Predictive Models of Insulin Resistance Derived from Simple Morphometric and Metabolic Measurements Related to Obesity in Baboons.

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Running Title: Simple Predictive Models of Insulin Resistance in Baboons

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Abstract

Non-human primates are valuable models for the study of insulin resistance and human obesity. In baboons, insulin sensitivity levels can be evaluated directly with the euglycemic clamp and is highly predicted by adiposity, metabolic markers of obesity and impaired glucose metabolism (i.e. percent body fat by DXA and hemoglobin A1C). However, a simple method to screen and identify obese insulin resistant baboons for inclusion in interventional studies is not available. We characterized a population of obese nondiabetic, insulin resistant baboons using the euglycemic clamp technique and used a multivariate linear regression analysis (after adjustment for gender) to test three different predictive models for insulin sensitivity. In the first model, abdominal circumference explained 63% of insulin mediated glucose uptake. The second model, which included fasting plasma insulin (log transformed) and abdominal circumference, explained 69% of insulin-stimulated glucose uptake. The third model, which contained abdominal circumference and 1/log (FPI+FPG), explained 70% of insulin sensitivity. In baboons, simple morphometric measurements of adiposity/obesity, i.e. abdominal circumference, plus baseline markers of glucose metabolism, i.e. fasting plasma glucose and insulin, provide a feasible method to screen and identify overweight/obese insulin resistant baboons for inclusion interventional studies aimed to study human obesity and type 2 diabetes.

Introduction

Non-human primates are valuable models for the study of human disease, because of their close genetic and physiologic similarity to man (1-3). The baboon (*Papio hamadryas*) develops biochemical and molecular characteristics of the insulin resistance (metabolic) syndrome and type 2 diabetes (T2DM), as they progress from a lean to obese phenotype (4, 5), and represents a well established model for the study of the genetics of obesity(6). Recently, using the hyperinsulinemic euglycemic clamp technique, we characterized a baboon model of insulin resistance and identified key biochemical and molecular defects in the insulin signaling cascade in insulin target tissues (skeletal muscle and adipose tissue) (7). Our results demonstrated that insulin resistance is directly related with measurements of adiposity, with percent body fat measured by dual-energy X ray absorptiometry (DXA), providing the best predictor for insulin resistance. However, in large scale studies, the feasibility of DXA scan as a screening method to identify insulin resistant baboons is limited, because it is labor intense and expensive. At our institution, as part of the routine animal husbandry, all baboons receive a biannual health check. While sedated for this health check animals are also weighted and basic morphometric measurements are obtained, along with a blood chemistry panel for metabolic profiling (6). We sought to develop a simple screening strategy to identify lean insulin-sensitive and obese-insulin resistant baboons for inclusion in genetic, physiologic and pharmacologic studies of obesity and insulin resistance using morphometric and biochemical markers of adiposity and glucose metabolism.

Methods.

Nineteen adult nondiabetic baboons (9 females and 10 males) with varying degrees of adiposity and insulin sensitivity comprised the study population. For inclusion, sedated

baboons were evaluated with morphometric measurements including weight, crown to heel length, BMI, abdominal circumference (measured with a flexible non-stretchable measuring tape at a level midway between the lower rib margin and iliac crest) and a biochemical panel during the course of a scheduled health check. On a separate day under general anesthesia, baboons received a 2-hour 60 mU/m^2.min hyperinsulinemic euglycemic clamp after an overnight fast (~12 hour), as previously reported (7, 8). Fasting plasma glucose (FPG) was measured by the glucose oxidase method (Beckman Glucose Analyzer 2, Beckman-Coulter, Fullerton, CA) and fasting plasma insulin (FPI) concentration was determined using a commercial radioimmunoassay (Diagnostic Products, Los Angeles, CA) prior to start the insulin infusion and at 10-15 minutes intervals throughout the insulin clamp procedure. Insulin sensitivity was calculated as the mean glucose infusion rate during the 90-120 min time period of the insulin clamp, reflecting the insulin-stimulated rate of glucose disposal (Rd). Using the FPG and FPI, we also calculated the quantitative insulin sensitivity check index (QUICKI) as 1/(log insulin + log glucose) (9). Since baboons show a marked gender dimorphism in some morphometric and metabolic parameters, all analyses were adjusted for gender differences and variables with a non-normal distribution were log transformed prior to analysis. In order to determine the predictive value of metabolic and morphometric measurements, we constructed three different models using a stepwise linear regression analysis, with Rd as the dependent variable. In the first model, abdominal circumference, adjusted for gender, was the independent variable. In the second model, we included abdominal circumference and FPI (log transformed) as the independent variables. Lastly, we generated a third model using the abdominal circumference and the QUICKI as independent variables. Statistical analyses were performed using SPSS 15 (SPSS Inc. Chicago, IL). A p value of <0.05 was considered to be statistically significant.

