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Development of Metabolic Function Biomarkers in the Common Marmoset, *Callithrix jacchus*

Toni E. Ziegler^{*1}, Ricki J. Colman¹, Suzette D. Tardif², Megan E. Sosa¹, Fredrick H. Wegner¹, Daniel J. Wittwer¹, and Hemanta Shrestha¹

¹Wisconsin National Primate Research Center, University of Wisconsin, Madison, Wisconsin, 53715

²Department of Cellular & Structural Biology, Barshop Institute for Longevity & Aging Studies, University of Texas Health Science Center at San Antonio, Texas, 78245

Abstract

Metabolic assessment of a nonhuman primate model of metabolic syndrome and obesity requires the necessary biomarkers specific to the species. While the rhesus monkey has a number of specific assays for assessing metabolic syndrome, the marmoset does not. Furthermore, the common marmoset (*Callithrix jacchus*) has a small blood volume that necessitates using a single blood volume for multiple analyses. The common marmoset holds a great potential as an alternative primate model for the study of human disease but assay methods need to be developed and validated for the biomarkers of metabolic syndrome. Here we report on the adaptation, development and validation of commercially available immunoassays for common marmoset samples in small volumes. We have performed biological validations for insulin, adiponectin, leptin, and ghrelin to demonstrate the use of these biomarkers in examining metabolic syndrome and other related diseases in the common marmoset.

Keywords

marmoset; *Callithrix jacchus*; metabolic syndrome; obesity; insulin; adiponectin; leptin; ghrelin

INTRODUCTION

The common marmoset, *Callithrix jacchus*, has many advantages for modeling metabolic dysfunction associated with obesity throughout the lifespan as well as such dysfunctions that may be caused by aging, itself. These small New World monkeys are adult by two years of age, have a high rate of fertility and have a short life-span (~16 years). Marmosets show metabolic dysfunction as well as frank diabetes associated with high body weight by six to eight years of age [Tardif et al. 2011]. More recent studies indicate that insulin resistance may occur as young as one year of age in animals that are already obese as juveniles [S.D. Tardif, personal communication]. Dietary changes such as increased glucose in the diet can lead to rapid obesity and these changes are different from results where marmosets are fed high fat diets that increase the incidence of atherosclerosis [Wachtman et al. 2011]. Marmosets display the typical indices of obesity found in humans when they weigh above

*Address correspondence to: Toni E. Ziegler, Ph.D. Wisconsin National Primate Research Center 1202 Capitol Court University of Wisconsin Madison, WI 53715 ziegler@primate.wisc.edu 608-263-3507.

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the 80th percentile [Tardif et al. 2009]. Obese individuals exhibit dyslipidemia and altered glucose metabolism.

Full development of the marmoset as a model of metabolic dysfunction has, however, been stymied by the lack of established assays for most of the relevant adipose, gut and pancreatic hormones. There has been a lack of known immunoassays using antibodies that reliably cross-react with marmoset metabolic hormones. Additionally, important hormones and biomarkers of metabolism have not been validated in marmosets to show a lack of interference with other substances in the biomatrix. The limited blood volume found in marmosets also necessitates the use of assays sensitive enough to measure the determinants in very small quantities of sample. In order to fully develop this model, hormones such as insulin, adiponectin, leptin, and ghrelin, need to be validated in the marmoset.

