SIRT3: Molecular Signaling in Insulin Resistance

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Abstract

This study aims to show the relationship of different states of insulin resistance on abundance of Sirt3, a mitochondrial deacetylase, during exercise. This deacetylase is responsible for activating other proteins responsible for regulating cell energy homeostasis. Six subjects of varying levels of insulin resistance underwent a bout of exercise. Muscle biopsies were taken before and after exercise and the amount of Sirt3 measured and compared. Results of the study indicated that there was a negative correlation of insulin sensitivity and amount of Sirt3. This is hard to interpret with current literature.

Introduction

Sirtuins are a group of regulatory molecules which deacetylate amino acid residues in order to activate other proteins involved in energy homeostasis. Sirt3 is a sirtuin of specific interest as it is located within the mitochondria and its activity is thought to be influenced by metabolic by-products and regulatory proteins. It may play an important role in the pathophysiology of prevalent diseases such as diabetes mellitus and metabolic syndrome. This study looked at the relationship between Sirt3 activity and level of insulin resistance. The hypothesis was that insulin sensitive muscle would have a higher amount of SIRT3 and thus be more adaptable to changing energy needs in the body.

Subject Characteristics	
Sex(male/female)	4/1
Age, yr	31.6±4.7
BMI, kg/m^2	28.0±2.0
Body Weight, kg	85.3±7.7
Fat mass, kg	21.7±2.5
M, mg/kg/min	6.4±1.1
FPG, mmol/l	4.9±0.1
FPI, pmol/l	47.4±11.4
HbA_{1C}, %	5.2±0.1
Total Cholesterol, mmol/l	190.2±10.7
Triglycerides, mmol/l	147.8±34.2
HDL, mmol/l	52.0±9.8
LDL, mg/dl	113.6±9.2
SBP, mmHg	127.0±4.9
DBP, mmHg	80.2±5.5
Resting heart rate, beats/min	66.6±3.2
Maximum heart rate, beats/min	186.6±3.9

Table 1: Subject characteristics are baseline values expressed as means SE. BMI, body mass index; FM, fat mass; M, glucose disposal rate; FPG, fasting plasma glucose; FPI, fasting plasma insulin; Hb A1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure. Maximum heart rate estimated at 200 – age of subject.

Methods

Each subject underwent a euglycemic hyperinsulinemic clamp to determine insulin sensitivity, described as the M-value. On a separate day, the subject performed a 48 minute exercise protocol on a cycle ergometer. Muscle biopsies were taken before exercise and 5 hours after. Mitochondria were isolated from the muscle and stored. SDS-polyacrylamide gel electrophoresis was performed to isolate Sirt3. The quantity of Sirt3 protein was determined using chemiluminescence and band intensity quantification. The pre- and post-exercise quantity of protein was compared to the predetermined M-value

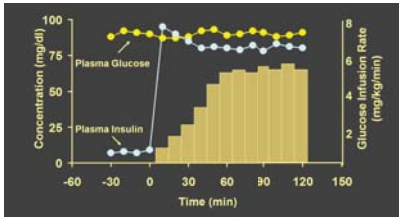

Figure 1: Representation of euglycemic hyperinsulinemic clamp. Exogenous insulin and radiolabeled glucose are infused through a cannulated forearm vein. Plasma insulin and glucose are intermittently measured. M-value is determined as the amount of continuous glucose infused to maintain euglycemia for a constant hyperinsulinemic infusion. In this example, M-value = 5.5.

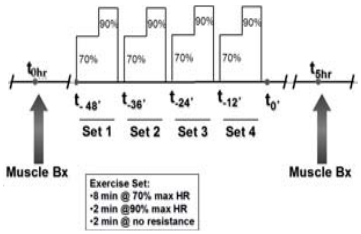

Figure 2: Representation of exercise protocol (not to scale). Before engaging in exercise, a biopsy was taken of the vastus lateralis under local anesthesia using a Bergstrom cannula. The subject then underwent a bout of cycling for eight minutes at 70% of estimated maximum heart rate, followed be 2 minutes at 90%, and finally 2 minutes of rest. This was repeated 3 times. A final biopsy was taken at 5 hours following completion of exercise.

Quantification of Sirt3

Quantification of Sirt3 protein showed mixed results. Only one (GC48) had an increase in Sirt3 protein after exercise. The rest of the samples had decreased levels of Sirt3 following exercise.

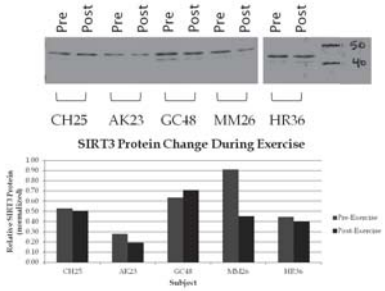

Figure 4: Effect of exercise on SIRT3 quantity before and after exercise in non-diabetic subjects. Muscle biopsies were taken pre- and post-exercise and quantity of SIRT3 measured using SDS-PAGE and band density quantification

Sirt3 and M-Value

The relative change in quantity of Sirt3 was compared to the previously determined M-Value. A moderate correlation in decreased Sirt3 and insulin sensitivity was demonstrated.

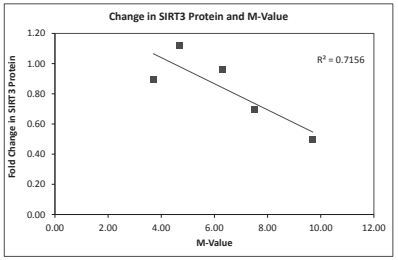

Figure 5: Graph showing relationship of change in Sirt3 value compared to M-value. The pre-determined M-value was plotted against the fold change of Sirt3 protein before and after exercise. This showed a moderate negative correlation with an r^2 value of 0.7156.

Discussion and Conclusions

Cellular energy production involves a complex interplay of regulatory molecules and their substrates. The activity of these processes is based on a variety of different factors, including abundance of nutrition and physical activity of the cell. Inability to appropriately regulate the breakdown of energy results in a disruption of energy homeostasis and is likely a large contributor to disease states such as metabolic syndrome and diabetes.

The purpose of this study was to determine whether our previous findings that insulin resistant muscle had a reduced gene expression response to exercise extended to the main mitochondrial sirtuin, Sirt3. Sirt3 deacetylates numerous mitochondrial proteins and may contribute to metabolic flexibility in fuel choice. We found instead that Sirt3 protein abundance decreased 5 hours after exercise. Furthermore, the decrease was more pronounced in insulin sensitive muscle. Therefore, the concept of "exercise resistance", as we defined it (De Filippis), does not apply to Sirt3, the abundance of which changes more after exercise in insulin sensitive muscle.

There are several explanation that could be responsible for the findings we encountered. It is possible that 5 hours is not a sufficient time after exercise to see an increase in Sirt3 that is responsible for the cell's response to energy homeostasis. Further, it could be that the deacetylation reaction is completely independent of Sirt3 and performed by another Sirt or a completely different mechanism.

Acknowledgements

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