Results.

In our study population, the Rd spanned a wide range of insulin sensitivity. Using a cut point for Rd <5 mg/kg.min to screen for insulin resistant primates we identified 9 insulin resistant baboons range and 10 insulin sensitive baboons. In baboons, similar to humans, there was a marked gender dimorphism in percent body fat, with higher values in females compared to male baboons (12% vs. 6% respectively, $p<0.05$).

Regression Analysis and Predictive Models of Insulin Sensitivity.

In model 1, abdominal circumference was inversely correlated with insulin sensitivity levels after adjusting for gender ($r= - 0.72$, $p<0.001$). The regression analysis showed a high degree of correlation between the predicted and measured levels of insulin sensitivity ($r= 0.795$, $r^2=0.6336$, $p<0.01$). Overall, abdominal circumference explained approximately 63% of tissue sensitivity to insulin (Figure 1-A). Since the increase in abdominal circumference is directly correlated with percent body fat and visceral obesity in baboons (6, 7), this finding suggests that, similar to humans, a pattern of central adiposity predicts the presence of an insulin resistance (metabolic) syndrome in the baboon. Inclusion of the FPI concentration (log transformed) to abdominal circumference in model 2 (Figure 1-B), increased its predictive power in the regression analysis (measured Rd vs. predicted Rd, $r=0.835$, $r^2=0.698$, $p<0.001$) and explained 69.8 % of insulin-stimulated glucose disposal. When plasma glucose was included to calculate the QUICKI index in the third model, a further increase in the power of model was obtained ($r= 0.839$, $r^2= 0.7051$, $p<0.001$), explaining 70.5% of insulin sensitivity (Figure 1-C).

For simplicity, we generated a gender specific predictive scale, using the abdominal circumference model, to predict insulin sensitivity (Figure 2-A). With this model, an

abdominal circumference >55 cm for males and >65 cm for females is highly predictive of the presence of insulin resistance in baboons.

Discussion

In the present study, we evaluated the predictive value of simple markers of adiposity for the presence of insulin resistance (measured with the gold-standard euglycemic insulin clamp) in a population of adult nondiabetic baboons. Abdominal circumference is a simple and feasible measurement to predict insulin resistance in this nonhuman primate model. Our results support previous observations which demonstrated that baboons become insulin resistant as they develop a central obese phenotype (10). During weight gain baboons accumulate fat in the abdominal area (both visceral and subcutaneous) and the abdominal circumference is correlated with abdominal fat content and percent body fat. There are no definitive cut points for the diagnosis of obesity in baboons. Therefore, our analysis included baboons exhibiting a wide range of adiposity, measured both by waist circumference and by percent body fat with DXA. Moreover, when surrogate and simple markers of glucose metabolism and insulin sensitivity (such as FPG, FPI and the QUICKI assessment) are used in conjunction with abdominal circumference, the predictive power of the model increases and explains the majority (~70%) of the variation in insulin sensitivity. Our study has several strengths: (i) the study population is a well characterized group of baboons from the morphometric, biochemical and molecular standpoint with clear cut differences between the lean, insulin sensitive and the obese, insulin resistant baboons; (ii) this nonhuman primate model mimics closely findings in humans that relate the presence of a central obese phenotype with impaired glucose metabolism and insulin resistance (11-13), indicating the presence of the insulin resistance (metabolic) syndrome equivalent in the baboon. One potential weakness of our analyses is that we studied a relative small number of primates. However, we included

baboons with both gender male and female and with a wide spectrum of insulin sensitivity, abdominal circumference and percent body fat. Our findings are in agreement with other reports (6, 14) and covered the range from the lean, insulin-sensitive to overweight/obese, insulin-resistant baboons. Our findings also demonstrate that insulin resistance develops as baboons evolve from a lean to an overweight/obese phenotype.

