in the case of food provisioning) between humans and baboon raiders could result in cross-infection (Drewe, *et al.* 2012).

Besides transmissible diseases that are harmful to both humans and baboons, primates that reduce their physical activity in the presence of human-supplied food abundance are self-selecting conditions similar to those they would be forced to live under in controlled obesity or health trials. Few studies have examined this hypothesis. Altmann *et al.* (1993) studied baboons living on a tourist lodge garbage dump in Amboseli National Park, Kenya. It was found that provisioned female baboons had 50% greater body mass than wild-feeding female baboons, with fat mass being dramatically different between the two groups. The difference in female body fat percentage between the groups is comparable to the difference between lean and obese humans, however no

animals had fatness levels in the range of laboratory animals that would be considered obese. The lodge-feeding group that travelled less than 4km per day had an average of 23.2% body fat. The wild-feeding group that travelled 8-10 km per day had an average of 1.9% body fat. The fattest male baboons were the youngest individuals, where the average percentage of body fat was 16,4% compared to 6,2% for wild-feeding males. This means that there was less of a difference between garbage-dump fed males and wild-fed males, compared to females (Altmann, *et al.* 1993).

Kemnitz, *et al.* (2002) studied the relationship between food availability and metabolic physiology in the Kenyan Amboseli baboons discussed above. Amboseli lodge-feeding animals showed three times higher blood insulin levels than wild-feeding animals, and males presented higher total cholesterol. Similar trends were found in the other provisioned study troop in Masai Mara, where insulin concentration was twice as high and total cholesterol was higher than in wild feeding groups. The physical inactivity of the provisioned groups could possibly have shifted body composition from lean tissue to fat. Increases in cholesterol indicators in lodge males, and increases in HDL-Cholesterol in both males and females are indicative of diets with high cholesterol and saturated fats. Although there was a significant insulin and cholesterol increase in lodge fed animals, these increases were not as extreme as those found in captive study animals. Elevated serum insulin and cholesterol concentrations influence the development of cardiovascular disease and can be manipulated with diet and exercise. These results show that calorically rich human food, although highly accessible and high energy per unit, results in lowered physical activity and possible insulin resistance, that could therefore be harmful to the health of free-ranging baboons (Kemnitz, *et al.* 2002).

Another, more physiological, explanation for habitual raiding of human foods by primates may involve addiction. The sugar present in many of the foods raided by primates from dumps, crops or urban areas could potentially result in sugar-addiction, as in humans. Sugar causes the release of both opioids and dopamine, and therefore would be expected to have the potential to cause addictions (Avena, 2007). Addictive drugs activate dopamine-containing neurons that process behaviour reinforcement, and a variety of foods such as sugar, saccharin and corn oil cause the increases in dopamine in the *nucleus accumbens* that are associated with addiction behaviour (Avena, *et al.* 2008). High fructose corn syrup, present in most artificially sweetened drinks, does not

cause the satiation that its caloric content should, and therefore can lead to obesity. In some circumstances sugar can cause behavioural and neurochemical changes in rats that resembles substance abuse (Avena, *et al.* 2008). Behaviour changes include bingeing, withdrawal signs, and increased sensitivity to psychostimulant drugs. However, although sugar bingeing does contain some of the effects of substances of abuse, it does not contain all of them, and the effects are not as severe as those seen with drugs (Avena, 2007).

When fat and sugar are combined, as seen in many human foods, consumption appears more compulsive than with sugar alone, and results in both increased consumption and weight gain (Ziauddeen, *et al.* 2012 ; Johnson & Kenny, 2010). Withdrawal symptoms, demonstrated with sugar, have not been demonstrated for fat/sugar combination diets, but it has been noted than this combination causes a reduction in dopamine levels for standard food, but not palatable food, indicating standard food is no longer as 'satisfying' (Ziauddeen, *et al.* 2012). The translation of these animal models to human "food addiction" is not successful, due to great variation in neuroimaging results, and a weak overlap between binge eating disorder and addiction (Ziauddeen, *et al.* 2012). Therefore although not certain, there is a possibility that primates that eat human food over sustained periods could become addicted to some degree to the sugar or sugar/fat content of the food.

Insulin Resistance and GLUT-4

Insulin resistance is the failure of adipose, liver, skeletal muscle or cardiac muscle tissues to respond to insulin secretion in a normal way (Chavez, *et al.* 2008). It results in impaired glucose metabolism and its slow clearance in peripheral tissues (Chavez, *et al.* 2008). Many common metabolic disorders have insulin resistance as a characteristic feature, such as obesity, type 2 diabetes and hypertension (Chavez, *et al.* 2008, Cai, *et al.* 2004). These manifestations are generally named the cardiometabolic syndrome (Higgins, *et al.* 2010), the development of which is linked to exposure to a Western Diet (that is, high sugar and high fat) in both human and animal model studies (Higgins, *et al.* 2010; Cordain, *et al.* 2005). How diet can induce cardiometabolic syndrome is not fully understood, but animal models are useful for experimentation (Higgins, *et al.* 2010). Nonhuman primates specifically are large and long-lived and provide a model that is

both physiologically and genetically similar to humans (Wagner, *et al.* 2006). The similarity in disease susceptibility means primates have been used to study cardiometabolic syndrome indications such as coronary artery disease, obesity, and type 2 diabetes (Chavez, *et al*. 2009). Baboons in particular are useful for these studies as baboon and human proteins are similar in structure, are similar in chromosomes and similar in genetic loci. There is a high degree of similarity in chromosome-banding patterns between baboons and humans, and they share 96% of DNA variation (Comuzzie, *et al.* 2003).

The fact that baboons can become spontaneously obese in the wild was demonstrated by Altmann et al. (1993) lending strength to the idea than baboons are susceptible to not only obesity, but coronary heart disease and type 2 diabetes (Comuzzie, *et al.* 2003; Higgins, *et al.* 2010). Increasing adiposity is associated with increased risk of heart disease and type 2 diabetes in primates as well as humans, and similarly to humans, the majority of fat gained by baboons occurs in the trunk region (Comuzzie, *et al.* 2003). Higgins *et al* (2010) found that baboons gain fat mass and show alterations to their biochemistry that are consistent with metabolic disorders in as little as eight weeks. Increases in haemoglobin A_{1C} (%HbA_{1C}) suggest changes to glucose cycling may have developed after only 8 weeks of a high fat/high sugar diet. Cholesterol was unaffected over this short period, but triglyceride levels were elevated as result of the high monosaccharide diet. The rate of fat gain on this diet suggests that obesity would be achieved in a very short time, as well as possibly the onset of cardiometabolic syndrome (Higgins, *et al.* 2010).

Transporter protein isoforms GLUT-1 and GLUT-4 are facultative in the process of glucose uptake, which becomes impaired with insulin-resistance (James & Piper, 1994). These proteins make an aqueous pore across the cell membrane, through which sugar (in the form of glucose) can move (Bryant, *et al.* 2002). GLUT-1 maintains basal glucose requirements, when insulin is not present, while GLUT-4 is stored intracellularly and is used to transport glucose across the cell membrane in the presence of insulin (Camps, *et al.* 1992). Muscle and adipose tissues have developed a specialised glucose transport system, in which the rate of glucose transport can be rapidly increased, which is essential during exercise due to a higher metabolic demand (Bryant, *et al.* 2002). A dysfunction in this glucose uptake in muscle and fat cells contributes to the onset of the

cardiometabolic syndrome (Bryant, *et al.* 2002). Both exercise and insulin cause the recruitment of GLUT-4 to the cell surface, resulting in an increase in the cell surface levels of this protein (James & Piper, 1994).

The GLUT-4 pathway is yet to be completely resolved, as it involves numerous complex processes such as signal transduction and vesicle transport. When insulin binds to a muscle or fat cell, it triggers a cascade of signalling events, which end with the translocation of GLUT-4 from the interior of the cell to its surface, in order to transport glucose into the cell (Bryant, *et al.* 2002). After insulin binds to its receptor on the cell surface, a conformational change occurs, triggering the activation of its tyrosine-kinase domain. After activation the receptor phosphorylates nearby substrates, such as the insulin receptor substrates IRS-1 and IRS-2, as well as c-Cbl. These phosphorylated proteins recruit effector molecules such as p85/p110 type PI3-Kinase to their location, activating the Akt and PKCζ pathways, which then results in the translocation of GLUT-4 to the cell membrane (Bryant, *et al.* 2002; Shao, *et al.* 2002). The c-Cbl-CAP pathway has also been implicated in GLUT-4 translocation in adipocytes (Bryant, *et al.* 2002).

GLUT-4 defects are of particular interest in diabetes studies, as there is a down-regulation of GLUT-4 expression in adipose tissue in obese human individuals, as well as an up-regulation of it's expression in skeletal muscle under exercise (Huang & Czech, 2007; Kahn, 1996). This indicates that GLUT-4 may be of vital importance in the path towards insulin resistance. GLUT-4 protein and gene expression is likely changed by both exercise and diet (Lee, *et al.* 2002). Exercise training in carbohydrate-fed rats showed an increase in GLUT-4 protein and mRNA expression (Lee, *et al.* 2002), and these changes in the levels of GLUT-4 may be significant as they could translate into changes in glucose tolerance. Enhancing GLUT-4 expression may therefore be a viable therapy strategy (Huang & Czech, 2007). Shao, *et al.* (2002) found that the coupling between IRS-1 and p85α (usually stimulated by insulin) was inhibited in gestational diabetes mellitus in mice, and therefore GLUT-4 translocation to the cell membrane was decreased significantly, which ultimately contributes towards insulin resistance in skeletal muscle. Leguisamo *et al.* (2012) found that GLUT-4 expression levels were lower in heart tissue, adipose tissue, and skeletal muscle in obese rats under a MSG diet. Whole-body insulin resistance accompanied this reduction in GLUT4 protein content, indicating its role as a fundamental mechanism for glucose uptake (Leguisamo, *et al.*

2012). Additionally, when the GLUT-4 gene in mice is disrupted there is a decrease in GLUT-4 expression in adipose tissue and skeletal muscle, which did not lead to obesity but to reduced glucose uptake, hypertension and diabetic histopathologies similar to those seen in humans with diabetes (Stenbit, *et al.* 1997).

