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Effect Modification Investigation Using SAS® - A Model Building Exercise

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ABSTRACT

The positive association of adiposity with insulin and negative association with adiponectin have been documented along with evidence that adiponectin and insulin are inversely associated. The purpose of this study was to determine the influence of adiponectin on insulin and its potential to modify the relationship between insulin and percent body fat (%BF) in 441 black and white 14-19 y olds. An exercise in model building will be presented showing the progression from a model of the insulin and %BF relationship to the addition of the modification variable of adiponectin. Statistical graphics will be used to help understand the nature of the relationships among the variables. Results show a significant %BF*adiponectin interaction such that the positive relationship between %BF and insulin was attenuated in those with higher %BF **and** higher adiponectin. These findings suggest that higher adiponectin levels may be protective to individuals with high %BF by increasing glucose tolerance.

INTRODUCTION

The prevalence of Americans becoming overweight and obese has steadily increased over the years among males and females of all ages, all racial/ethnic groups and all educational levels. Obesity is a predisposing factor for a number of cardiovascular risks including insulin resistance and type 2 diabetes mellitus. The positive relationship between obesity and insulin resistance is well documented. Moreover, an unprecedented rise in the prevalence of insulin resistance is emerging in overweight young people. Characteristic progression from a healthy state of glucose tolerance to a diabetic one follows gain of fat mass and includes compensatory hyperinsulinemia. Therefore, higher fasting insulin levels often positively correlate with higher adiposity[1].

Precise linking mechanisms of this relationship are not fully understood. Recent studies have established adipose tissue as a dynamic, endocrine organ with the capacity to secrete a number of active proteins, termed adipokines. The biological activity of these adipokines may be an important connection between adiposity and insulin resistance. Adiponectin, a protein hormone secreted solely from adipocytes[2], is believed to provide vascular protection via regulation of metabolism, lipids, glucose and inflammation. While other known adipose-derived hormones are increased with larger fat mass, adiponectin production and circulating levels are often found to be the same or lower in obese persons[3]. There is also evidence that circulating adiponectin and insulin are inversely associated and that adiponectin has a cardiovascular protection effect, in part by increasing insulin sensitivity[4].

The purpose of this study was to determine the influence of adiponectin on fasting insulin, while accounting for %BF in black and white, male and female adolescents. To accomplish this, we used SAS to explore the data with descriptive statistics and correlations, tested our variables for normality and transformed insulin, created a model of the insulin and %BF relationship, and then progressed the model by adding the modification variable of adiponectin. We also used statistical graphics to facilitate understanding of the relationships amid the variables.

Subjects were 14-19 year-old black and white, male and female teenagers recruited via flyers sent to high schools in the Augusta area. Subjects were apparently healthy, had no contraindications to any study procedures, and were taking no medications that might affect the results. Analyses were conducted on 441 subjects for whom adiponectin, %BF and insulin were available. Fasting blood samples were drawn for analyses. Insulin was measured in duplicate aliquots of sera by specific radioimmunoassay at the University of Alabama and plasma adiponectin levels were measured in duplicate using ELISA kits at the Medical College of Georgia (LINCO Research, Inc.). %BF was derived from fat mass and total mass obtained using dual-energy x-ray absorptiometry.