Although our results were obtained in baboons, they are likely applicable to other non-human primates, since the myriad of metabolic abnormalities associated with an obese phenotype is well documented across primate species (15-19).

In summary, abdominal circumference is a major determinant of insulin sensitivity in both male and female baboons and its association with fasting measurements of glucose metabolism and insulin sensitivity, i.e. FPI and FPG, provide a useful instrument to screen primates to identify insulin sensitive and insulin resistant for inclusion in large scale protocols designed to examine the effects of dietary and pharmacological interventions in the study of obesity, insulin resistance and type 2 diabetes.

Acknowledgments

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Figure Legends and Figures

Figure 1. Linear regression models to predict insulin resistance in adult nondiabetic baboons using (A) abdominal circumference, (B) abdominal circumference + log FPI, and (C) abdominal circumference + QUICKI, as independent variables. FPI= fasting plasma insulin, QUICKI= quantitative insulin sensitivity check index.

Figure 2. (A) Relationship between abdominal circumference (independent variable) and predicted rate of insulin-stimulated glucose disposal (Rd). (B) Suggested screening procedure to identify insulin-sensitive and insulin-resistant baboons using abdominal circumference.

Figure 1-A

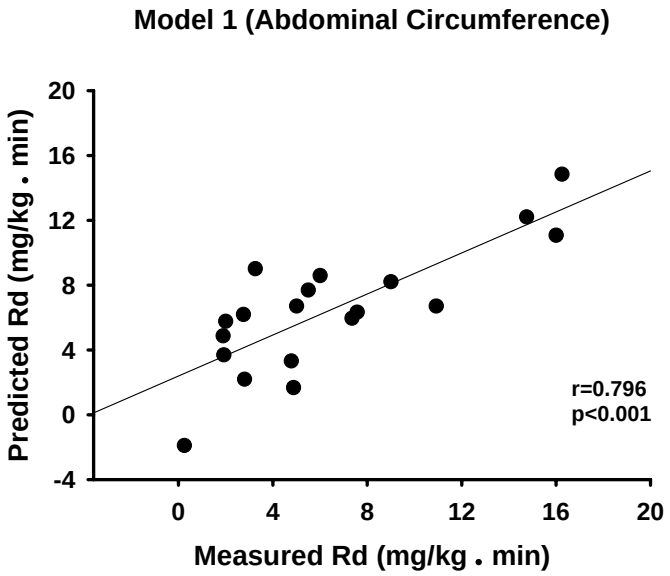


Figure 1-B

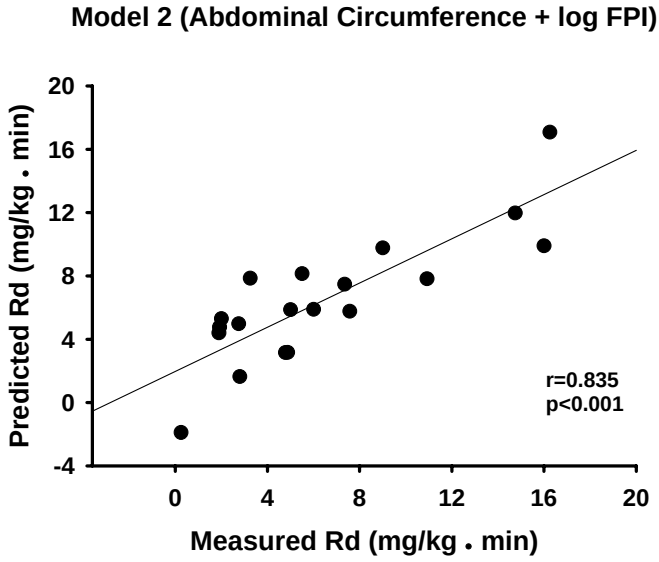


Figure 1-C

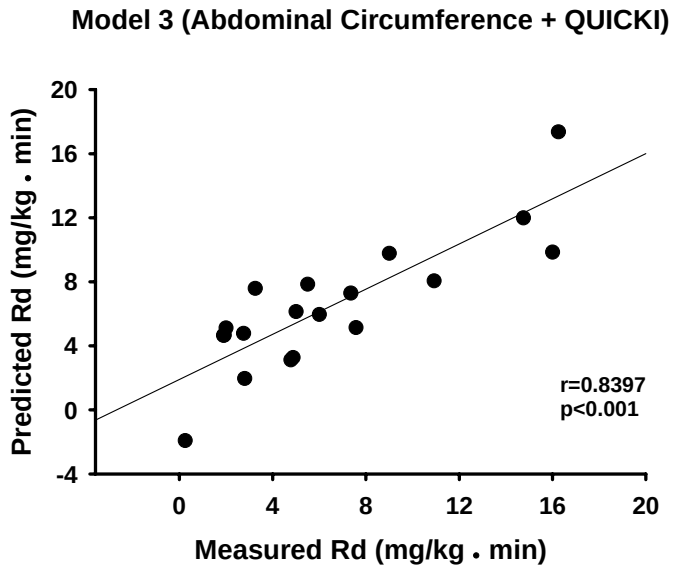


Figure 2-A

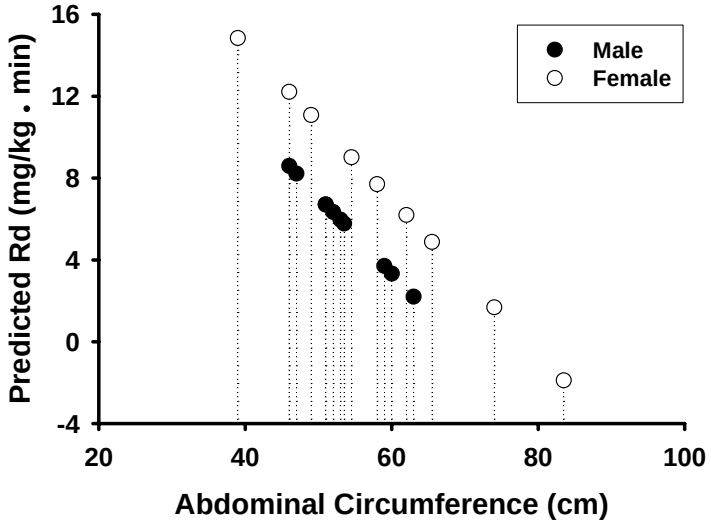
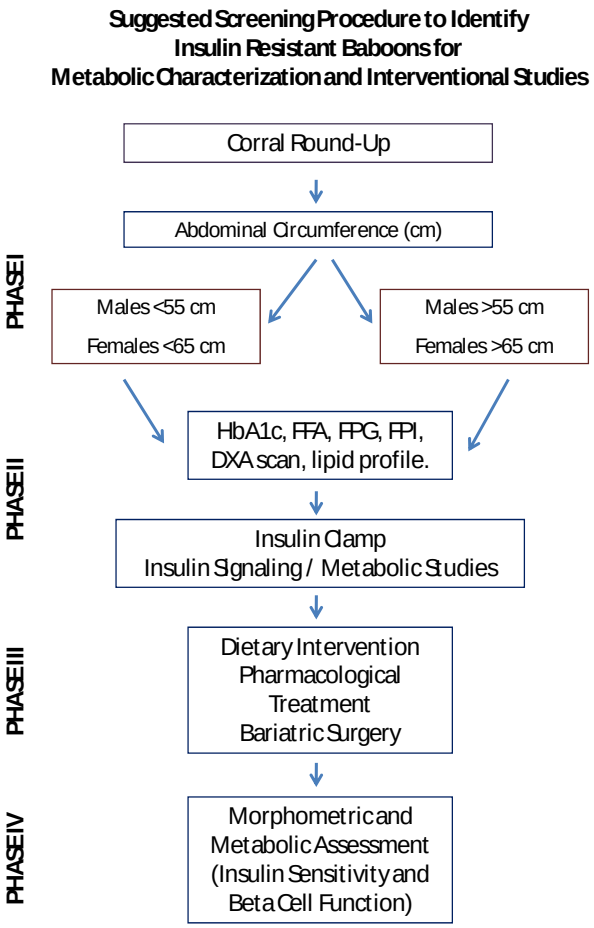
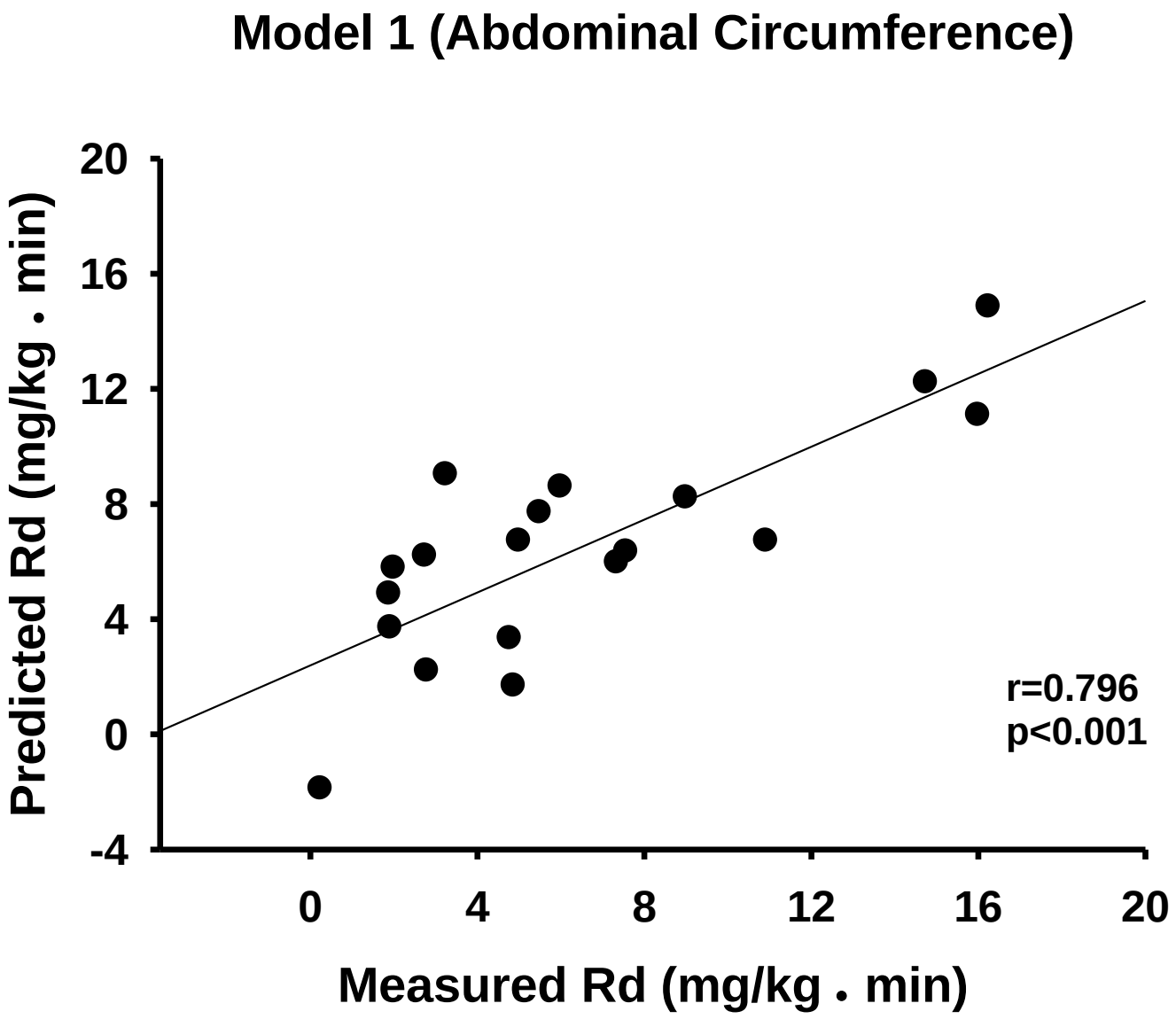