Due to insulin's role in regulating carbohydrate and fat metabolism by causing the uptake of glucose from the blood, insulin's response to glucose levels is one of the hallmarks of metabolic dysfunction. Glucose tolerance tests (GTTs) are used to determine how rapidly insulin causes the clearance of glucose from the blood. The immunogenicity of mammalian insulin of many species are highly conserved only differing in one to four positions with 92–98% identity [Hallden et al. 1986]. However, it was originally reported that in the squirrel monkey, *Saimiri sciureus*, insulin was not detectable following glucose-stimulations. Furthermore, New World monkey insulin did not have a high level of cross-reactivity in an insulin RIA using guinea pig anti-pork insulin antibody, whereas this assay detected rhesus monkey and chimpanzee insulin similar to humans [Mann and Crofford 1970]. This led to the belief that New World primates were like New World species of rodents, hystricomorph rodents, whose insulin sequences differ extensively from Old World mammals [Smith 1966]. However, purification and sequencing of squirrel monkey, owl monkey, *Aotus trivirgatus* [Yu et al. 1990], and more recently the common marmoset insulin [Wallis 2009], has indicated that for New World monkey species the amino acid sequence is highly conserved and differs from humans in five residues.

It is now well established that adipose tissue serves as an important endocrine gland [Ahima 2006]. One of the hormones secreted by adipocytes is adiponectin. It is one of the main regulators of carbohydrate and fat exchange, produced exclusively in fat tissue [Oranskiy et al. 2012]. Obesity is associated with a decrease in circulating adiponectin [Kiortsis et al. 2001] but while weight loss has been shown to increase adiponectin, there are conflicting results [Christou et al. 2012]. This may be because there are known to be several multimeric forms of adiponectin that circulate in human [Waki et al. 2003] and rhesus monkey [Bobbert et al. 2008]. These forms are thought to have different biological activity. High molecular weight adiponectin (HMW) is the most bioactive [Guerre-Millo 2008]. Current commercial assays offer the measurement of medium molecular weight adiponectin (MMW), and total adiponectin (Total). HMW has been shown to predict future weight gain [Hivert et al. 2008] and the ratio of HMW to Total has been found to be associated with obesity in children [Aknc et al. 2012]. However, it is unclear in some conditions such as coronary atherosclerosis whether HMW or the ratio of HMW/Total adiponectin provides the more accurate prediction of metabolic condition [Liang et al. 2006].

Another adipocyte hormone, leptin, is involved in regulating energy intake and expenditure by influencing appetite and metabolism. Leptin signals a feedback system regulating body weight and energy balance [Friedman 2002]. Leptin is positively correlated to body weight. It is secreted in direct proportion to the amount of stored body fat and therefore serum leptin responds to fluctuations in body fat [Considine et al. 1996]. Additionally, leptin interacts with the brain to modulate food intake and energy expenditure [Penicaud et al. 2012]. Leptin has been measured in rhesus monkeys using a human leptin radioimmunoassay [Colman et

al. 1999; Ramsey et al. 1998]; [Michopoulos and Wilson 2011] but not in marmoset monkeys.

Ghrelin, a 28 amino acid peptide hormone, is primarily produced in and secreted from the gastric mucosa. Ghrelin acts at the level of the hypothalamus, initiating signaling cascades leading to changes in food intake, and is involved in appetite and energy homeostasis. Ghrelin acts in both the short-term control of food intake at single meals and in long-term body-weight regulation [Cummings 2006]. Ghrelin levels rise before meals and fall rapidly after ingestion [Wren et al. 2001]. Additionally in its role in energy homeostasis, ghrelin levels inversely correlate with measures of adiposity and with alterations in body weight [Cummings 2006].

The following report describes the methods used to adapt and validate commercially available immunoassays for insulin, adiponectin, leptin, and ghrelin for use in common marmoset serum samples at small volumes. Unsuccessful attempts with commercial kits are also reported. Assays were developed and biological validations implemented to demonstrate the validity of these assays for marmoset serum.