The reduction of GLUT-4 in adipose tissues is relatively well accepted, while its reduction in skeletal muscle remains more contentious. For example, Kahn (1996) found that GLUT-4 levels are decreased in adipose cells but not skeletal cells in human obesity and type 2 Diabetes, as well as in hyper-insulinemic rodents. In high fat diets, adipose tissue showed decreased expression of GLUT-4, while in skeletal muscle this remained normal (Kahn, 1996). Similarly Zierath et al. (1997) found that a high fat diet can lead to reduced glucose transport from decreased expression of GLUT-4 in adipose tissue of rats, while in muscle there is no initial change in GLUT-4 content. Therefore, in skeletal muscle, insulin resistance may be a response to impaired insulin signalling or defects in GLUT4 translocation and fusion to the membrane, and not to total GLUT-4 content of the muscle (Zierath, *et al.* 1997). Whatever the case may be, GLUT-4 appears to be highly significant in the development of insulin resistance, whether due to changes in GLUT4 levels, or alterations in the translocation and fusion of GLUT4 to the plasma membrane either in adipose tissue or skeletal muscle.

The baboons of the Cape Peninsula

Chacma baboons (*Papio ursinus*) on the Cape Peninsula exploit diverse food sources, both natural and human-provisioned, and experience great heterogeneity of habitat (Hoffman & O'Riain, 2012). This includes an urbanized landscape in the lowlands, agriculture at mid-elevations and indigenous, low-quality fynbos habitat at the higher elevations (Hoffman & O'Riain, 2012). Fynbos and marine organisms are exploited as part of the baboons natural diet; while alien vegetation, agricultural foods such as grapes, refuse and raided household/tourist food items are anthropogenically-added sources (Hoffman & O'Riain, 2012). This raises concerns of the possible detrimental effects of this food, such as obesity and insulin resistance, discussed previously.

The Cape population of Chacma baboons consists of 12 troops, and all troops interact with humans to some degree or another. Only one troop is not known to raid for human

food (Hoffman & O'Riain, 2012). This one troop is the only troop that does not experience human-baboon conflict (Hoffman & O'Riain, 2012*b)*. The size of the Cape Peninsula's baboon population does not exceed the available space, and therefore there is no ecological reason for a regulation of their numbers (Hoffman & O'Riain, 2012*b*). The loss of access to low-lying land due to urbanization, as well as the close proximity of sleeping sites and urban areas are the best explanations for the high level of conflict, given that population levels are not ecologically too high (Hoffman & O'Riain, 2012*b*). The time troops spend in human-modified habitat varies amongst troops and ranges between 0.1% and 99%. Troops living mainly in human habitats reduce their daily path length significantly (Hoffman & O'Riain, 2012). The troops that spend most time in a urban habitat also have smaller home ranges, and increases in group size and density, presumably due to the spatially concentrated, calorically rich food source they access (Hoffman & O'Riain, 2012). A focal study on one female baboon resident in Tokai forest demonstrated that human foods contributed 8% to the subjects total energy intake, while exotic plant species such as pine nuts contributed 44% (Johnson, *et al*. 2013). The human foods that were consumed by this subject consisted of one or two macronutrients and thus provided an unbalanced diet. This indicates that human foods may contribute a small percentage to the diet of this particular Chacma baboon, but given that females of low rank participate in raiding significantly less, and the small sample size of the study, mean this result may not be generalizable (Johnson, *et al*. 2013).

The increasing conflict with humans (as a result of baboon utilization of human food resources) ultimately leads to injury and mortality of Cape Peninsula baboons, despite the population being protected by legislation (Kaplan, *et al.* 2011). Monitoring is the main mitigation method employed, but the program is costly (Kaplan, *et al.* 2011). Since raiding can be classified as a foraging strategy, it has associated costs and benefits. Therefore techniques that manipulate the ratio, such that costs of raiding outweigh benefits, may be effective (Strum, 1994). The intelligence of primates makes preventing raiding extremely difficult. Euthanizing primates is not a long-term solution, as troops will continue to raid, just more cautiously (Strum, 1994), or neighbouring troops will move into the newly available area and continue the conflict (Hoffman & O'Riain, 2012*b*). Keeping animals away from crops and human areas is time-consuming and

expensive, and often of vital economic importance in rural areas (Strum, 1994). Enhancing the perception of risk is often unsuccessful as primates habituate quickly, and will learn that the cost is not real (Strum, 1994). Sound aversion and electric fencing have been used to reduce human-baboon conflict in many areas, but the fact that the spatial extent of Cape Town has doubled over the last 30 years together with the steady increase in the size of the baboon population, can only result in further conflict (Hoffman, 2011). Another alternative, providing supplementary food away from urban areas, did not result in a substantial reduction in the time spent in urban areas, except when combined with better waste management of the nearby urban areas (Kaplan, *et al*. 2011). Therefore it is unlikely that conflict will improve until the spatial overlap between human and baboon environments is reduced, by minimizing incentives (such as readily-available waste) to enter human areas (Kaplan, *et al*. 2011). Other strategies, such as prioritizing conservation of the remaining available low-lying areas, decreasing access to obvious hot-spots of conflict, and increasing the distance of sleeping sites from the urban area are also important management practices that need to be implemented (Hoffman & O'Riain, 2012*b)*.

This study aims to determine whether human food, obtained by frequent raiding activities, is negatively affecting the health of Cape Peninsula baboons. This research will enhance the current knowledge of urban baboons, which is important due to their tourism value and ecological importance. It will also increase knowledge about obesity and diabetes in primates closely related to humans, and further evaluate their usefulness as a model for metabolic abnormalities in humans. Finally, it may also provide the impetus for coming up with management solutions such as effective waste disposal or for the relocation of troops, if this population's health is suffering as a result of human contact.

It is predicted that calorically rich human food obtained from raiding, accompanied with reduced physical activity, is detrimental to the health of Cape Peninsula Chacma baboons. This is likely to manifest itself in symptoms of the cardiometabolic syndrome, either through obesity or insulin-resistance. The possibility of sugar/fat addiction enhances the chances of health problems for these baboons. Kemnitz, *et al*. (2002) provide precedent for spontaneous insulin-resistance in free-ranging baboons due to

their access to high fat, high-sugar human foods. It is therefore hypothesised that the raiding Cape Peninsula baboons will show signs of obesity, such as increased fat percentage, when compared to non-raiding baboons. It is also hypothesised that a reduction in total GLUT-4 transporter protein levels will occur in raiding Cape Peninsula baboons as an indication of dysfunction in the insulin-mediated uptake of glucose into skeletal muscle.

Methods

Study Animals

Chacma baboons (*Papio ursinus*) from troops around the Cape Peninsula are occasionally euthanized by Cape Nature, SANparks or the City of Cape Town for exhibiting aggressive behaviour towards humans, or for persistent aggressive raiding of homes. These carcasses are donated to the Department of Biological Sciences, University of Cape Town for research purposes. The baboons euthanized are usually aggressive males, and therefore urban baboons used in this study will be limited to: adult males, that have caused human-baboon conflict, were euthanized humanely and with reason, and live in the urban Cape Peninsula area as part of either urban troops or as solitary individuals. Any individuals that are juvenile, female or killed inhumanely will be excluded from this study. Ten adult male Peninsula baboons were used for this study.

The wild-feeding baboons (hereafter referred to as controls) were donated to UCT after a routine culling exercise performed on a farm near Pearston, Eastern Cape. These baboons only occasionally raid for farm products such as lucerne. These baboons were culled in the winter season, where fewer farm items are available. This population of baboons does not come into contact with human food, and they do not raid homes. Therefore individuals from this population are deemed to have a more natural diet than urban baboons from the Cape Peninsula. These baboons will be limited to: adult males or females with a wild diet that have seldom or never consumed human food, animals killed humanely by a registered veterinarian or licenced farmer, and donated to UCT for research purposes. No requests were made for animals to be killed for this project. Ten Cape Peninsula samples (all adult males) and 4 control samples (1 adult male and 3 adult females) were used for the GLUT-4 analysis, while eight Cape Peninsula and six control adult male baboons were compared using DXA scans. All necessary ethical clearance was received prior to the start of this study (Appendix, Figure 7).