EXPLORING THE DATA

CHECKING MODEL ASSUMPTIONS

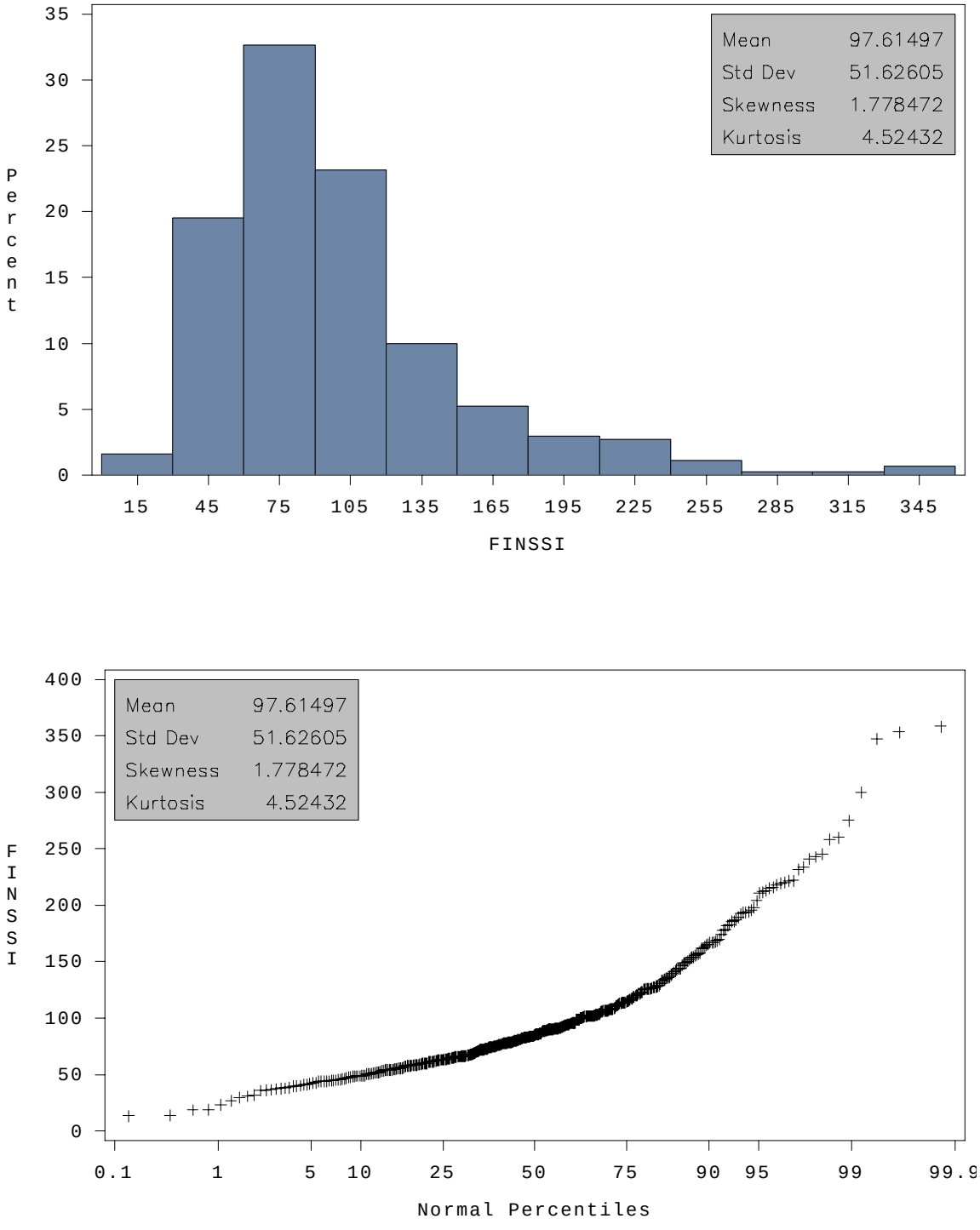
We used PROC UNIVARIATE to check the distribution of the dependent variable, fasting insulin, for normality and also looked at the log10 (l_finssi) and the natural log (ln_finssi) transformations. The results for the natural log transformation were similar to the log10 transformation and will not be presented.

```
proc univariate noprint;
   histogram finssi l_finssi ln_finssi / cfill=ligb;
      inset mean std="Std Dev" skewness kurtosis/ pos=ne cfill=ligr;
   qqplot finssi l_finssi ln_finssi;
```

```
inset mean std="Std Dev" skewness kurtosis/ cfill=ligr;
```