METHODS

Insulin Assay

Initial attempts to measure insulin did not provide for good validations of parallelism and accuracy. We first used the Human Insulin Specific RIA (radioimmunoassay) kit (Millipore, Billerica, MA) as it was currently in use for rhesus blood samples in Assay Services at the WNPRC and provided excellent results. Therefore, we selected the Porcine Insulin RIA (Millipore, Billerica, MA) as it uses a porcine insulin antiserum that is less specific than the human specific insulin antiserum but still crossreacts 100% with human, 100% with porcine and 90% with bovine insulin. We initially used 100 μ l sample volumes in duplicate but decreased volume to 50 μ l as the assay was sensitive enough to measure insulin in low levels in the marmoset serum. We followed the methods recommended in the procedural manual. Serially diluted pooled marmoset serum in assay buffer was parallel to the standard curve points ($t = -1.67$, $df = 26$, $P > 0.05$, Figure 1a). Accuracy of the assay was determined by adding previous assayed marmoset serum with known amounts of insulin to the standard curve points and determining the percentage of expected from observed results. The volume and concentration of insulin added to the standard curve points remains constant as concentration of the standard insulin changes. Accuracy was determined to be 98.89 % + 2.38 SEM, $N = 18$. Sensitivity of the assay was determined to be 0.25 μ U. The coefficient of variation (CV) for inter & intra, respectively, were 4.89 & 2.87 for 6 assays.

Two biological validations were also performed to establish that the assay was measuring marmoset insulin. First we performed GTTs on 12 marmoset males around 2 years of age (18 – 24 months). These males were selected as healthy animals with normal weights from the Wisconsin National Primate Research Center's (WNPRC) marmoset colony. For GTT, a baseline blood sample was taken from the femoral vein in the awake, manually restrained marmoset followed by an oral dose (5 ml/kg, via syringe) of a 40% sucrose solution. Blood samples were then collected at 15, 30, 60 and 120 minutes after glucose dosing, processed as serum, and frozen at -80°C for subsequent analysis. Second, a pancreas was removed from a marmoset at the time of euthanasia for another project. The pancreatic tissue was placed in 5 ml of culture media containing Hanks' salts, L Glutamine, 25 mM HEPES (Media199, GIBCO, Invitrogen, Grand Island, New York) for 10 minutes at 37°C in an incubator. One ml of the media was then removed placed in a vial and stored frozen until assay while 1 ml of D-Glucose 1.8 mg/ml was added to the pancreas. The tissue was agitated to mix the solution and incubated 10 minutes. This was repeated so that at 15, 30 and 60 minutes 1 ml

of media was removed for assay. Both the live marmoset samples and the pancreas-timed samples were measured for insulin concentration as described above. Glucose was measured by a glucometer in the serum samples of the 12 males to illustrate the insulin response to glucose challenge.

Adiponectin Assay

Our first selected adiponectin assay (RIA, Human adiponectin, HADP-61HK, Millipore, Billerica, MA) did measure adiponectin in the marmoset but the serial dilution of marmoset serum was not parallel to the human standards ($t = 3.4$, $P < 0.05$) and the accuracy showed an overestimation of adiponectin throughout the standard curve range (accuracy = 127.40 ± 2.25 s.e.m). We then selected an Adiponectin multimeric ELISA (enzyme labeled immunosorbent assay) for several reasons. First, the procedure required less marmoset serum for dilution and measurement then the RIA and therefore could be further diluted which would lessen the interference seen with the previous assay. Second, the use of an enzyme assay meant not having to use radioactivity and third, the ELISA was multimeric meaning that we could measure the variants of adiponectin as well as the total.

Adiponectin was measured independently as the high molecular weight (HMW) and then again as Total adiponectin (all forms of adiponectin). To measure HMW, samples were pretreated with Protease II to digest the low and mid-molecular weight adiponectin. Total adiponectin was measured by reducing multimeric adiponectin to dimers as per instructions and subsequent measurement by enzyme immunoassay (ELISA). We used the Adiponectin ELISA (#47-ADPHU-E01, ALPCO Diagnostics, Salem, NH) and followed the instructions. Samples were 10 μ l of marmoset serum and for the HMW the sample was diluted 1:2575. A protease buffer was used to dilute the sample and incubated at 37°C for 20 minutes. The samples were read using a microplate reader at 492 nm. Parallelism and accuracy were determined for the HMW adiponectin. Serial dilutions of marmoset serum in assay buffer were parallel to the standard curve with no differences between the slopes of the line ($F = 2.87$, $df = 1,6$, $P > 0.05$, Figure 1b). Accuracy was 94.97 ± 2.36 SEM. The CVs for HMW with 4 assays were 6.1 & 1.5. Total adiponectin was run at a dilution of 1:5151 due to the high concentration in the marmoset samples. Serial dilutions of marmoset serum in assay buffer were parallel to the standard curve with no differences between the slopes of the line ($F = 5.01$, $df = 1,6$, $P > 0.05$, Figure 1c). Accuracy was 105 ± 2.18 SEM. The CVs for Total adiponectin with 4 assays were 6.1 & 1.5.