Study Area

The Peninsula group used in this study all came from the Cape Peninsula area, located at the southwestern point of South Africa (Figure 1). This area consists of low-lying developed areas dominated by urbanization, plantations or vineyards at mid-elevations

and mountains with limited human influence (Hoffman & O'Riain, 2012). The mountains are predominated by Fynbos vegetation, which is nutrient poor. Marine resources such as shellfish are available along the coastline. Anthropogenic food sources include waste from dumps and homes, plantations, vineyards, and invasive alien vegetation (Hoffman & O'Riain, 2012). The climate is Mediterranean, with wet winters and dry summers. Total annual precipitation averages 788mm (SA Explorer, 2011). The control animals all came from around a farm near Pearston in the Eastern Cape province, South Africa (Figure 1). Pearston has a typical Karoo climate: very arid with dry winters and wetter summers. This area receives an average of 254mm of precipitation annually (SA Explorer, 2011). This area consists of agricultural fields and grazing pastures interspersed with natural vegetation in low-lying areas, while higher elevations have completely natural vegetation. Anthropogenic food sources are limited to agricultural products such as lucerne, and do not include plantations or much alien vegetation.

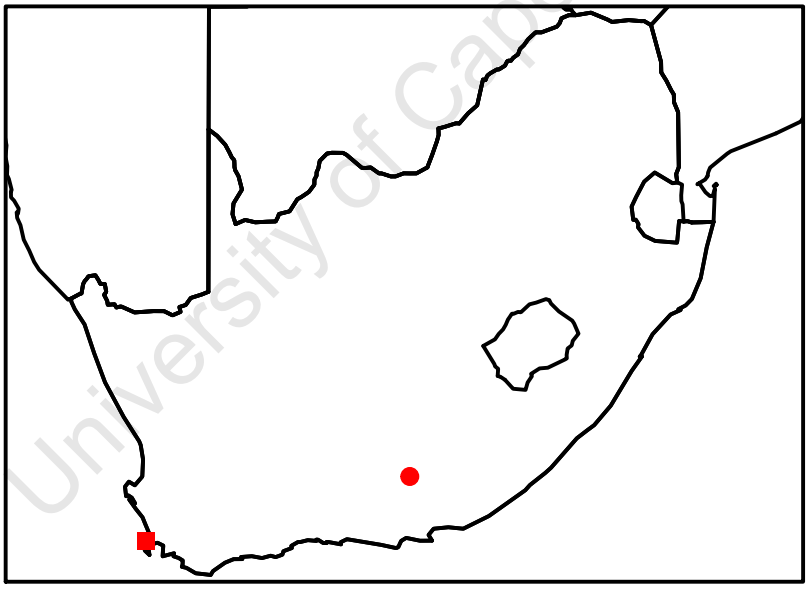


Figure 1: Map of South Africa, showing locations of the Peninsula group (square) and control group of baboons (circle)

Muscle biopsies

Upon receiving the baboon carcass, its physical characteristics were noted, such as sex, body condition and teeth condition. A muscle biopsy was taken as soon after death as possible, with the procedure based on Kohn *et al.* (2011), with some modifications. A

tissue block was taken from the *Vastus lateralis* muscle of each individual, at a sampling depth of 1 cm. The sample site was identified using anatomical markers, namely, half the distance between the hip joint and the knee joint (Figure 2). The muscle sample was removed with a scalpel after a portion of the skin was removed at this site. This muscle sample was then further cut up into smaller blocks, following the grain of the muscle, and immediately frozen in liquid nitrogen. This sample was stored at -270°C until further use.

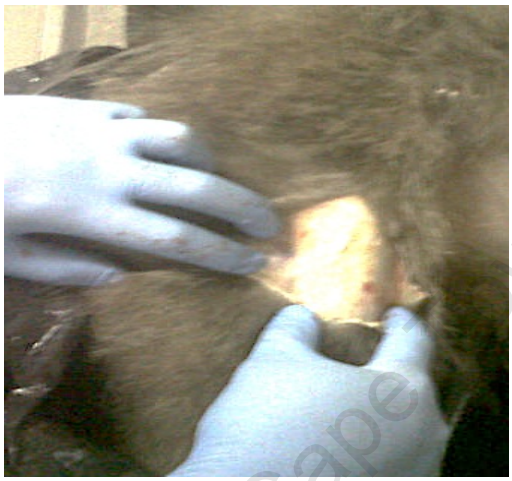


Figure 2: Example of biopsy site of the *Vastus lateralis* muscle, located at half the distance between the hip and knee joints, with hide removed to access muscle

DXA Scanning

Ten animals had been received and scanned prior to the start of this project (Peninsula group). The carcasses were frozen, if necessary, for no longer than 12 hours and then thawed, but otherwise used immediately in order to prevent cell lysis and fluid loss. The body composition was determined using a DXA (Dual Energy X-ray Absorptiometry) Scan, performed by a qualified radiographer at the Exercise and Sports Medicine research Unit, Department of Human Biology, University of Cape Town. The carcass was placed on the DXA bed, which had been covered in thick black plastic. The limbs were secured to keep the carcass in a supine position. The head of the baboon was positioned to face directly upwards, in order to promote the bulging of the chest for scanning accuracy (Figure 3). The scan was then performed and the total body mass (kg), lean mass (kg), fat mass (kg), bone mass (kg), the lean and fat mass of distinct body regions (kg), and finally, body fat (%) were recorded. The software used was designed for human scans (Version 13.4.1, Model Discovery W, S/N 80196). Photographs of the teeth of each individual were taken.

Figure 3: Positioning of baboon carcasses on DXA bed

Laboratory methods

Bradford Assay

A 33g portion of each frozen muscle sample was weighed out, ensuring its temperature was kept constant by continuous immersion in liquid nitrogen. This muscle sample (in homogenizing RIPA buffer at a 1mg:19μl ratio) was homogenized in two bouts of about seven seconds, using a Teflon tip. It was then sonicated on ice once at 33% for 10 seconds. The tissue homogenate was diluted 6-fold (1:5) in distilled water. A Bradford assay was performed in duplicate using an Elisa plate. A blank containing Bradford reagent and a 6-fold RIPA/water dilution was created. A Bovine Serum Albumin (BSA) solution was created using 25 μg of BSA and 5 ml of water and then protein concentrations of 0.5, 1.0,1.5 and 2.0 $\mu g/\mu l$ of this solution were made as standards in order to generate a standard curve. This will be used to determine the protein concentration of the 6x dilution of baboon tissue samples.

The wavelength of the Elisa spectrophotometer was set to 595 nm and shake of 0.01 seconds set. The absorbance values for the blanks, standards and the samples of unknown concentration were then recorded, each in duplicate. A standard curve was made using the absorbance for the 0.5,1.0, 1.5 and 2.0 standards, a trend line fitted with an intercept of 0, and the equation and R-squared value generated (Appendix, Figure 1). The diluted protein content of the muscle sample was determined by averaging the two absorbance values found for each sample, and then substituting this average value into

the equation generated from the standard curve. This value was then multiplied by six in order to find the undiluted protein concentration for each muscle sample (Appendix, Table 2).

Using the undiluted protein concentration value a 5 $\mu g/\mu l$ concentration of muscle homogenate to RIPA working buffer was created, This was then diluted to 2.5 $\mu g/\mu l$ concentration with a reducing sample buffer. Although standard practice is to boil this solution at 95℃ for 5 minutes, this was not done as the GLUT-4 protein is highly hydrophobic, and aggregates at high temperatures, meaning that much of the protein would not enter the gel and higher order oligomers would be observed (Abcam, 2013).

SDS-PAGE

A 10% polyacrylamide gel was run via electrophoresis, in order to separate the principal protein being investigated, here GLUT-4, according to its specific molecular weight of 45 kDa. A separating gel was added to the gel plates and set for 30 minutes, followed by a stacking gel and gel combs. Upon running the gel, the combs were removed and a running buffer was prepared to fill the inner buffer dam. The gel was then loaded with 12μl (30ug/ul) of the 2.5 ug/ul protein dilution. The gel was run at 120 V for 100 minutes.

Western Blot

The Western Blot procedure was used in order to transfer the protein from the gel matrix to a membrane for quantitation. A transfer buffer was prepared and used to incubate the gel for 5 minutes. The transfer buffer was also used to soak the sponges and filter paper to be used in the cassette. The PVDF membrane was cut to the appropriate size and transferred to 100% methanol for 10 seconds, then washed with distilled water and soaked in transfer buffer. The gel was placed on top of the soaked sponge and filter paper. The membrane was placed on top of the gel, and filter paper and sponge put on top of this. The cassette was then closed and transferred to the tank filled with transfer buffer. The tank was then put on ice and placed on a magnetic stirrer at 30V for 16 hours. Once removed, the membrane was stained with Ponceau S solution to check transfer. The membrane was then blocked with 5% TBS-T and BSA for one hour and 15 minutes at room temperature with shaking. After blocking, the membrane was washed

in TBS-T three times for five minutes. An overnight incubation with gentle shaking was done in a primary antibody diluted with a TBS-T/BSA buffer. An Abcam AntiGLUT-4 (Rabbit) antibody at a 1:5000 dilution was used (Abcam, 2013). After primary antibody incubation, the membrane was washed three times with TBS-T for five minutes each. A goat anti-rabbit HRP secondary antibody was used at a 1:1000 dilution in a buffer of 10ml TBS-T with 0.5 g BSA, for 1 hour with gentle shaking. After incubation the membrane was again washed in TBS-T three times, for 5 minutes each.

