Introduction

Resistin is a novel cysteine rich peptide hormone encoded at the RSTN gene secreted by the adipocyte. Since its discovery it has proposed link with obesity related insulin resistance and type 2 diabetes. It is unclear whether resistin production is increased or decreased in obesity. Expression of resistin levels have been reported to be decreased in white adipose tissue of several animal models of obesity. Additionally, Way et al reported that administration of PPAR-gamma ligands actually increased resistin expression in both obese mice and Zucker diabetic fatty rats.

The physiologic role of resistin in humans remains unknown. Recently, it is suggested that circulating resistin levels are not associated with obesity or insulin resistance in humans. However, the effects of weight loss on serum resistin concentration has not been studied. In order to better understand the physiologic role of resistin in human obesity, we measured the serum resistin concentration in subjects with severe obesity (before and after 6-months of dietary intervention) to test the hypothesis that serum resistin concentrations are elevated amongst individuals with severe obesity and weight loss would reduce these levels.

Abstract. – Background: Resistin has proposed link with obesity related insulin resistance and type 2 diabetes. The physiologic role of resistin in humans remains unknown. It is suggested that circulating resistin levels are not associated with obesity or insulin resistance in humans. However, the effects of weight loss on serum resistin concentration has not been studied. In order to better understand the physiologic role of resistin in human obesity, we measured the serum resistin concentration in subjects with severe obesity (before and after 6-months of dietary intervention) to test the hypothesis that serum resistin concentrations are elevated amongst individuals with severe obesity and weight loss would reduce these levels.

Methods: Seventy-one obese subjects (defined as BMI > 35 kg/m²) who were randomized to low fat (LF) vs low carbohydrates (LC) diets and who completed the 6-month follow-up were studied. Their baseline demographic information was collected and serum resistin, insulin, glucose were measured at baseline and at 6-months.

Results: Subjects in LC diet lost more weight than LF (-19.54 ± 7.87 lbs vs -7.83 ± 11.23 lbs., $p = 0.001$). Insulin sensitivity (HOMA) improved in LC group compared with LF group [-3.72 ± 9.84 (LC) vs +1.31 ± 7.31 (LF), $p = 0.006$]. Serum resistin levels did not decrease in either diet.

Conclusions: Our study found that despite a significant weight loss and improvement in insulin sensitivity there was no reduction in serum resistin concentration in morbidly obese men with metabolic syndrome suggesting that resistin does not play a central role in obesity related insulin resistance.

Key Words: Obesity, Resistin, Weight loss, Diet, Insulin.
Methods

Subjects
The design and method of this study has been reported before\(^8\). Briefly, the study was approved by the Institutional Review Board at the Philadelphia Veterans Affairs Medical Center (PVAMC), and an approved consent form was signed by each subject. Inclusion criteria were age \(\geq 18\) years and a body mass index (BMI) \(\geq 35\) kg/m\(^2\). Exclusion criteria were a serum creatinine > 1.5 mg/dl, hepatic disease, severe life-limiting medical illness, inability of diabetic patients to self-monitor glucose levels, active participation in a dietary program, or use of weight loss medications. Women, diabetic patients (either self claimed, on hypoglycemic agents or having fasting blood glucose \(\geq 126\) mg/dl on two separate occasions), and severely obese (BMI \(\geq 40\) kg/m\(^2\)) subjects were randomized separately (block randomization) to ensure approximately equal numbers in the two groups. Subjects and investigators were not blinded during the study.