Levels of Total, HMW (High Molecular Weight) and the Ratio of HMW to Total were compared with the body fat determined by quantitative magnetic resonance scans as previously reported [Tardif et al. 2009] in 30 male and female marmosets all at 12 months of age to determine which form of adiponectin would best correlate with body fat levels. These marmosets lived at the Southwest National Primate Research Center (SNPRC) and had been categorized as either obese or lean by the ratio of fat to weight. Additionally, HMW, Total and the ratio, were compared to the actual weight of the marmosets at the time of sampling. Marmosets were then compared in their adiponectin levels to whether they were lean or obese.

Leptin Assay

Initial validations were attempted with the Multi-Species Leptin RIA kit (#XL-85K, Millipore, Billerica, MA). With this assay we obtained parallelism ($t = 1.32$, $P > 0.05$) but the accuracy was poor (78.56 ± 3.17 s.e.m.). Additionally the assay was not very sensitive. Better results were obtained with the Human Leptin RIA (#HL-81K, Millipore, Billerica, MA). This assay uses 125 I leptin, a human leptin antiserum to determine the level of leptin in marmoset serum samples. The leptin antibody cross-reacts 100% with human leptin but less

than 0.2% of mouse leptin, human insulin, C-peptide and glucagon are non-detectable in this assay as reported in the leptin kit literature. The standards are derived from human leptin. We used 100 μ l sample volumes in duplicate and followed the methods recommended in the procedural manual for the RIA leptin kit. Serially diluted pooled marmoset serum in assay buffer was parallel to the standard curve points ($F = 1.42$, $df = 1,26$, $P = 0.24$, Figure 1d). Accuracy of the assay with marmoset serum was determined by adding known amounts of marmoset serum to the standard curve points and determining the percentage of expected from observed results. Accuracy was determined to be $99.88\% \pm 1.95$ SEM, $N = 18$. Sensitivity of the assay was determined to be 0.1ng/ml. The CVs for 2 assays were 9.19 & 6.71.

A biological validation was performed to determine if leptin levels would strongly correlate with weight or age in marmosets. Serum samples taken in the morning (09:30) from 10 healthy males between the ages of 2 and 13 years housed at the WNPRC marmoset colony were analyzed for leptin concentration according to the method describe above.

Ghrelin Assay

Marmoset serum was analyzed for ghrelin using Human Ghrelin RIA (# GHRA-88HK, Millipore, Billerica, MA). The assay kit uses an antibody specific to the biologically active form of ghrelin with the octanoyl group on Serine 3 and 125 I Ghrelin. The ghrelin antibody crossreacts 100% with human, rat and canine ghrelin and the 1–10 amino acid ghrelin variant. No significant cross-reactivity occurs with leptin, insulin, glucagon GLP-1 or other peptides tested. Sensitivity of the assay is 7.8 pg/ml. Due to the unstable nature of the active form of ghrelin, marmoset serum samples were kept on ice, acidified with 15 μ l of 1 N HCL and 3 μ l of Phenylmethylsulfonyl fluoride (PMSF) per 300 μ l serum as instructed for this assay. We used 100 μ l for marmoset serum. Initial tests for parallelism indicated that interference precluded the marmoset samples from paralleling the standards. Therefore, we diluted the sample and ran a 25 μ l amount of marmoset serum. Serially diluted pooled marmoset serum in assay buffer was parallel to the standard curve points ($F = 2.70$, $df = 1,20$, $P = 0.12$, Figure 1e). Accuracy of the assay with marmoset serum was determined by adding known amounts of marmoset serum to the standard curve points and determining the percentage of expected from observed results. Accuracy was determined to be $89.18\% \pm 2.43$ SEM, $N = 15$. The CVs for 2 assays were 15.33 & 6.79.