Protein Detection

An ECL reaction kit was used for protein detection. 1ml of peroxide and 1ml of luminol (1:1 ratio) was mixed, poured on top of membrane, and left for 1 minute. The membrane was then transferred to an X-ray cassette, and a piece of photo film of corresponding size placed on top, and left for 30 seconds to 1 minute in the darkroom. The film was then placed in developing solution and subsequently fixing solution, for 1-2 minutes each, until bands on film were obvious. The membrane was then re-probed with Alpha-Tubulin to standardize each well. A harsh stripping buffer was used, and incubated for 30 minutes at 55°C. The membrane was then washed in water, then methanol for 1 minute, and then washed with TBS-T. It was then re-blocked using a TBS-T and BSA buffer, with gentle shaking, for one hour. The primary Alpha-Tubulin antibody was used to incubate the membrane overnight using its individual specific concentration, and the secondary HRP antibody was used for 1 hour incubation at a 1:5000 dilution. The membrane was washed three times in TBS-T for five minutes and then developed using an ECL kit as above. Both the Alpha-Tubulin and sample protein films were then scanned and digitized using UNSCANIT software. The GLUT-4 pixel values were then normalized to the Alpha-Tubulin value from the corresponding well, by dividing the GLUT-4 pixel total by the alpha-tubulin pixel total for that lane. The GLUT-4 was readily detected at 45 kDa. The alpha-tubulin formed heterodimers of alpha + beta tubulin at 100kDa (Abcam, 2013) which were used in lieu of clear bands of alpha-tubulin at 55 kDa.

Statistical analysis

A general linear model was run in STATISTICA on the results from the DXA scans in order to find any significant differences in physical characteristics between the Peninsula and control baboons. This is similar in approach to doing a Student's 2-sample t-test on each variable. All variables were normally distributed. Only adult males from each group were analysed, comprising of 6 control animals and 8 Peninsula animals. The variables compared between the two groups were total weight (kg), total body fat mass (kg), total body lean mass (kg), fat mass (%), trunk fat mass (kg) trunk fat mass (%), trunk lean mass (kg), and bone mineral content (BMC) (kg), and lean mass (%) which was total lean body mass converted to a percentage of total weight. The means and standard deviations of each variable were also found.

The normalized GLUT-4 levels (in total pixels) were compared for the Peninsula (n=10) and control baboons (n=4 each replicated twice). A Mann-Whitney U test was used to compare the GLUT-4 levels of these two groups, due to the distributions not being normal but similar variances.

Results

DEXA scans

The results of the DXA scans on adult baboons from the control and Cape Peninsula groups show that the peninsula baboons have higher mean values for all variables analyzed than the control group (Table 1). The GLM analysis found that there was a significant difference in three of these physical variables between the control and Cape Peninsula groups namely total weight (kg), total body lean mass (kg) and BMC (kg) (Table 2). Lean mass as a percentage of total weight was not quite significant. The values were consistently lower for control individuals than for peninsula individuals for all three of these significant variables (Figure 4), except for lean mass (%) where control baboons had higher values than peninsula baboons. An example of the results from the DXA scans is shown in Figure 5.

Table 1: Means and standard deviations of DXA scan variables for control (n=6) and peninsula (n=8) Chacma baboon groups

	Control		Peninsula	
Variable	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
Total weight (kg)	28,47	2,14	31,56	1,76
Total body fat mass (kg)	3,27	0,81	3,84	0,76
Total body lean mass (kg)	24,17	1,37	26,42	1,34
Fat mass (%)	11,37	2,09	12,11	2,00
Trunk fat mass (kg)	2,00	0,64	2,31	0,76
Trunk lean mass (kg)	13,70	1,35	14,49	1,17
BMC (kg)	1,03	0,06	1,30	0,07
Trunk fat mass (%)	14,32	3,58	15,93	5,17
Lean mass (%)	48,06	1,64	45,88	2,19

Table 2: GLM results testing the difference in DXA scan variables between control and peninsula Chacma baboon groups, with significant variables in bold print.

Variable	f-value	p-value
Total weight (kg)	8,793	**0,012**
Total body fat mass (kg)	1,828	0,201
Total body lean mass (kg)	9,422	**0,010**
Fat mass (%)	0,458	0,511
Trunk fat mass (kg)	0,656	0,434
Trunk lean mass (kg)	1,363	0,266
BMC (kg)	55,764	**<0,001**
Trunk fat mass (%)	0,429	0,525
Lean mass (%)	4,143	0,065

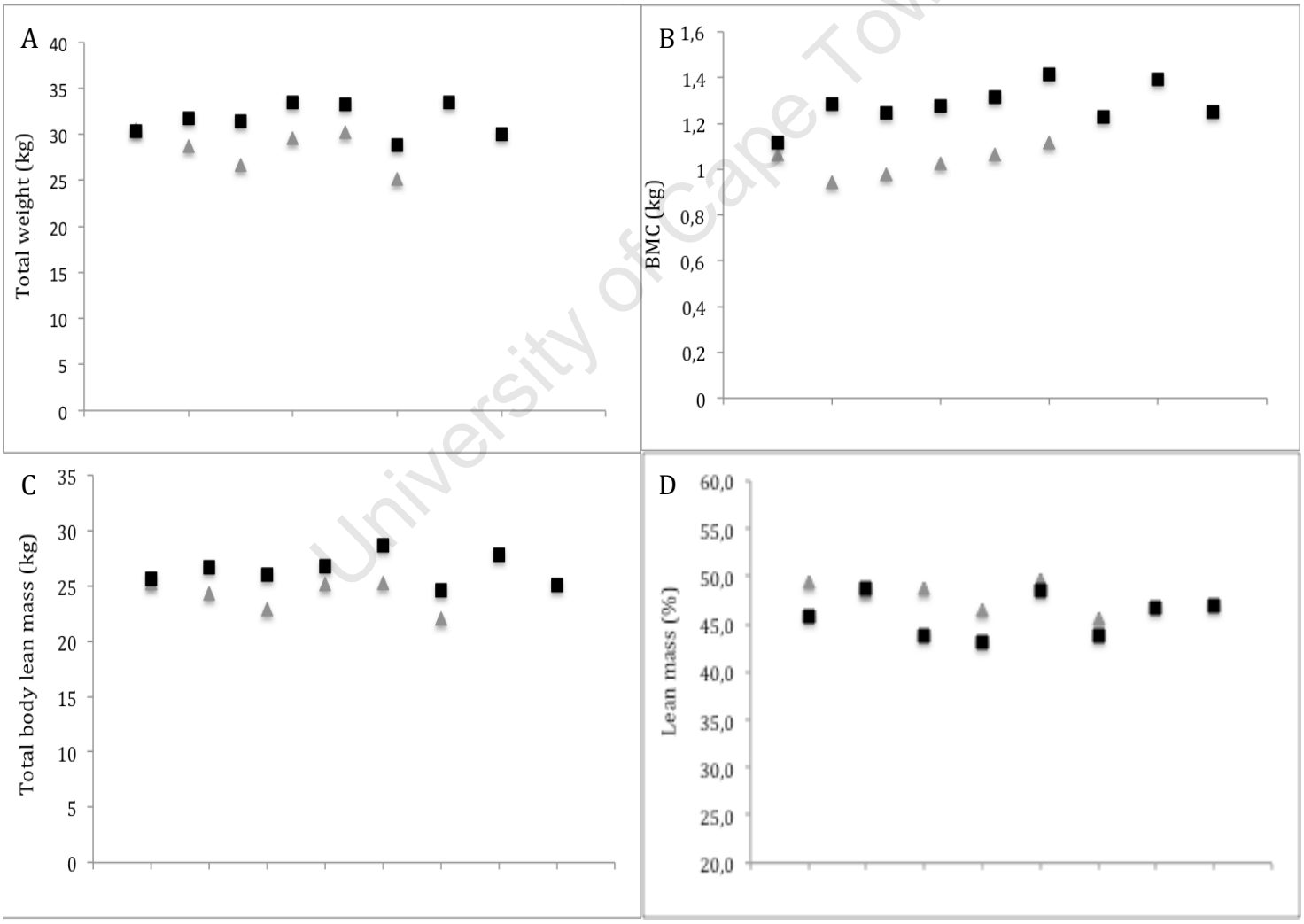
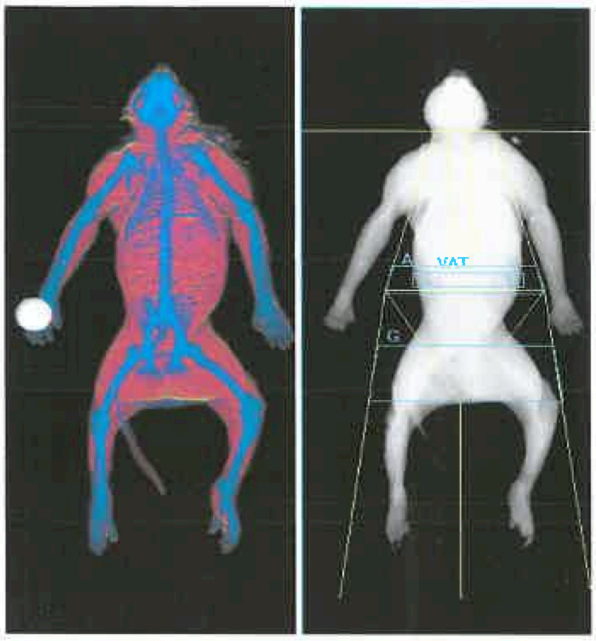
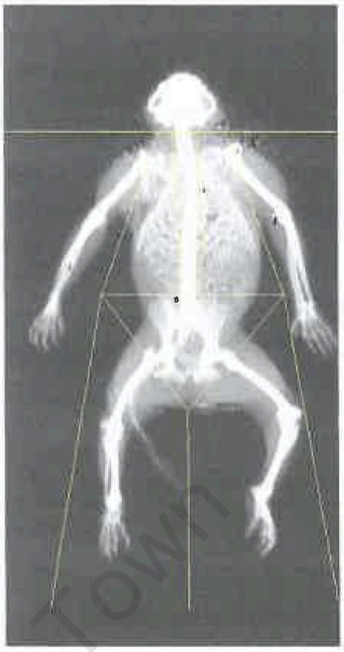