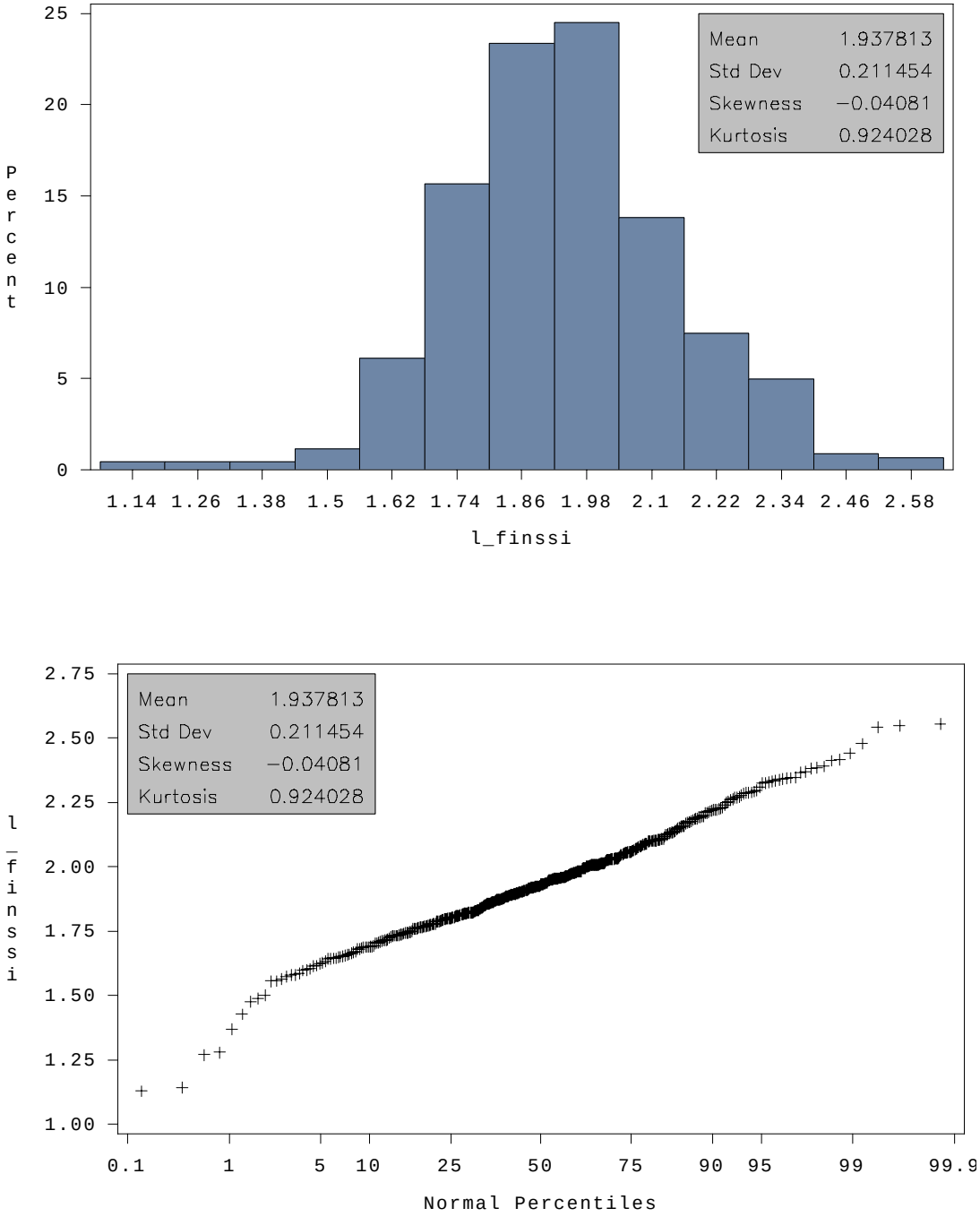
As seen in Figure 1 the distribution of fasting insulin is skewed to the right and the Q-Q plot shows a curved pattern with slope increasing from left to right.

Figure 1. Fasting Insulin – PROC UNIVARIATE Results



The log10 transformation provides a more symmetrical distribution (Figure 2) and the values for skewness and kurtosis are closer to the normal distribution values of zero. The Q-Q plot indicates that there might be some outliers in both tails since all but a few points fall on a line. These values were within biological possibility so they remained in the dataset. All analyses will use log10 fasting insulin as the dependent variable.