Since daily fluctuations of human serum ghrelin reflect ghrelin's pattern as a meal-initiating hormone we took sequential blood samples from marmosets to determine if the assay would reflect ghrelin's role as a meal-initiating hormone. Five male marmosets (1.6–2.0 years) were sampled at three times during the day: 11:30 as a midmorning sample; 13:30 as a pre-meal sample and at 15:30 as a late afternoon sample without food restriction, (2 hr postmeal). An additional six marmosets, both male and female, with ages varying from 2.3 to 8.4 years of age were fasted for 16 hours overnight before the first blood collection (8:30 am), fed a normal meal, then the next blood samples was taken two hours after feeding (11:00 am). To test for ghrelin's role in long-term effects such as varying inversely with weight, we collected blood samples from 18 male and female marmosets (1.6 to 10 years of age) to correlate with their weights selected to show a large weight variation (332 to 509 grams) taken on the same day. This last group of marmosets were not food-deprived before samples were taken but did not have a major meal prior to sampling (blood sample at 11:00).

For all animal studies at the Wisconsin marmoset colony, the research complied with protocols approved by the University of Wisconsin, Graduate School's AUCUC. For the study requiring the marmosets at Southwestern, the protocols were approved by the University of Texas Health Science Center at San Antonio's ACUC committee. The research adhered to the legal requirements of performing research in the United States. Additionally,

this research adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non Human Primates.

Statistical analyses

The biological validations used several statistical tests. All correlations were performed, as Pearson's parametric, as the data were normal, and Student's t-tests were used to compare two data sets, 2-sided. Analysis of Variance, ANOVAs, were performed as 1-way, parametric. Significance was reported as $P < 0.05$.

RESULTS

Insulin

Insulin showed a normal response curve following glucose dosing for the 12 marmoset males (Figure 2, upper panel). No increase over baseline occurred in the 15 and 30-minute samples while insulin was increased at 60 minutes. Insulin levels declined for all marmosets by 120 minutes. We validated this response in the marmoset by determining the pancreatic response to glucose independent of other factors in the blood that might interfere with measurement. The lower panel of Figure 2 depicts the pancreatic insulin response to the glucose added to the culture media. Insulin was elevated by 10 minutes and reached peak levels by 30 minutes of incubation. Insulin had returned to baseline by 60 minutes.

Adiponectin

Total adiponectin levels were significantly negatively correlated to the fat levels in the combined male and female 12-month-old marmosets (Pearson $r = -0.37$, $P = 0.04$, $n = 30$). The HMW values showed a trend towards significance ($r = -0.36$, $P = 0.07$, $N = 30$) while the ratio of Total to HMW was nonsignificant ($r = 0.12$, $P = 0.52$, $n = 30$). When male and females were examined separately for their correlation of fat to Total adiponectin, females showed no correlation (Pearson $r = 0.09$, $P = 0.74$, $N = 18$) while Total adiponectin did negatively correlate with fat in males ($r = -0.96$, $P = 0.0001$, $N = 9$). Total adiponectin levels also negatively correlated to the marmoset weight (Pearson $r = -0.44$, $P = 0.04$, $N = 30$). As with the Fat level of a marmoset, weight did not correlate with HMW ($r = -0.29$, $P = 0.11$, $N = 30$) or the ratio of Total to HMW ($r = -0.08$, $P = 0.69$, $N = 30$). When lean marmosets were compared to obese marmosets, Total levels were significantly different ($t = 2.83$, $P = 0.01$, $N = 13,14$, Figure 3) however HMW ($t = 1.50$, $P = 0.14$) or the Ratio of Total/HMW ($t = 0.57$, $P = 0.57$) did not show significant differences.