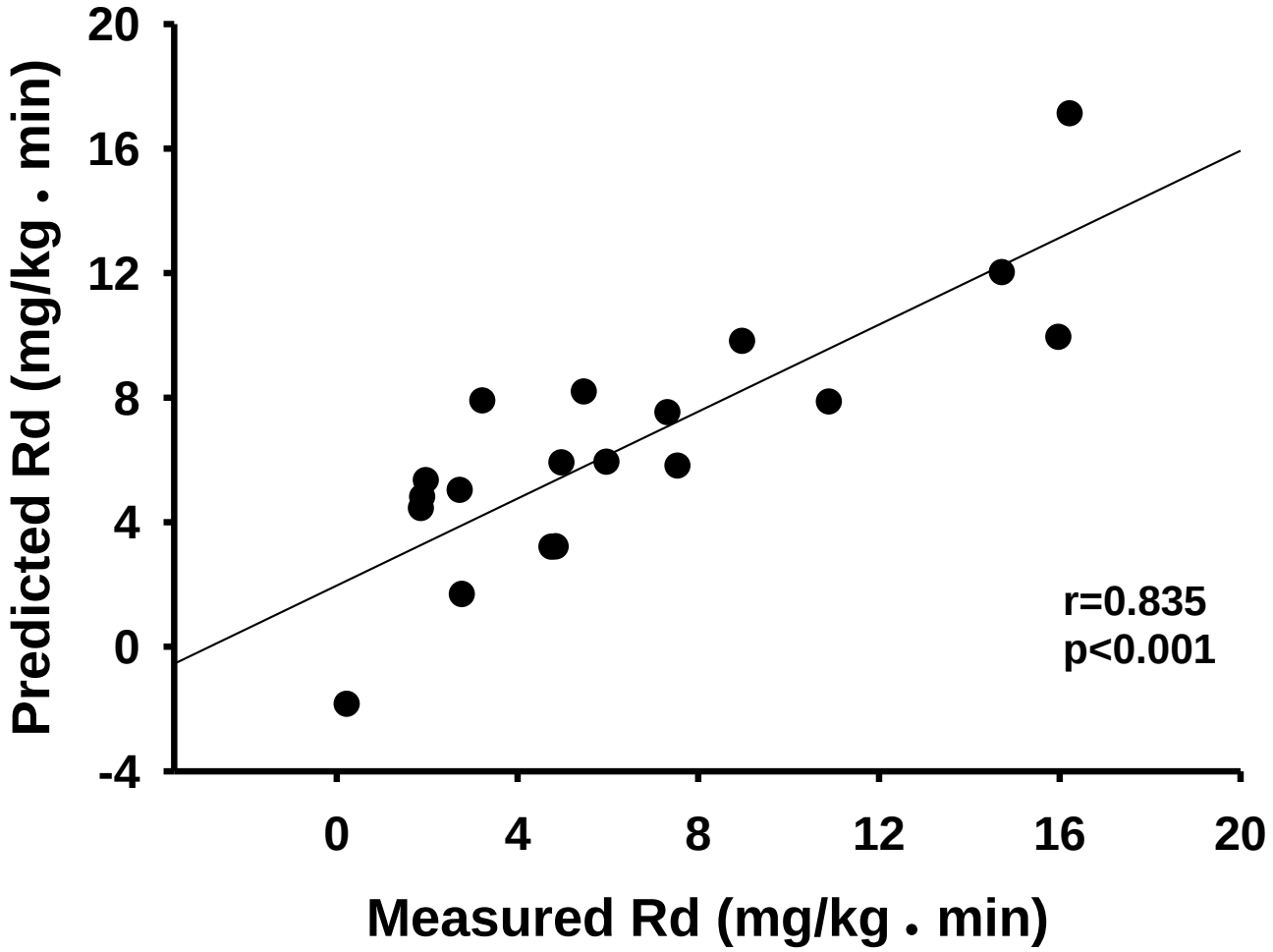
Figure 2-B


Suggested Screening Procedure to Identify
Insulin Resistant Baboons for
Metabolic Characterization and Interventional Studies
Corral Round-Up
Abdominal Circumference (cm)
PHASE I
Males <55 cm
Females <65 cm
Males >55 cm
Females >65 cm
PHASE II
HbA1c, FFA, FPG, FPI,
DXA scan, lipid profile.
Insulin Clamp
Insulin Signaling / Metabolic Studies
PHASE III
Dietary Intervention
Pharmacological
Treatment
Bariatric Surgery
PHASE IV
Morphometric and
Metabolic Assessment
(Insulin Sensitivity and
Beta Cell Function)