Figure 2. Fasting Insulin, log10 transformed – PROC UNIVARIATE Results



SAMPLE DESCRIPTIVES

We then performed an analysis of covariance using PROC GLM to assess age, race and sex comparisons for sample description using the following code:

```
proc glm;
  class race sex;
  model l_finssi perfat adipo = age race sex race*sex / solution;
  means race sex race*sex;
  lsmeans race*sex / pdiff stderr adjust=tukey;
run; quit;
```

Table 1. Subject characteristics and race/gender subgroup comparisons

Level of RACE	Level of SEX	N	AGE		INSULIN*		%BF		ADIPONECTIN	
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
White	Boy	120	16.3	1.2	94.7	53.6	19.6	8.6	15.7	7.9
White	Girl	127	16.0	1.2	84.9	41.4	29.4	6.9	21.7	9.8
Black	Boy	102	16.0	1.3	104.6	58.5	16.9	8.4	14.9	10.0
Black	Girl	92	16.5	1.2	111.2	49.8	30.0	8.4	15.6	8.5
Significance			NS		↓ with Age, p=0.003 Blacks>Whites, p<0.0001		Girls>Boys, p<0.0001		White Girls>others, p=0.002	

***** log transformed prior to analysis

Results of our race and gender subgroup comparisons for %BF, insulin and adiponectin are quite consistent with the literature. Among children and adolescents, the percent and degree of overweight is rising in both males and females. However, being overweight is currently more prevalent in the female adolescent population. Moreover, studies examining the impact of race and ethnicity on adiposity also show a higher prevalence of overweight in black subjects than age and sex-matched white subjects[5-7]. We observed only a gender-specific difference in %BF and not a significant difference between races in our population.

It is interesting to note that although %BF was not significantly different between the black and white subjects in our study, black subjects had significantly higher levels of insulin. Previous reports have established that insulin resistance and resulting hyperinsulinemia are more prevalent in blacks than whites matched for age and %BF[8]. Although it is well-established that obesity is associated with insulin resistance, the mechanism whereby adipose tissue modulates insulin sensitivity remains controversial[9]. Our findings suggest that the regulatory mechanisms may be different for blacks than they are for whites.

In general, males have a higher prevalence of atherosclerosis than females, suggesting a possible role of gender in the manifestation and progression of CVD. One possible explanation is that males have less circulating protective factors than matched females. For instance, clinical studies often report higher levels of adiponectin in females than males[10]. Recent reports suggest a testosterone-dependent mechanism for the inhibition of adiponectin release from adipocytes[11]. Studies have also found that adiponectin levels are lower in blacks than whites[12, 13]. Therefore, our finding that white girls have higher levels than all other groups is consistent with previous findings.

Next, we computed Spearman Correlations using PROC CORR to investigate the bivariate relationships among the dependent variable of insulin and the independent variables of age, %BF and adiponectin. We chose Spearman's rank-order correlation coefficient because it is a distribution-free test and makes no assumptions concerning the shape of the distribution from the sample data. The correlations are shown in Table 2.

Table 2. Spearman Correlation coefficients

Spearman Correlation Coefficients, N = 441 Prob > |r| under H0: Rho=0				
	INSULIN	**ADIPONECTIN**	**AGE**	**%BF**
INSULIN	1.00	-0.28 <.0001	-0.12 0.01	0.42 <.0001
ADIPONECTIN	-0.28 <.0001	1.00	0.05 0.34	-0.06 0.22
AGE	-0.12 0.01	0.05 0.34	1.00	-0.03 0.59
%BF	0.42 <.0001	-0.06 0.21	-0.03 0.59	1.00

Obesity has been described as the central causative component in the development of metabolic disorders related to CVD including hyperinsulinemia[14]. Studies in adults have found a strong association between increased adiposity and insulin resistance[15]. Therefore, our finding of a significant positive correlation between fasting insulin and %BF in adolescents is not surprising. Our results also show the negative correlation between fasting insulin and adiponectin. One potential explanation for this is the proposed role of adiponectin to increase insulin sensitivity and glucose tolerance[16-19]. It is important to note that while our results show significant relationships between our dependent variable of insulin and our independent variables, we found no significant relationships between our independent variables.

MODELING THE INSULIN-%BF RELATIONSHIP

Analysis of covariance (ANCOVA) is a strategy for analyzing data where, in addition to classification variables, one or more continuous variables (i.e. covariates) are measured on each experimental unit. ANCOVA can be thought of as a methodology to compare a series of regression models. Rather than building a regression model for each level of the classification variables, ANCOVA is a strategy for making decisions about the form of the models using all of the data by using interactions between classification and continuous variables. In our case we investigated the relationship between %BF and insulin and wanted to check to see if that relationship was the same for the race-sex subgroups.

There are several ways to approach the process of determining the form of the covariate part of a model.