Leptin

Weight in grams measured at the time of the serum sample, were tested for correlation with their corresponding leptin concentration by Pearson. Weights in the males ranged from 300 to 517 grams while leptin ranged from 0.33 to 1.17 ng/ml. Figure 4 presents the data indicating a significant and strong correlation between weight and leptin ($r = 0.87$, $P = 0.002$, $N = 9$). No correlation was found between age and leptin ($r = 0.24$, $P = 0.53$) or between age and weight ($r = 0.08$, $P = 0.84$, $N = 9$).

Ghrelin Assay

Mean ghrelin levels were highest at the pre-feed time interval and lower at the pre and postfed times (Figure 5). However, not all males showed the same pattern and the changes were not significant (1 way ANOVA, $F = 0.71$, $df = 2,4$, $P = 0.52$). The males and females who were sampled fasted and then after feeding did not show changes in ghrelin levels to reflect the expected ghrelin changes with satiety (t-test, 2-sided, $t = 0.44$, $df = 5,5$, $P = 0.67$). However, weights of individual males and females did show the expected long-term effect

of an inverse correlation with weight (Pearson $r = -0.63$, $n = 18$, $P = 0.005$). Marmosets were then divided into high weight (over 300 grams which represents average and elevated weight, $n = 12$) and Low weight (under 300 grams, indicates a very thin marmoset, $n = 5$) and tested for significant differences in ghrelin levels. There were significant differences between the two groups ($t = 4.96$, $df = 15$, $P = 0.0002$) with the low weight marmosets showing the highest levels of ghrelin (Figure 6).

DISCUSSION

The metabolic hormones, insulin, leptin, adiponectin and ghrelin, were successfully validated for the common marmoset. For as little as 400 μ l total, all four metabolic hormones can be analyzed via commercially available RIA and ELISA. We were able to show that each assay could be validated to show no interference and that the assay was accurately measuring the hormone of interest in marmoset serum. Furthermore, we also demonstrated a biological validation for each hormone to confirm the expected physiological response to the hormone.

Measurement of insulin is critical to assessing metabolic regulation. In the glucose tolerance test, insulin responded predictably to the glucose challenge. Cultured pancreas tissue also responded to the glucose challenge with a rise in insulin. As expected, the pancreas responded more rapidly and with higher insulin increase than was found for the blood samples. The glucose was able to reach the pancreas immediately in the cultured pancreatic tissue and showed almost instant release. This experiment isolated the insulin response to glucose to the pancreas and reflects the selectivity of the assay for insulin when measuring in the blood where other compounds could react. Recent research comparing the pancreas of marmosets to humans found many similarities in the microstructure and the glucose transporter expression indicating that the common marmoset is an ideal model for diabetes research and regenerative medicine [Mohanasundaram et al. 2011]; [Plentz et al. 2012]. This is the first validation reported for an insulin assay for marmosets.

While HMW adiponectin is considered the most bioactive form, we found that Total adiponectin showed the expected significant negative effect with both fat and weight and with obese measurements. HMW showed a trend towards a negative correlation with the fat values but not with weight. The ratio of HMW/Total did not correlate with either measurement of body condition. Thus, Total adiponectin may be the best method for examining adiponectin's role in insulin resistance and protection from obesity in marmosets. In humans, lower adiponectin levels are associated with increased insulin resistance and obese people have significantly less circulating Total [Weyer et al. 2001]; [Kovacova Z 2012]. While Total levels are lower in plasma samples from obese than non-obese humans, the secretion of HMW versus Total is higher in visceral fat than in blood samples and subcutaneous adipose tissue and more indicative of obesity consequences. Since our study had young but definitively obese marmosets, Total appears to be the best method of assessing the difference. When assessing changes in adiponectin with dietary changes in non-obese individuals, HMW, or the ratio of HMW to Total, may be more reflective of the influence of fats on the form that is released into the circulation. Further work in this area may provide for a better understanding of the forms of adiponectin and its release from adipose tissue.