Study Design
The diet groups met separately in weekly 2-hour group teaching sessions for the first 4 weeks, followed by monthly one-hour sessions, all led by individuals with expertise in nutritional counseling. Subjects received a diet overview handout, instructional nutrition labels, sample menus and recipes, and a book on counting calories and carbohydrates. No specific exercise program was recommended. The low carbohydrate (LC) group was instructed to restrict carbohydrate intake to \(\leq 30\) grams per day\(^9\). No instruction on restricting total fat intake was given. Vegetables and fruits with a high fiber-to-carbohydrate ratio were recommended. The Low Fat (LF) group received instruction in accordance with National Heart, Lung and Blood Institute (NHLBI) obesity management guidelines\(^9\) including caloric restriction to create a 500-calorie per day deficit, with \(\leq 30\) percent of these calories derived from fat. The quality of the counseling sessions was assessed by questionnaire administered to 15 subjects from each diet group.

Weights were measured initially and after 6 months on a single calibrated scale (SRScales\(^{TM}\), SR Instruments Inc., Tanawanda, NY. Serum insulin concentration was measured by radioimmunoassay (Laboratory Corporation of America, Raritan NJ). Insulin resistance was estimated by the by Homeostasis Model Assessment [HOMA, fasting serum insulin (in micro units per liter) \(\times\) fasting plasma glucose (in millimoles per liter)/22.5]. The human resistin ELISA (Biovendor, Inc) had an intra-assay cv of 2.4% and inter-assay cv of 5%.

Dietary Intake
Dietary compliance was estimated by a previously validated\(^10\) 24-hour recall of dietary consumption obtained by interview at baseline and 6 months, which was analyzed with Nutribase nutrition management software (CyberSoft, Inc, Phoenix, AZ). As reported in our previous paper, our subjects low carbohydrate dieters experienced a non-significant reduction in calories and a significant reduction in percentage of carbohydrates and increase in percentage of protein and fat compared with low fat dieters\(^8\).

Statistical Analysis
For comparison of continuous variables between the two groups, we calculated the changes from baseline to 6 month in each subject and compared the mean changes in the two diet groups using an unpaired t-test\(^11\). We assessed the normality of the distribution of all variables before using the t-test. Insulin and glucose levels were skewed and were therefore log transformed for analysis. Dichotomous variables were compared by chi-square analysis\(^11\). Linear regression and two-way analysis of covariance models were used to correct for potentially confounding variables and to identify interactions between variables and diet group assignment\(^11\). All p-values were two sided and a P-value of 0.05 or less was considered to be statistically significant. Analysis was performed by using SPSS software (version 10.0).

Results

Baseline Characteristics
Thirty-nine subjects from LC and 32 from LF diet groups completed the study. The baseline characteristics are given in Table I. There were no differences between weights,
HOMA and serum resistin concentrations at baseline between these groups. The subjects were predominantly African American males (52%) with the mean age of 54 ± 9 years.

There were no differences between the baseline resistin and other anthropometric parameters between subjects who completed the study versus subjects who dropped out.

With regard to the baseline values per dietary group, for LF vs LC for weight (295 ± 52 lbs vs 294 ± 47 lbs), HOMA (7.45 ± 8.59 vs 9.08 ± 11.91) and resistin (11.1 ± 10.31 ng/ml vs 10.43 ± 6.10 ng/ml) were not significantly different between groups, respectively (Table I).

### Changes in Weights, Insulin Sensitivity and Serum Resistin Concentration

Subjects in LC lost more weight than LF (-19.54 ± 7.87 lbs vs -7.83 ± 11.23 lbs., *p* = 0.001). Insulin sensitivity (HOMA) increased in LC compared with LF [-3.72 ± 9.84 (LC) vs +1.31 ± 7.31 (LF), *p* = 0.006]. Resistin increased in both diets but the increase was not significant between diets [+0.29 ± 8.08 ng/ml (LF) vs +2.74 ± 4.48 ng/ml (LC), *p* = 0.131]. Within diet groups, changes were significantly different in LF for weight (*p* < 0.001) and in LC for weight, HOMA, and resistin (*p* < 0.001) See (see Figure 1).

No correlation was found between changes in HOMA, weight or resistin in LF group. Only in the LC diet were we able to demonstrate a correlation of weight and HOMA with no correlation of resistin to either of these variables.