We chose to first test the hypothesis that all of the within race-sex subgroup slopes for the regression of insulin on %BF were equal to zero by using the following code:

```
proc glm;
  class sex race;
  model l_finssi =age race sex race*sex perfat*race*sex/solution;
  means race sex race*sex;
  lsmeans race sex race*sex;
  run; quit;
```

The three-way interaction along with the SOLUTION model option provides estimates of the slopes for each race-sex subgroup as seen in Table 3.

Table 3. Parameter estimates for three-way interaction

Parameter	Estimate	Standard Error	t Value	Pr > |t|
PERFAT*RACE*SEX 1 1 (White boys)	0.017481	0.00183	9.55	<.0001
PERFAT*RACE*SEX 1 2 (White girls)	0.008494	0.00220	3.87	0.0001
PERFAT*RACE*SEX 2 1 (Black boys)	0.016539	0.00202	8.19	<.0001
PERFAT*RACE*SEX 2 2 (Black girls)	0.011929	0.00212	5.63	<.0001

We fail to reject the hypothesis that all of the slopes are equal to zero since the Type III p-value for the three-way interaction was p<0.0001 (Table 4).

Table 4. Model results for testing that all subgroup slopes are equal to zero

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AGE	1	0.16719854	0.16719854	5.76	0.0169
RACE	1	0.03239571	0.03239571	1.12	0.2915
SEX	1	0.00314968	0.00314968	0.11	0.7421
RACE*SEX	1	0.01916991	0.01916991	0.66	0.4170
PERFAT*RACE*SEX	4	5.94764160	1.48691040	51.19	<.0001

Since we have determined that there is at least one significant slope we then test the hypothesis that the different race and sex subgroup slopes are equal using the following code:

```
proc glm;
  class sex race;
  model l_finssi =age race sex race*sex perfat perfat*race
                  perfat*sex/solution;
  means race sex race*sex;
  lsmeans race sex race*sex;
  run; quit;
```

We reject the hypothesis that the slopes are the same for boys and girls but fail to reject the hypothesis for blacks and whites based on the results of the ANCOVA analysis seen in Table 5. There is a significant interaction between %BF and sex. This model accounts for 36% (model R^2) of the variation in fasting insulin. The model containing only age, race and sex accounted for 6% of the variation in fasting insulin.

Table 5. Model results for testing that the subgroup slopes are equal

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AGE	1	0.16694739	0.16694739	5.74	0.0170
RACE	1	0.06389524	0.06389524	2.20	0.1388
SEX	1	0.00296533	0.00296533	0.10	0.7495
SEX*RACE	1	0.00665613	0.00665613	0.23	0.6325
PERFAT	1	5.22643119	5.22643119	179.85	<.0001
PERFAT*RACE	1	0.00694506	0.00694506	0.24	0.6252
PERFAT*SEX	1	0.32200403	0.32200403	11.08	0.0009