As expected, leptin showed a significant correlation with body weight. Two of the marmosets had very high weights and could be classified as obese. These marmosets had the highest levels of leptin. The human leptin RIA allows for analysis of marmoset leptin for future studies of metabolism.

While we were able to validate the ghrelin assay for marmoset serum, we did not see the expected pattern of ghrelin fluctuations throughout the day with food intake that has been reported for humans [Wren et al. 2001]. Nor did we see differences between a fasted and post-parandial sample collected. Further investigation is needed to understand why the marmosets did not show the expected pattern. It is possible that our sample collection timing was not appropriate to show the ghrelin changes with food expectation or feeding. Perhaps the post-fed sample should have been taken within an hour after feeding or that the fasting sample should have been taken earlier in the morning when the marmosets were expecting food. In humans, it has been shown that ghrelin levels can fall to trough-like levels within one hour after eating [Cummings et al. 2001] and we had waited two hours. It is also possible that since marmosets eat throughout the day, instead of at specific time points as most humans who have been studied for their ghrelin patterns do, that ghrelin levels will not show an anticipatory rise prior to the main feed of the day. More importantly, our final study on ghrelin levels and weight did reflect the relationship between and ghrelin in that higher weight marmosets had significantly lower levels of ghrelin. A recent study in humans has shown that ghrelin is negatively correlated with weight in normal weight men as well as change in weight and that the relationship is positive in obese humans [Cummings et al. 2001; Wadden et al. 2012]. Additionally, ghrelin plays a role in insulin sensitivity [Dezaki et al. 2004; Qader et al. 2005] and may be a counteraction to insulin resistance in obese individuals who may be heading towards diabetes. Since many of the studies where marmoset ghrelin levels would be examined, the relationship to weight and obesity might provide an indicator of the progression towards obesity.

Marmosets gain weight readily with high fat or high sugar diets reaching obese weights [Wachtman et al. 2011]. Now we have the tools to examine the metabolic hormones and how these are changing in relationship to varied diets. The assays validated and used in the current study were easily accessible and can be run routinely in most laboratories. These assays also take into account the limited blood volume is available from marmosets.

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Figure 1.

Parallelism graphs for: insulin, adiponectin, leptin, and ghrelin. Pooled marmoset serum was serially diluted and run against the hormones used to create the standard curve.

Figure 2.

Insulin response to glucose in the marmoset. The upper panel represents the mean $\pm$ s.e.m. for 12 male marmosets for serum glucose levels and endogenous insulin response to the GTTs (glucose tolerance tests). Insulin responded to the glucose challenge between 30 to 60 minutes following the challenge. The lower panel represents the release of insulin from a marmoset pancreas cultured *in vitro*. Insulin responded to the glucose added to the media by 20 minutes and showed peak levels by 30 minutes.

Figure 3. Significant differences in Total adiponectin levels between lean and obese marmosets, $P < 0.05$. The lean versus obese determination was made by comparing the fat content, as measured by quantitative magnetic resonance scans, to the weight of the marmoset.

Figure 4.

Ghrelin levels at three time-points in five male marmosets: midmorning was two hours prior to the main feed of the day, pre-meal was right at the time of feeding, and post-meal was two hours past feeding. Although the mean $\pm$ SE showed the expected profile of ghrelin highest around the main feed, the results were not significant.

Figure 5. Ghrelin levels (pg/ml) were significantly different between low weight (< 300 grams) and high weight (> 300 grams) in the serum of 18 male and female marmosets.

Figure 6.