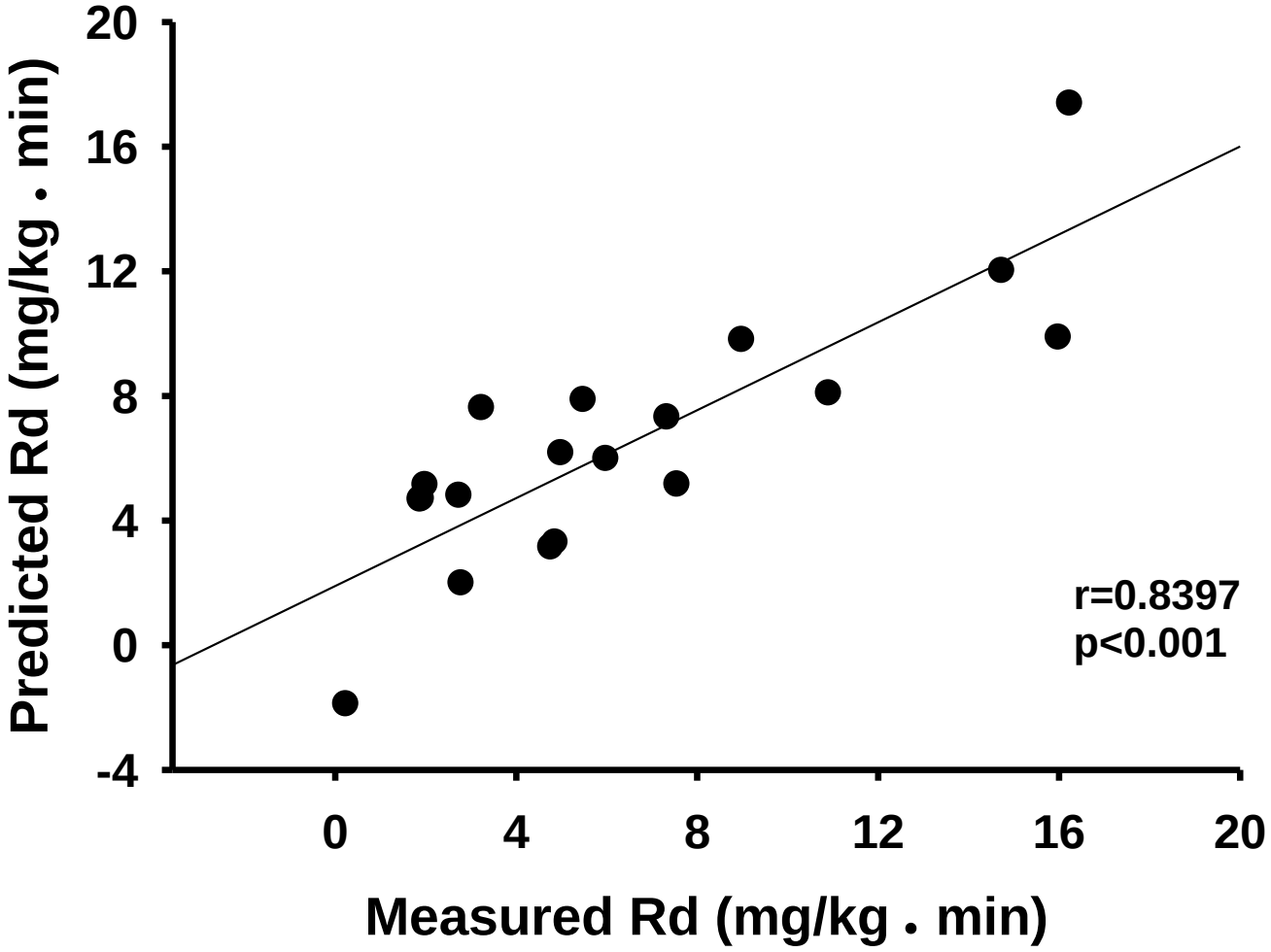
5

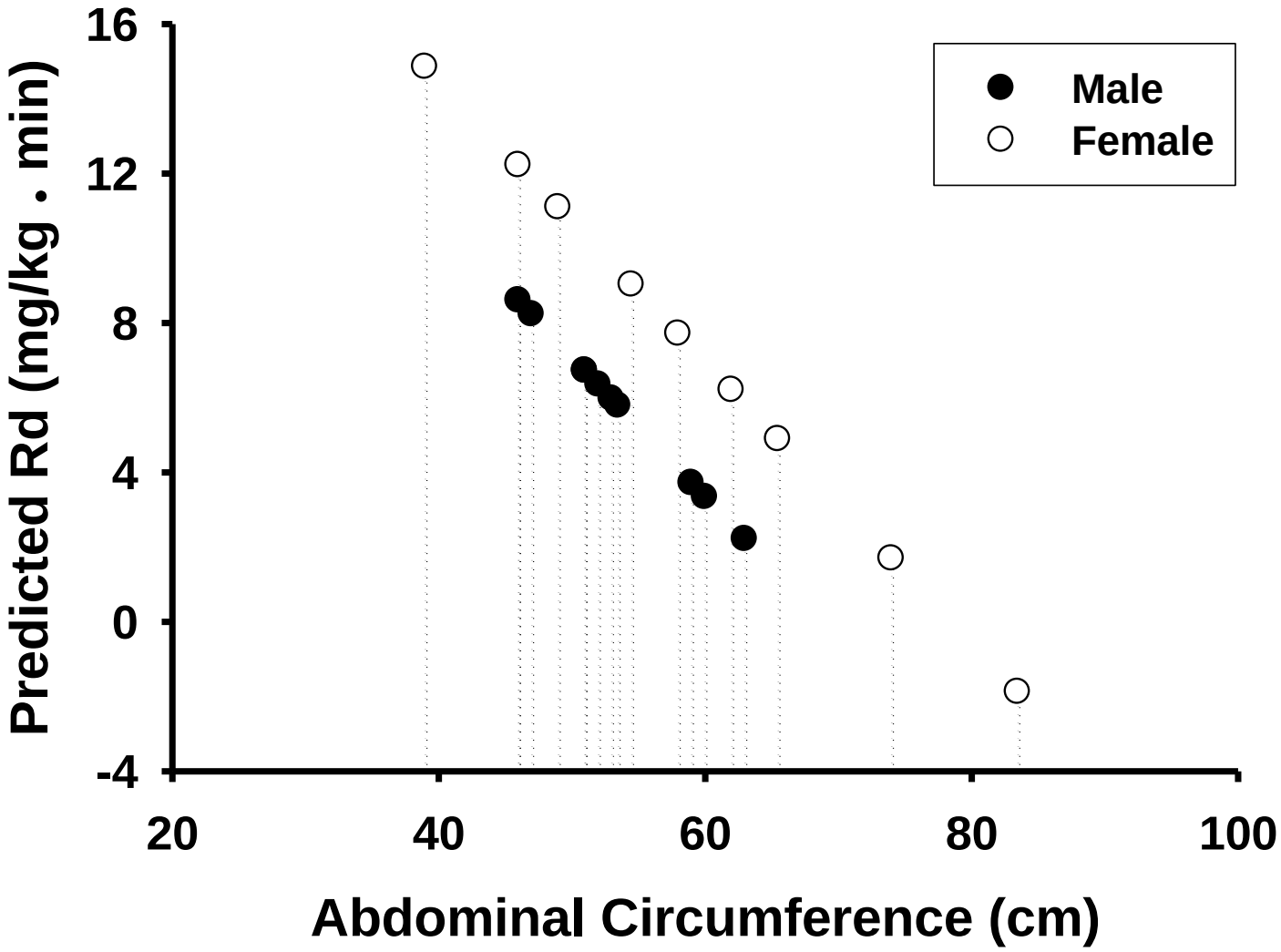

Model 1 (Abdominal Circumference)
Predicted Rd (mg/kg . min)
20
16
12
8
4
0
-4
0
4
8
12
16
20
Measured Rd (mg/kg . min)
r=0.796
p<0.001

Model 2 (Abdominal Circumference + log FPI)



Model 3 (Abdominal Circumference + QUICKI)




Predicted Rd (mg/kg . min)
16
12
8
4
0
-4
20
40
60
80
100
Abdominal Circumference (cm)
Male
Female

Suggested Screening Procedure to Identify Insulin Resistant Baboons for Metabolic Characterization and Interventional Studies