### Discussion

We found that severely obese subjects with a high prevalence of diabetes and metabolic syndrome who were randomized to LC diet lost more weight than individuals randomized to LF calorie restricted diet in a 6 month pe-
period\(^7\). However, despite this weight loss and an improvement in insulin sensitivity we did not find any reduction in serum resistin levels. This is the first study in humans that has studied the effects of weight loss on serum resistin concentrations. Also, we did not find any correlation between severity of obesity and serum resistin concentrations.

Prior animal data have shown conflicting results regarding the role of resistin as a link between obesity and insulin resistance. While Steppan and others\(^1,2\) have shown higher circulating resistin levels in genetic and diet induced models of obese mice and have shown worsening of insulin resistance with exogenous resistin administration, others have not found any such associations. In humans, Nagaev et al\(^12\) found that resistin was expressed only in very low levels in human fat cells with no difference in expression in normal, insulin resistant, and Type 2 diabetic individuals.

Our findings are in agreement with other studies in humans that demonstrated no association between mRNA and protein expression and insulin resistance or obesity\(^5,6\). Also, despite significant weight loss and an improvement in insulin sensitivity in obese subjects on low carbohydrate diet we did not see any reduction in serum resistin levels. On the contrary, resistin levels appear to increase with weight loss though did not reach statistical significance.

While our data supports the notion that resistin does not play a central role in the insulin resistance associated with obesity, it remains possible that it may have other autocrine and/or paracrine functions.

It is also possible that inclusion of morbidly obese men may be too restricted a study population to fully understand the role of resistin and insulin resistance. However, an initial report by Lee et al\(^5\) reported a cross sectional study of 123 middle aged overweight women (BMI of 30.9 ± 5.5 kg/m\(^2\)) vs 118 lean healthy subjects that serum resistin levels did not correlate with markers of obesity or insulin resistance. Also, a recent study\(^6\) showed resistin concentrations were not different amongst non-obese (BMI: 25 ± 4.3 kg/m\(^2\)). Obese (BMI: 33 ± 2.5 kg/m\(^2\)) and obese individuals with type 2 diabetes (BMI: 34 ± 2.4 kg/m\(^2\)). Serum resistin concentration was not related to the percent of body fat, BMI or fat cell size and demonstrated a weak relationship between resistin and insulin sensitivity in non-obese subjects.

There is also the issue of the actual ELISA used to measure serum resistin levels. While the Biovendor assay is a highly specific ELISA, with no cross reactivities to mouse resistin or other human cytokines, it is a newly designed assay and may still be in the process of being fully developed. There is some contradictory information regarding the exact normal values of serum resistin levels that need to be sorted further in the future. However, further studies by Pfutzer et al\(^13\) evaluated three commercially available resistin ELISA with different epitopes (Phoenix, Biovender, Immunodiagnostik), all three passed successfully standardized technical validation procedure with inter and intra assay variability less than 10% and 15% respectively. They proved to be different with regard to calibration and reference range which may be linked to different antibody specificity. No correlation was seen between any of the resistin assay and BMI and clinical measures for insulin resistance\(^13\).

Fain et al\(^14\) found that resistin release in human explants of adipose tissue was highly variable over 48 hours (3-158 ng/g). It is possible that serum resistin levels may fluctuate and sampling at various times during the 24 hour period may be necessary to further understand its secretion. However, Pfutzer et al\(^13\) did not find any correlation between fasting serum resistin level and clinical measures of obesity and insulin resistance.

In conclusion, our study found that despite a significant weight loss and improvement in insulin sensitivity there was no reduction in serum resistin concentration in morbidly obese men with metabolic syndrome suggesting that resistin does not play a central role in obesity related insulin resistance. Given the newness of the ELISA assay, the restricted population, and the unknown kinetics of resistin, more studies are needed before the role of resistin in insulin sensitivity and obesity can fully be defined.

References

Serum resistin is not associated with obesity or insulin resistance in humans.

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