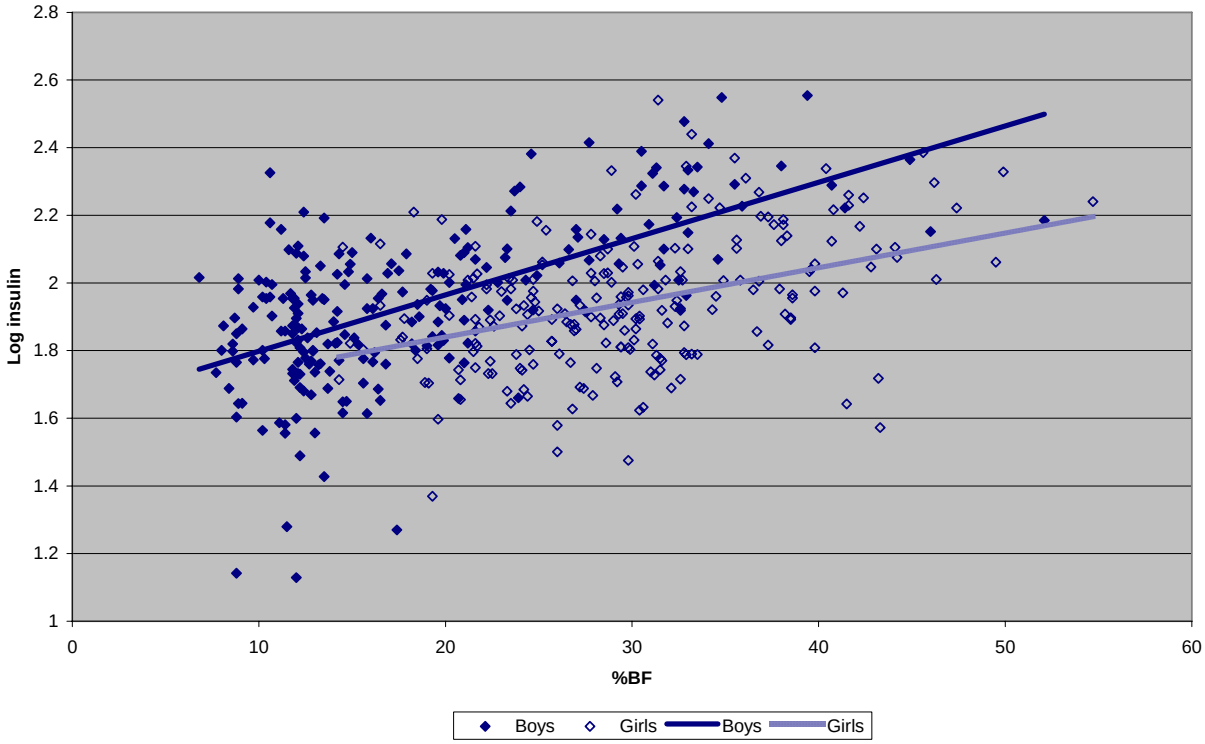
Using the estimates from this model that are shown in Table 6 we can compute the estimate of the slope of the relationship between insulin and %BF for each sex. The slope is larger and the relationship is steeper (i.e. more positive) for boys ($\hat{\beta}$=0.0103+0.0068=0.0171) than for girls ($\hat{\beta}$=0.0103+0=0.0103).

Table 6. Parameter estimates for the %BF by sex relationship

Parameter	Estimate	Standard Error	t Value	Pr > |t|
PERFAT	0.010271942	0.00152555	6.73	<.0001
PERFAT*SEX 1 (Boys)	0.006780607	0.00205088	3.31	0.0010
PERFAT*SEX 2 (Girls)	0.000000000	.	.	.

This relationship is shown graphically in Figure 3.

Figure 3. Plot of the %BF*Sex interaction for fasting insulin (p=0.001)



This differential relationship between adiposity and insulin levels for boys and girls has been shown by other researchers[20]. The girls in this study have overall higher levels of %BF than the boys (Table 1), but at comparable high levels %BF the boys show higher levels of insulin.

MODELING THE EFFECT MODIFICATION OF ADIPONECTIN

Now that we had established a relationship between adiposity and insulin levels in our data we wanted to see if the addition of adiponectin to the model would modify this relationship.

First we would like to determine the relationship between insulin and adiponectin while adjusting for the %BF relationship. We failed to reject the hypotheses that the race-sex subgroup slopes are equal (analyses not shown) so we then fit a common slopes model using the following code:

```
proc glm;
  class race sex;
  model l_finssi= age race sex race*sex perfat perfat*sex adipo /solution;
  means race sex race*sex;
  lsmeans race sex race*sex;
  run; quit;
```

There was a significant negative relationship between adiponectin and insulin and the relationship between %BF and insulin is the similar to the model before the addition of adiponectin. The addition of adiponectin accounts for about 1% of the variance of fasting insulin (model R^2=0.37).

Table 7. Parameter estimates from the model with the addition of adiponectin

Parameter	Estimate	Standard Error	t Value	Pr > |t|
PERFAT	0.009499628	0.00154084	6.17	<.0001
PERFAT*SEX 1 (Boys)	0.007053032	0.00203841	3.46	0.0006
PERFAT*SEX 2 (Girls)	0.000000000	.	.	.
adipo	-0.002490694	0.00091604	-2.72	0.0068

The modification effect of adiponectin is tested by adding the interaction between %BF and adiponectin to this model using the following code:

```
proc glm;
  class race sex;
  model l_finssi= age race sex race*sex perfat perfat*sex adipo
                    adipo*perfat/solution;
  means race sex race*sex;
  lsmeans race sex race*sex;
  run; quit;
```

There is a significant interaction between %BF and adiponectin (Table 8) that accounts for an additional 1% of the variation in fasting insulin (model R^2=0.37). This relationship was not different for the race-sex subgroups (analyses not shown).