Figure 4: Scatterplots of DXA scan variables a) total weight (kg), b) BMC (kg), c) total body lean mass (kg) and d) lean mass (%) for control individuals (n=6) (grey triangles) and peninsula individuals (n=8) (black squares), adult males only





Body Composition Results

Reg	Fat Mass (g)	Lean + BMC (g)	Total Mass (g)	% Fat	%Fat Percentile YN AM
L Arm	223	2012	2235	9.99	
R Arm	221	2072	2293	9.63	
Trunk	2266	15875	18141	12.5	
L Leg	289	2896	3186	9.08	
R Leg	268	2774	3041	8.80	
Subtotal	3267	25629	28896	11.3	
Head	521	2332	2853	18.3	
Total	**3788**	**27961**	**31750**	**11.9**	
Android (A)	274	2054	2328	11.8	
Gynoid (G)	517	5132	5648	9.1	

Figure 5: Example of DXA scan output of a peninsula baboon (BOB 3), showing body composition by colour, where yellow indicates fat, red indicates muscle and blue indicates bone, with body composition results, and x-ray of skeleton on right

Teeth condition

The teeth from the peninsula baboons were observed to be in worse condition than the teeth from the control wild feeding baboons, more often showing missing teeth or decay (Figure 6). Four out of nine Peninsula baboons showed signs of teeth damage, while 1 out of 4 control baboons showed signs of teeth damage, probably due to old age.

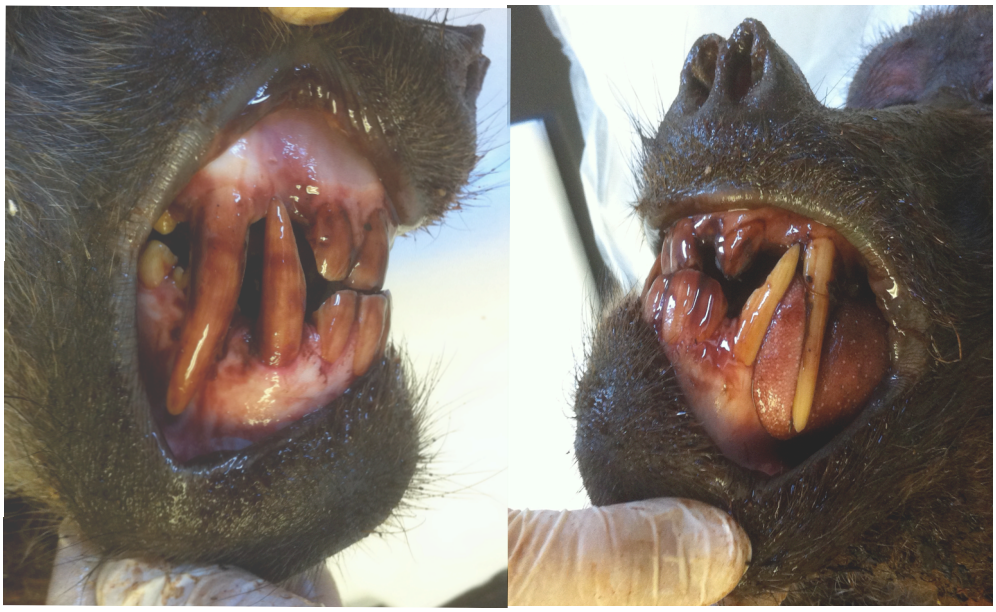


Figure 6: Example of teeth photographs taken while doing scans of a peninsula baboon, showing tooth condition

GLUT-4 levels

The average normalized GLUT-4 level was 24,1% higher in the Peninsula group than in the control group of baboons (Figure 7), however this difference was not significant **(U_{17}=27, Z=-1,111, p=0,274).** Therefore GLUT-4 levels are not reduced in Peninsula baboons eating human food, when compared to wild-feeding controls (Figure 8).

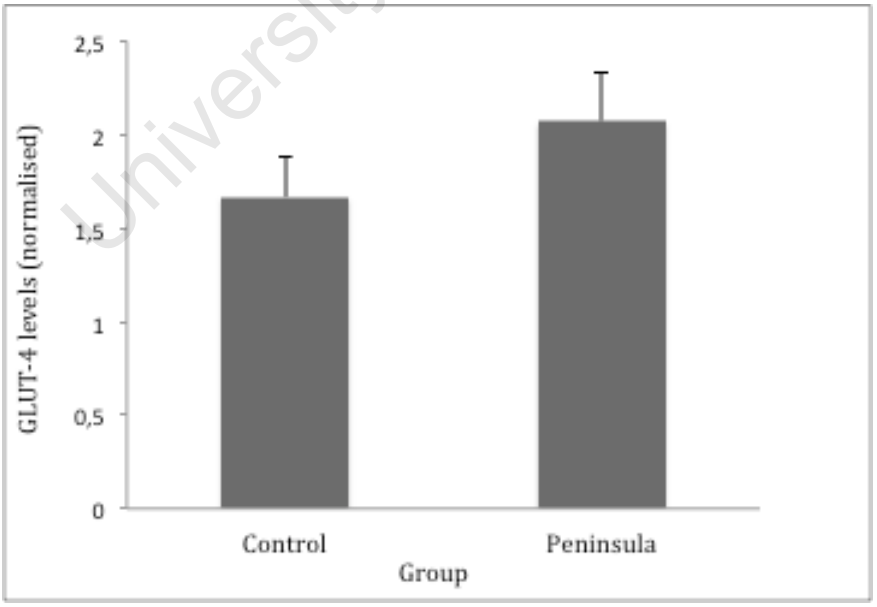


Figure 7: Average GLUT-4 levels in total pixels (normalised against alpha-tubulin) for control (n=4 each replicated twice) and Peninsula baboons (n=10)

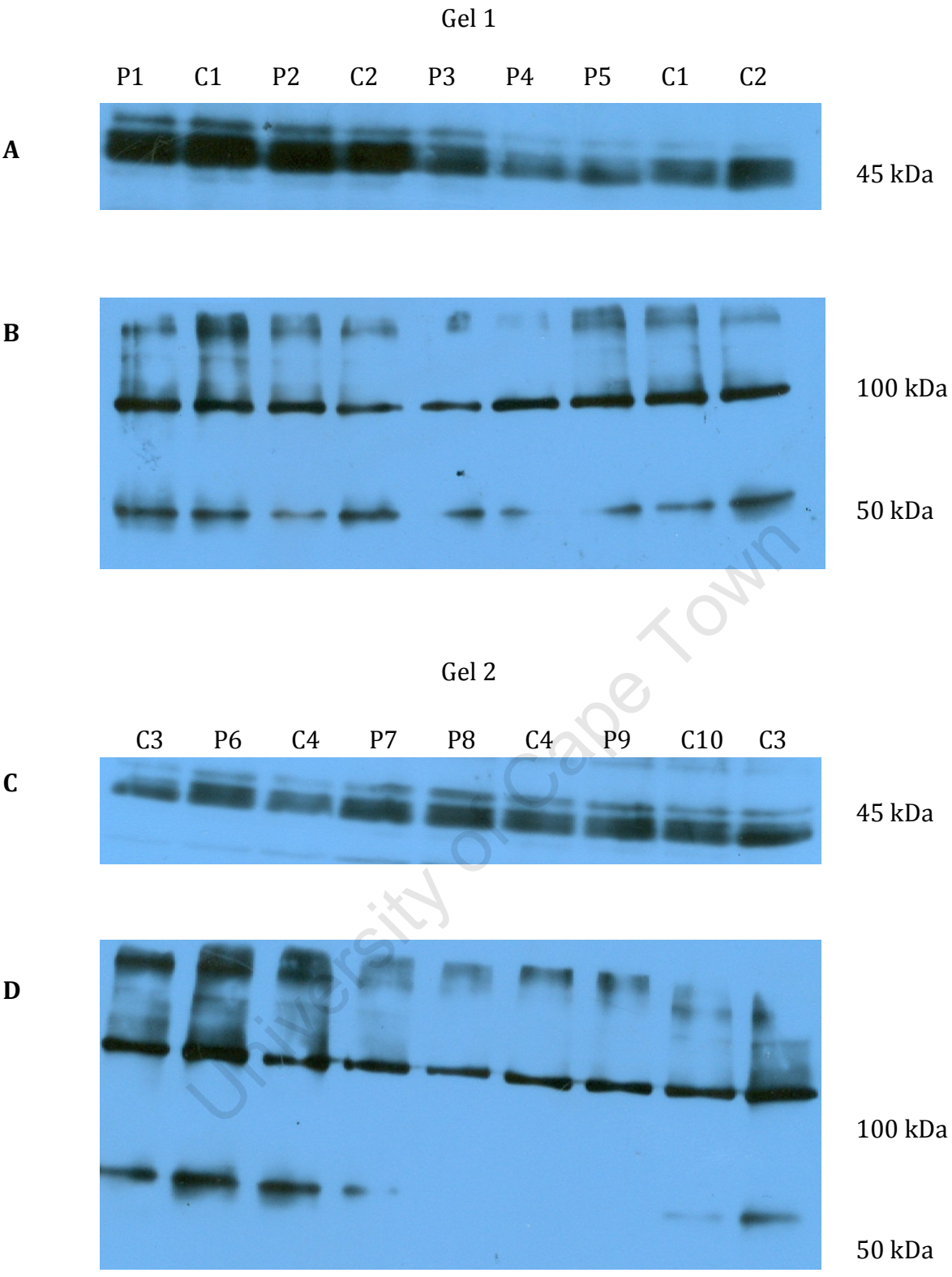


Figure 8: GLUT-4 western blots gel 1 and 2 (A & C) at 45 kDa, re-probed with alpha-tubulin (B & D) for normalization. Alpha-tubulin blots formed alpha and beta heterodimers at 100kda, which were used for normalization. C1-4 are the control baboon samples (each replicated twice) and P1-10 are the peninsula baboon samples

Discussion

Chacma baboons from the Cape Peninsula differed significantly in total weight (kg), bone mineral content (kg) and total body lean mass (kg) from control wild-feeding animals, but not in any measure related to fatness. The methods used in this study were one of the first to attempt to answer questions relating to the health of free-ranging but urban-foraging baboons. For example, this is the first time DXA scanning technology has been used on free-living baboons. Hence results may be slightly imprecise, due to problems associated with the newness of this technique. GLUT-4 protein transporter levels were not significantly different between the two groups, and any metabolic abnormalities arising from the Cape Peninsula baboon's diet cannot be conclusively commented on, particularly because of small sample size. Peninsula baboons showed slightly higher GLUT-4 levels, although this was likely an artefact of methodology. Tooth decay seemed more advanced in Peninsula baboons, than control baboons, but low sample size and confounding factors such as age and environmental variables make this difficult to assess.

DXA scan results

The three variables that varied significantly between control and Peninsula groups (total weight, BMC and total body lean mass) are all related in some way to the overall size of the animal. Because no variable associated with fatness differed between groups it can be assumed that being overweight was not in fact the reason for the significant differences in weight measurements. Therefore it may be that the Peninsula baboons are larger but not fatter than the control wild-feeding baboons. Peninsula baboons were heavier than control baboons and this is similar to the results of Altmann*, et al.* 1993 where both male and female semi-provisioned Yellow Baboons *(Papio cynocephalus)* had significantly higher body mass than wild-feeding baboons. Altmann et al. (1993) also found the mean fatness levels of garbage–feeding female baboons in Kenya was 23,2% while wild-feeding female baboons had a mean fatness of 1,9 %. One garbage-feeding male had a percentage body fat of 16,4% and the wild-feeding males ranged from 0,8 to 9,3%. Therefore females differed more in body mass than males, possibly because young males disperse and would be exposed to conditions different from the environment in which they matured (Altmann, *et al.* 1993). Wild-feeding baboons in our study had a mean fat mass of 11,37% while Peninsula baboons had a mean fat mass of

12,11%. Therefore none of the adult males scanned in this study approached the fatness levels of the female baboons studied by Altmann et al. (1993).

The lack of significant differences in DXA variables related to fat was also contrary to Higgins et al. (2010), where animals on a fast-food type diet gained fat mass, trunk fat mass, and percentage fat mass. Mean percentage fat mass after exposure to this diet was 10,8 %, which was still less than the two groups in this study. Most of the weight-gain of these animals occurred in the trunk region, consistent with trends found in humans (Higgins, *et al* 2010). This indicates that the diet of the baboons on the Cape Peninsula may not be causing the animals to become overweight as expected, or that both of the groups in this study have higher fatness levels than the other studies because of prolonged access to either human food or exotic plant food. Although the control baboons did not have access to human food, they did have access to agricultural foodstuffs such as lucerne, which is high in protein, and would be more calorically concentrated than their wild-diet.

Increasing body weight in adult baboons has been found to correlate to increases in total fat mass and increasing waist circumference (indicating weight gain is due to a gain in fat tissue), as in humans (Comuzzie, *et al.* 2003). This was not the case with the baboons of this study, as fatness indices did not show any difference between groups, while total weight was greater in Peninsula baboons. Body weight is also positively correlated to BMD (Bone mineral density), which is bone mineral content divided by bone area (Baumgartner, *et al.* 1996). Bone mineral is affected by mechanical stress that may be due to increased weight-bearing, such that heavier individuals would have a higher BMC. This correlation has been found in older humans of both sexes. Interestingly, muscle is more closely associated with BMC than fat is (Baumgartner, *et al.* 1996), which confirms the trend of larger size (higher BMC) but not fat of Peninsula baboons in this study. Also, control animals showed a nearly significant higher lean mass (%) than peninsula animals, indicating that the control animals were more 'wiry' than the peninsula animals, as a result of their assumed tougher lifestyle and greater distances travelled than peninsula baboons, which have been found to have reduced their path lengths significantly when living in a human-modified habitat (Hoffman & O'Riain, 2012). Baboons that raid for crops in other parts of Africa show similar trends: they grow faster, reach maturity sooner and achieve higher final weights than

individuals who do not raid for crops (Strum, 1994). Raiding males also have greater consort success as young adults immigrating to a new troop due to their larger size and better condition (Strum, 1994).

Environmental factors also play a role in weight variation between populations. A comparison on Chacma baboon populations from around southern Africa found that baboons in wetter habitats had a tendency to be larger than those in drier areas (Barrett & Henzi, 1997). The most influential factors for body weight were mean annual rainfall and temperature. Male weights were found to be more sensitive to rainfall than female weights, and can vary widely among populations, showing differences of up to 6kg. There was also greater sexual dimorphism in populations in low rainfall areas because of the greater decline in male weights as rainfall decreases. A linear relationship may exist between weight and rainfall and temperature, where as rainfall and temperature increases, so does body weight (Barrett & Henzi, 1997). Dunbar (1990) found that male and female baboon body weights were a quadratic function of rainfall, meaning that animals from very dry or very wet habitats were larger than those from areas of intermediate rainfall. The reason for large animals in dry areas is suggested to be because animals could only live in very dry areas if there were pockets of rich habitat available to them (Dunbar, 1990). Rainfall was also found to be an important predictor of size and shape of Vervet monkey skulls (size of skulls being a proxy for body size), with male skull size being especially affected by this variable (Cardini, *et al.* 2007). Rainfall is a good indicator of habitat productivity, and thus higher rainfall via greater habitat productivity leads to larger male size in Vervet monkeys (Cardini, *et al.* 2007). Although rainfall does therefore seem to be an important factor influencing size and morphological variation among populations of primates, it is likely to be influenced by many complex, interacting factors such as phylogenetic history, the insularity of the population, stochastic processes and diet (Cardini, *et al.* 2010). Therefore the warmer, wetter conditions of the Cape Peninsula likely result in larger baboons, when compared to the control animals from an arid Karoo environment as a result of greater primary productivity.

Higher natural primary productivity as a result of higher rainfall on the Cape Peninsula is probably not the only factor resulting in a larger body size. The troop size of the Karoo

control group are likely to be smaller than the large troops of the Cape Peninsula which have a mean troop size of 34 individuals, and a density of 12.1 baboons/km^2 (Hoffman &O'Riain, 2011). The potential troop size of this arid region may be more comparable to that of the Kuiseb Canyon in Namibia, with a density of 5.3 baboons/km^2 (Hoffman &O'Riain, 2011). Troop size would therefore be an indicator of the richness of the environment, and food availability (Chapman & Chapman, 2000), as provisioned troops on the Cape Peninsula maintain large troop sizes even with a greatly reduced home range (Hoffman & O'Riain, 2011) indicating how anthropogenic food sources such as garbage and plantations deregulate troop size from natural constraints. As there are no remaining predators in the area where the control baboons come from, large groups would be more costly than beneficial because of increased food competition (Chapman & Chapman, 2000). With smaller troop size and lower density there may be less competition between males, meaning that large size may not be under as intense sexual selection as in a region with high baboon density and therefore greater competition between males both within and outside of the troop, although an increased number of females available may lessen competition. Larger size in a more competitive environment may reflect the benefit of size to males that are in competition for alpha social status (Alberts, et al. 2006).

Trouleshooting

DXA scanning

The DXA scan results used to test for differences between groups are likely to not be completely accurate. This is mainly to do with the fact that some of the baboon carcasses scanned had expanded due to gas build-up in the gut, perhaps due to a slightly longer time between death and scanning. This may influence the area reading of the animals and therefore measurements such as BMC, which relies on the area reading. The carcasses that had this problem were all from the control group however, and the fact that the Peninsula baboons still had a significantly higher BMC than the controls may indicate that the area change did not change the overall result between the groups. Also, the DXA scans were performed with software designed for a human body shape, which may influence the appropriateness of the algorithms used to calculate the variables.

Baboons have a curved spine in relation to humans as well as being nearly half of the region of the human spine (Sheng, *et al.* 2010), and therefore may require changes to the DXA software. The positioning of the animals on the DXA bed may also influence then readings. The peninsula baboons and the first four controls were scanned lying on their backs, with limbs taped into a supine position, as with Higgins *et al.* (2010). The other control animals that were received at a later date were scanned in a different position, lying on their stomachs because rigor mortis made it difficult to straighten the limbs properly. This may have affected the results of the scanning.