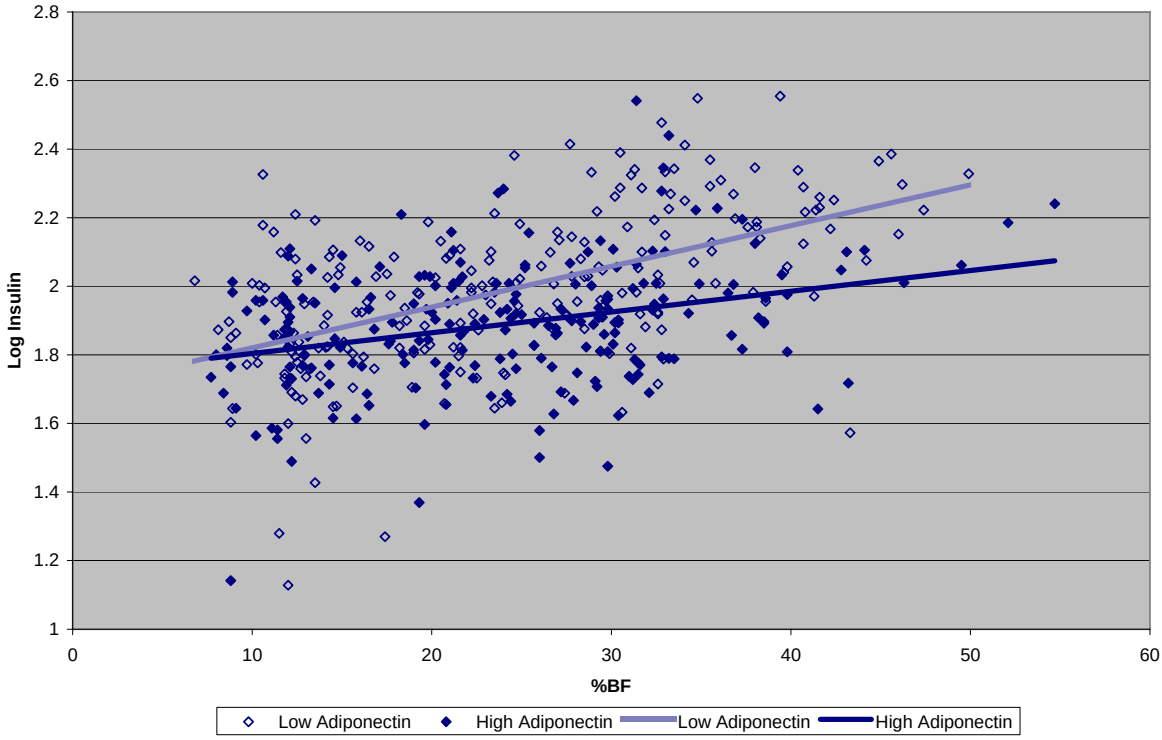
Table 8. Model results for the addition of the %BF by adiponectin interaction

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AGE	1	0.15068478	0.15068478	5.31	0.0217
RACE	1	0.76496100	0.76496100	26.95	<.0001
SEX	1	0.01080904	0.01080904	0.38	0.5375
RACE*SEX	1	0.00333686	0.00333686	0.12	0.7318
PERFAT	1	2.39606738	2.39606738	84.43	<.0001
PERFAT*SEX	1	0.30774263	0.30774263	10.84	0.0011
adipo	1	0.01618854	0.01618854	0.57	0.4505
PERFAT*adipo	1	0.11821039	0.11821039	4.17	0.0419

The effect of an interaction between two continuous variables is difficult to interpret since it represents a three dimensional relationship between the dependent variable (insulin) and the two continuous covariates (%BF and adiponectin).

There are a few ways to graph this relationship in two dimensions to help with this interpretation. Since we were interested in the modification effect that adiponectin might have on the %BF – insulin relationship we chose to dichotomize adiponectin at the median and plot the relationship between %BF and insulin for high and low levels of adiponectin. The resulting plot is shown in Figure 4.