GLUT-4

No significant differences in GLUT-4 levels were found between control and Peninsula animals. This may be due methodological problems, because the diet of the Peninsula baboons is not causing any metabolic abnormalities or because of small sample size. It is unlikely that a small difference would have been detected if it existed, because the procedure for analyzing western blots may not be highly accurate. Although western blotting is a proven technique it is often hampered by poor reducibility and a lack of accurate quantitation, as well as the long time between start and result (Protein Simple, 2011). These problems hampered this study as GLUT-4 control samples that were repeated in the same gel often had widely different values, and high standard errors.

Additionally the samples used as controls consisted of three females and one male, which is not ideally comparable to the all-male Peninsula group. Very limited amount of literature tested GLUT-4 levels/expression between sexes, and therefore this factor may not be essential. For example, male and female mice showed similarly reduced cell-surface GLUT-4 content in insulin-stimulated conditions under a high-fat diet, 35% and 50% reduction respectively (Zierath, *et al.* 1997). Methodology problems mainly involved normalizing against alpha-tubulin. Because no clear alpha-tubulin bands were achieved at 55 kDa, what was assumed to be a heterodimer of alpha + beta tubulin at 100 kDa was used instead. The reason for this was probably because the muscle samples were not boiled, as required for GLUT-4 (Abcam, 2013), which affected the denaturing required for getting alpha-tubulin bands. GLUT-4 normalization for baboon muscle samples therefore requires further optimization, however it remains an applicable protein for the study of potential metabolic abnormalities.

Management implications and future research

Baboons rank as among the most sugar-sensitive non-human primates, and will prefer water containing some form of sugar to normal water, at concentrations even lower than concentrations detectable by humans (Laska, *et al.* 1999). Sucrose was the most attractive sugar to baboons, as in humans, followed by fructose and glucose. This supports the hypothesis that baboons use sweetness as a criterion for food-selection (Laska, *et al.* 1999). This unexpectedly high sensitivity to sweetness will undoubtedly be contributing to the raiding behaviour of baboons on the Cape Peninsula, quite apart from the current work on addiction to sugar and fats that indicate that sugar causes increases in dopamine in the *nucleus accumbens* that is associated with addiction behaviour (Avena, *et al.* 2008). Additionally baboons and other Old-world primates respond to artificial sweeteners such as aspartame while New-World primates and lemurs do not (Glaser, *et al*. 1995).

Because of this preference for sweetened foods, baboon management on the Cape Peninsula is extremely complex. Combining this preference for human foods with ease of access means that raiding is likely to continue. Because of their dexterity and intelligence, deterrents work only for a short period of time. If future research finds that metabolic abnormalities such as reduced GLUT-4 levels are common within the Cape Peninsula population this may provide the impetus needed for long-term strategies such as complete relocation to take place. A study on two Olive baboon (*Papio anubis*) troops relocated to a wild area due to high conflict with humans indicates the potential for success (Strum, 2005). Relocation to another area has been shown to reduce human-induced mortality, and increase natural mortality via predation and disease (Strum, 2005). Birth-rates of relocated groups did not differ from indigenous groups and after time introduced troops were not more vulnerable to disease than indigenous groups. As a whole, relocated groups appeared to have adapted to a novel environment suitably (Strum, 2005). Interactions among newcomers and indigenous troops will cause increased competition, and therefore carrying capacity of an area must be considered in order for relocation to succeed. Relocations for other species of primates (Rhesus macaques (*Macaca mulatta*), Orangutans (*Pongo pygmaeus*), Red howlers (*Alouatta seniculus*) and Golden lion tamarins (*Leontopithecus rosalia*) to name a few, suggests

that the troops are able to adjust and reproduce as successfully as indigenous groups, provided intact units are released. These studies indicate that relocated primates show stages in the adjustment process after release; that death rate has a more significant effect than birth rates and that survival is sensitive to fluctuations in the short term (Strum, 2005). Therefore entire baboon troops have the potential to be relocated to a safer environement, and this approach could become an important management tool for the Cape Peninsula population.

The potential for future research on this conservation problem is very large. Metabolic abnormalities such as lowered GLUT-4 levels requires further study, as there was not enough time for full optimization of the methodology, but given sufficient time, very interesting conclusions could be reached. Other markers, such as IRS-1 could be used as an alternative to GLUT-4 (Shao, *et al.* 2002). Because sex may play a role in fatness levels and therefore metabolic abnormalities, it would be interesting to see a comparison between females from the Peninsula and a control area, to find whether they show similar trends in fat levels to the Kenyan baboons studied by Altmann *et al.* (1993). Additionally other indicators of the metabolic syndrome could be studied, such as looking for mitochondrial abnormalities (Chavez, *et al.* 2008), reduced oxidative capacity (Rimbert, *et al.* 2004), or muscle fibre typing (He, *et al.* 2001) between sedentary or obese populations and active populations.

Conclusion

The results from the DXA scans indicating that the baboons of the Cape Peninsula are larger but not fatter than wild-feeding controls is an interesting outcome, likely reflecting more nutritious, better quality and less seasonal food resources as a result of the higher rainfall, exotic plantations and human food of this area. It is thought that more intense competition due to a higher density (although the density of baboons in the control area is unknown) may also increase sexual selection for larger size. BMC is positively correlated to weight, and therefore it is as expected that higher BMC accompanies the greater weight of the Peninsula baboons in comparison to control baboons. The teeth condition of the Peninsula baboons is also thought to be worse than that of the control baboons, likely as a result of their non-natural diet. No difference was found in GLUT-4 levels, most likely as a result of methodological issues, and therefore

any metabolic abnormalities associated with this change in diet could not be detected. Further research into GLUT-4 levels is suggested, using larger sample sizes and controls from different localities. If GLUT-4 levels were found with future research to differ between groups, with Peninsula baboons having lowered levels, management strategies should be implemented that acknowledge the possibility of insulin resistance as a result of diet. If the Cape Peninsula baboon's health is being negatively affected by their current diet, manifesting as metabolic abnormalities, relocation may be a viable strategy in order to reduce conflict and potential negative health effects.

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Appendix

Bradford assay results

Table 1: Absorbance values (AU) from Elisa Plate, taken at 595 nm, for standards of BSA/ water solution,

Standards (g/l)	Absorbance 1 (AU)	Absorbance 2 (AU)	Average absorbance (AU)	Standard Error (St.dev/sqrt(n))
0,5	0,109	0,149	0,129	0,020
1,0	0,338	0,293	0,315	0,023
1,5	0,450	0,506	0,478	0,028
2,0	0,625	0,736	0,681	0,056

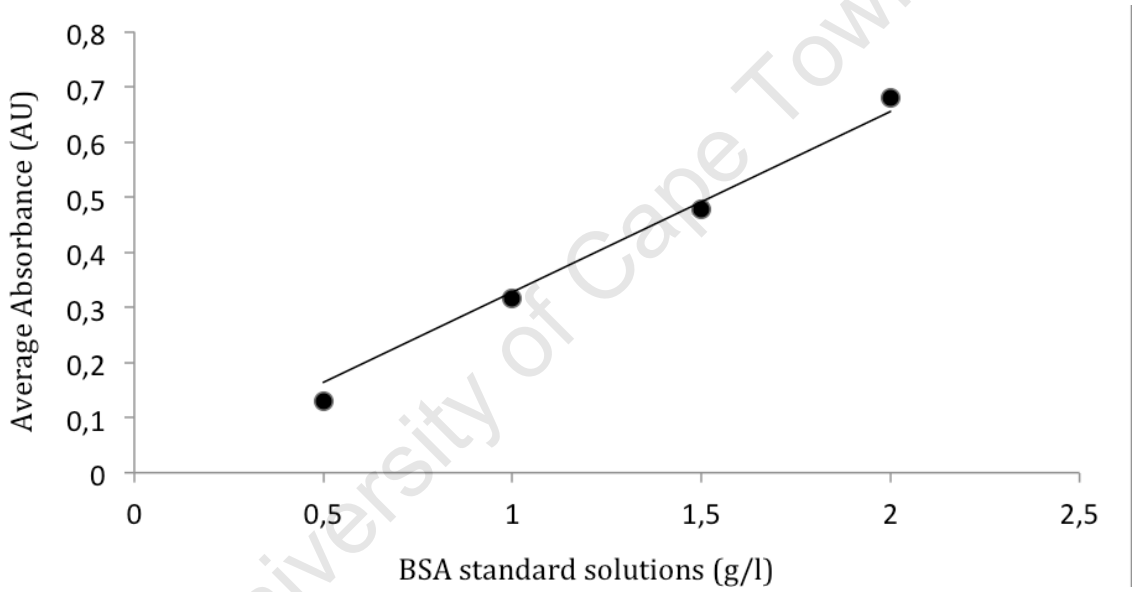

Figure 1: Average absorbance of standard BSA solutions (g/l), with trend-line of y=0,3277x fitted to the 0 intercept (R-squared = 0,987)

Table 2: Absorbance values (AU) for muscle samples performed in duplicate, with their average, and protein concentrations found using the standard curve equation (both diluted and undiluted)

Sample	Absorbance 1 (AU)	Absorbance 2 (Au)	Average absorbance (AU)	Protein Concentration diluted (ug/ul)	Protein Concentration undiluted (ug/ul)
C1	0,621	0,532	0,577	1,76	**10,56**
C2	0,744	0,613	0,679	2,07	**12,42**
C3	0,402	0,439	0,421	1,28	**7,70**
C4	0,609	0,583	0,596	1,82	**10,91**
P1	0,516	0,504	0,510	1,56	**9,34**
P2	0,473	0,559	0,516	1,57	**9,45**
P3	0,495	0,532	0,514	1,57	**9,40**
P4	0,499	0,600	0,549	1,68	**10,06**
P5	0,504	0,516	0,510	1,56	**9,34**
P6	0,623	0,581	0,602	1,84	**11,02**
P7	0,422	0,556	0,489	1,49	**8,95**
P8	0,516	0,445	0,481	1,47	**8,80**
P9	0,591	0,661	0,626	1,91	**11,46**
P10	0,546	0,625	0,586	1,79	**10,72**

SDS-PAGE and Western Blot trouble-shooting

GLUT-4 troubleshooting

GLUT-4 western blotting required about 3 gels of optimization, for both primary and secondary antibody dilutions, blocking time, and exposure. Loading the proteins into the gel required practice due to the presence of gel fragments in the well. The gel ran at too high a temperature during one gel due to a problem with the running buffer. Figures 2-4 illustrate the results of some of these problems. Figure 4 was the best GLUT-4 result achieved, but no alpha-tubulin bands were achieved, and therefore it could not be used.