Figure 4. Plot of the %BF*adiponectin interaction for fasting insulin (p=0.043)



There is a positive relationship between %BF and insulin but higher levels of adiponectin serve to blunt this relationship at higher levels of %BF. There is still a significant interaction between %BF and sex but the relationship between adiponectin and %BF is not different for boys and girls.

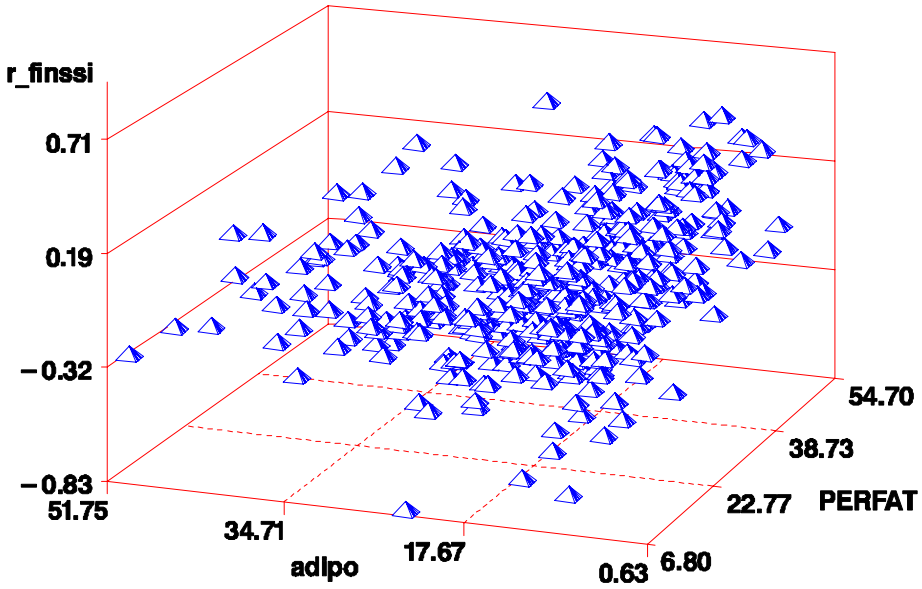
The three dimensional relationship between adiponectin and %BF with insulin is shown in Figure 5. In this plot insulin has been adjusted for age, race, and sex differences and the residuals from that analysis are what are used in the plot. High levels of %BF are again shown to be associated with higher levels of insulin, but this relationship is blunted at higher levels of adiponectin.

The following SAS code was used to produce the 3-D plot:

```
options ps=50 ls=97;
goptions reset=all fontres=presentation ftext=swissb htext=1.4;

proc g3d data=resid;
      title1 'Figure 5. Three dimensional scatter plot showing relationship
      between';
      title2 'adiponectin and %BF with insulin (adjusted for age, race and
      sex)';
  scatter adipo*perfat=r_finssi / grid noneedle color='blue';
  run; quit;
```

Figure 5. Three dimensional scatter plot showing relationship between adiponectin and %BF with insulin (adjusted for age, race and sex)



CONCLUSION

As persons become overweight and obese, their risk of developing cardiovascular disease also increases. However, precise linking mechanisms are not fully understood. What we do know is that higher %BF predisposes one to insulin resistance, even in children and adolescents. The purpose of this study was to determine relationship between %BF and fasting insulin levels in black and white, male and female adolescents. Additionally, we examined the modification potential of a vascular protective adipokine, adiponectin, on this relationship. To facilitate understanding of these relationships, we used SAS to present a model-building exercise and demonstrate the progression from a model of the %BF and insulin relations to the addition of the modifier adiponectin. We have also used statistical graphics to help illustrate these relationships.

Results of our study show that black subjects had higher levels of insulin than the white subjects, despite having similar %BF. Girls had higher levels of %BF than the boys and white girls had higher adiponectin levels than all other groups. Moreover, there was positive relationship between %BF and insulin which was stronger in females than in males. With further statistical investigation, we also were able to show a significant %BF*adiponectin interaction such that the positive relationship between %BF and insulin was attenuated in those with higher %BF and higher adiponectin. These findings suggest that higher adiponectin levels may be protective to individuals with high %BF by increasing glucose tolerance.

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