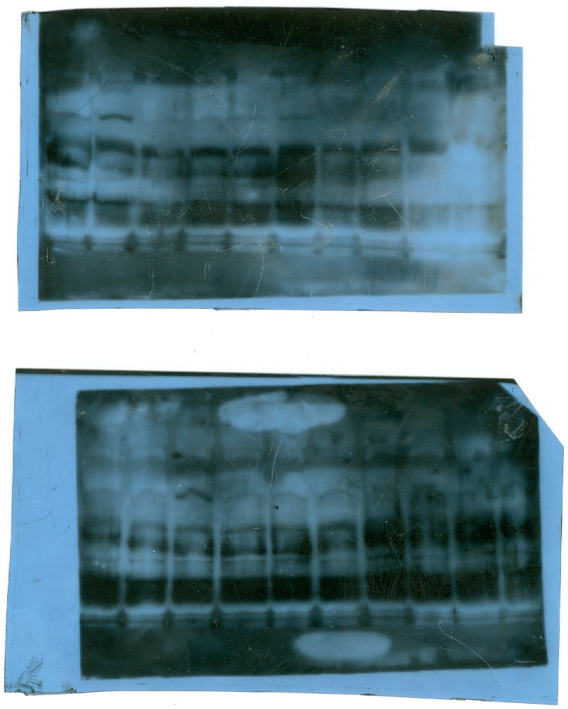

Figure 2: Western blot GLUT-4 film, gel 1&2, on control and peninsula baboon samples, attempt 2, with a primary antibody dilution of 1:2500 and developed for 15 seconds, blocking 1 hour

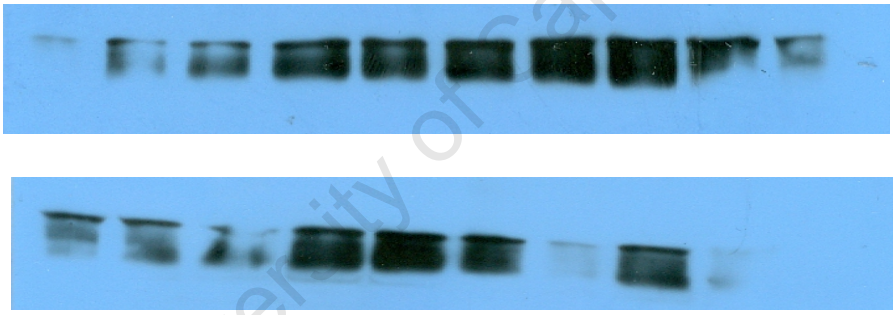

Figure 3: Western blot GLUT-4 film, gel 1&2, on control and peninsula baboon samples, attempt 4, with a primary antibody dilution of 1:5000 and developed for 30 seconds, blocking 1 hour in BSA TBS-T solution

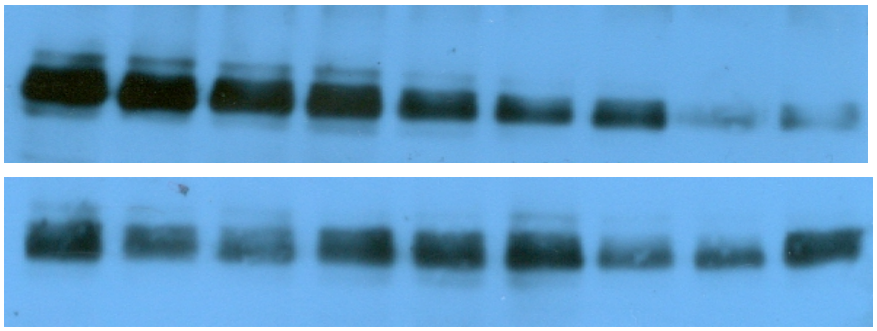

Figure 4: Western blot Glut-4 film, gel 1& 2,of control and peninsula baboon samples, attempt 8, with primary antibody dilution of 1:5000, developed for 1 min 30 seconds, blocking for 1 hour 30 mins in BSA, TBS-T solution

Alpha-tubulin troubleshooting

Alpha-tubulin required another 3 gels after GLUT-4 results were achieved to optimize, and by the final attempt no single useful bands were achieved (Figure 5,6). The only alpha- tubulin that had bands in all lanes were at 100kDa, which were alpha-beta heterodimers. Antibody problems were the cause of some of the failures, such as old antibody and antibody of unknown concentration. In all gels either no bands were found, or bands were achieved most strongly at 100 kDa instead of at 55 kDa. The suspected cause for the overall failure of Alpha-tubulin was the fact that the samples could not be boiled for GLUT-4 bands to be achieved, but alpha-tubulin may require boiling in order to break the bonds forming heterodimers.

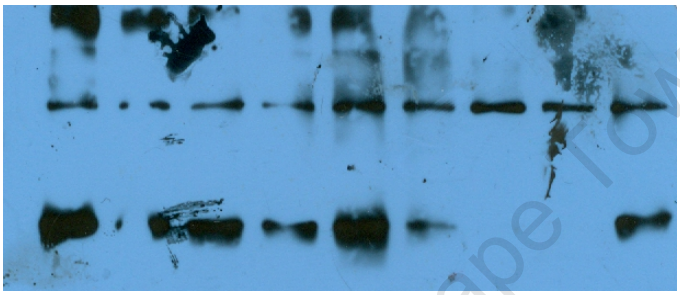

Figure 5: Western blot Alpha-tubulin film on control and peninsula baboon samples, gel 1, (c1-2, p1-5), with primary antibody dilution of 1:500, developed for 3 minutes, blocking for 1 hour 30 mins in BSA, TBS-T solution

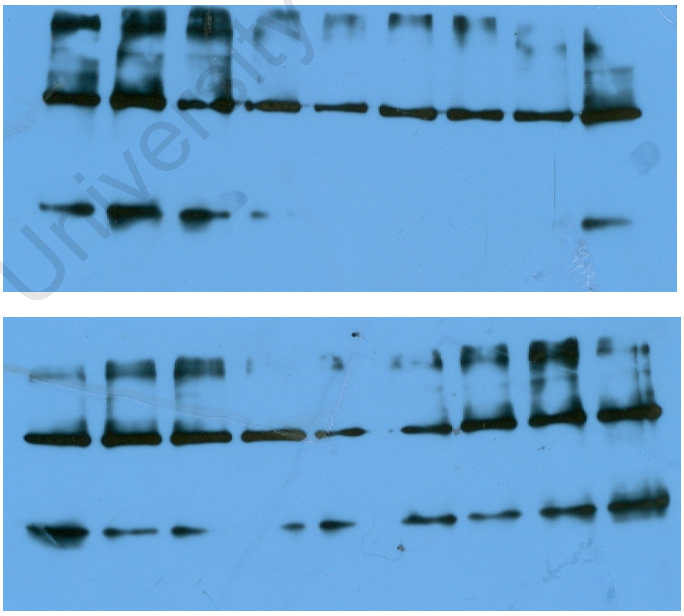

Figure 6: Western blot Alpha-tubulin film on control and peninsula baboon samples for gels 1&2 (c1-4, p1-10), with primary antibody dilution of 1:1000, developed for 3 minutes, blocking for 1 hour 30 mins in milk, TBS-T solution

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06 March 2013

Dear Dr Reed

Application for use of animals in research: protocol number 2013/V6/NEW

At a meeting on 28 February 2013, the Science Animal Ethics Committee (SFAEC) considered your application for ethics clearance for the study titled *The incidence of insulin resistance and type II diabetes in urban- and wild-feeding baboons*.

As this proposed research activity makes use of tissues collected opportunistically from dead baboons that will not be killed for the specific research purpose, the committee has concluded that SFAEC animal ethics clearance is not required. In accordance with the UCT Policy on the use of Non-Human Primates, this matter has been referred to the Senate Animal Ethics Committee for advice. I shall communicate the SAEC feedback as soon as this is received.

The SFAEC does, however, wish to advise you that:

1. Authorization from the Science Faculty Biological Safety Committee (BSC) may be required, as there is a risk of pathogen transfer from the baboon carcase/tissues to humans with the result that many organizations treat such carcase/tissues as biohazard risks and insist on special facilities for processing such materials. In this regard, you are encouraged to liaise with Dr Laura Roden concerning BSC clearance.
2. A special permit from CapeNature may be required for this work, and it is recommended that you contact the permit division at CapeNature to ensure that possible permit requirements are complied with.

Best wishes

Gary Bronner
Chair: SFAEC

Figure 7: Ethical clearance