Inhibition of mTOR Does Not Improve *In Vivo* Insulin Action in Ob/Ob or High Fat Fed Mice

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Abstract

The mammalian target of rapamycin (mTOR) is a Ser/Thr kinase that controls cellular growth and has been suggested to play a role in the development of insulin resistance. Rapamycin is a highly specific inhibitor of mTOR, but its effects on *in vivo* insulin action are not established. The purpose of the present study was to determine whether or not rapamycin can improve glucose tolerance and insulin tolerance in genetically obese and mice fed a high fat diet (HFD). Ob/Ob (OB) and wildtype (WT) mice were subjected to intraperitoneal (IP) injections rapamycin or 0.9% saline. Two hours following rapamycin or saline injections all mice were subjected to an IP glucose tolerance test or an IP insulin tolerance test. Blood glucose was measured via tail bleeds at baseline, 15, 30, 45, 60, and 90 min after the injection of either glucose or insulin. Glucose tolerance in OB mice was significantly lower than in WT mice ($P < 0.0001$). Rapamycin did not significantly alter glucose tolerance in OB or WT mice ($P = 0.168$). Insulin tolerance, a measure of insulin sensitivity, was significantly lower in OB compared with WT mine ($P = 0.0004$). Rapamycin did not alter insulin tolerance in OB or WT mice ($P = 0.238$). Identical experiments were conducted in normal mice following five months of HFD or a standard chow diet (CON). Glucose tolerance was similar in mice fed the HFD compared to mice consuming the CON diet ($P = 0.483$) but insulin tolerance was significantly reduced ($P = 0.041$), indicating the HFD produced insulin resistance. Rapamycin did not significantly alter glucose tolerance ($P = 0.127$) or insulin tolerance ($P = 0.702$) in mice consuming the HFD or CON diet. In summary, acute rapamycin treatment does not improve glucose tolerance or insulin tolerance in genetically obese mice or in normal mice following five months of a HFD.

Introduction

- Type 2 diabetes is a metabolic disease associated with obesity and hyperinsulinemia (i.e., insulin resistance).
- Skeletal muscle insulin resistance is thought to be a primary defect in type 2 diabetes.
- The mammalian target of Rapamycin (mTOR) is a nutrient sensitive and insulin responsive Ser/Thr kinase that controls cell growth.
- Cell culture studies demonstrate that mTOR promotes insulin resistance by the Ser phosphorylation and degradation of IRS-1 [1, 2].
- It is not known if rapamycin, a highly specific inhibitor of mTOR, can improve *in vivo* insulin action.

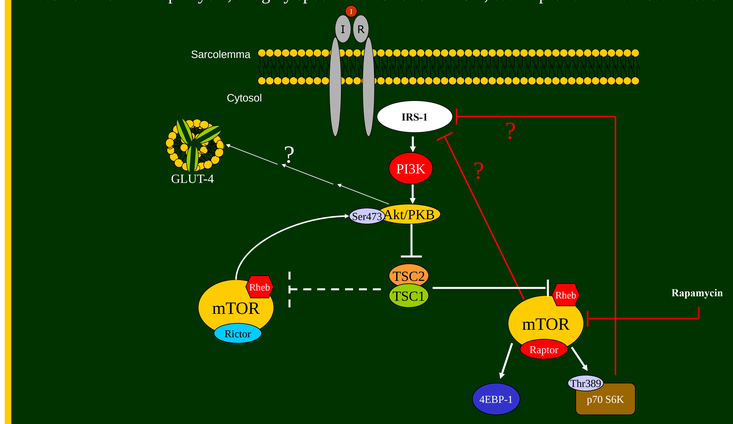


Figure 1. mTOR Signaling Pathway

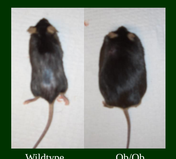
Purpose

The purpose of the present study was to determine the role of mTOR is insulin resistance and type 2 diabetes. We accomplished this by using Ob/Ob genetically obese (OB) mice and mice fed a high fat diet (HFD).

Methods

Animals

- Ob/Ob (n = 8) vs. Wildtype (WT, n = 8)
- C57Bl/6 male mice fed HFD (n = 16) vs. standard chow diet (n=16)



Wildtype Ob/Ob

Intraperitoneal glucose and insulin tolerance testing

- Rapamycin dose: 5.0 mg/Kg
- Glucose dose for GTT: 1.0 g/Kg
- Insulin dose for ITT: 0.25 U/Kg
- Blood was collected from the tail vein and glucose was assessed by glucometer (One Touch Ultra)

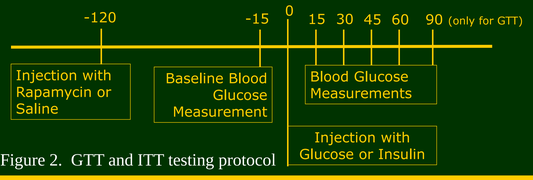


Figure 2. GTT and ITT testing protocol

Muscle Processing and Immunoblotting

- 15 minutes following IP injection of insulin (25 U/Kg) or saline quadriceps muscles were removed rapidly frozen in liquid nitrogen.
- Frozen muscles were powdered and homogenized in cell extraction buffer (Biosource).
- Skeletal muscle extracts were prepared and subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting experiments.
- Immunoblots were probed with the pThr389 S6K antibody, a phospho-specific antibody that recognizes only phosphorylated Thr389 of S6 Kinase (S6K). Thr389 is the mTOR phosphorylation site.
- pThr389 S6K immunoblots were stripped and reprobed to measure total amounts of S6K.

IRS-1 Assay

- Total amounts of IRS-1 were assessed by a commercially available ELISA (Biosource).

Statistical Analysis

- The effect of rapamycin on glucose tolerance and insulin tolerance was assessed by a 2X2 ANOVA with repeated measures (changes in glucose from 0-90 min).
- The effect of genotype (Ob/Ob vs. WT) and insulin (saline vs. 0.25 U/Kg insulin) on mTOR activity was assessed by a 2X2 ANOVA.
- The effect of diet (HFD vs. standard diet) and insulin (saline vs. 0.25 U/Kg insulin) on mTOR activity was assessed by a 2X2 ANOVA.
- The effect of genotype on IRS-1 expression was assessed by an unpaired t-test.

Results

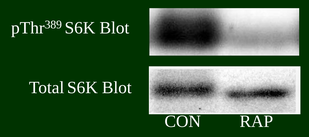


Figure 3. The effect of rapamycin (5 mg/Kg) on the phosphorylation of S6K on Thr389 in quadriceps muscles from C57Bl/6 male mice. Muscles were excised and frozen in liquid nitrogen 15 minutes following insulin injection.

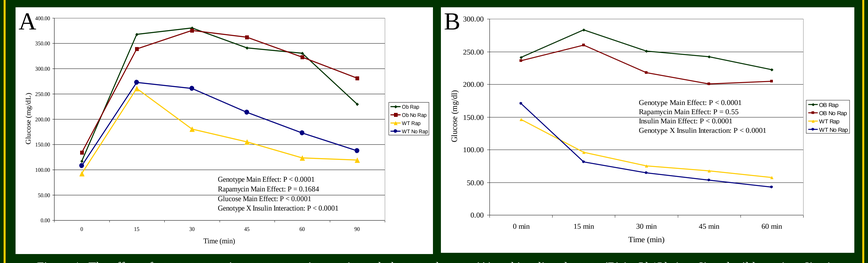


Figure 4. The effect of acute rapamycin treatment on intraperitoneal glucose tolerance (A) and insulin tolerance (B) in Ob/Ob (n = 8) and wildtype (n = 8) mice.

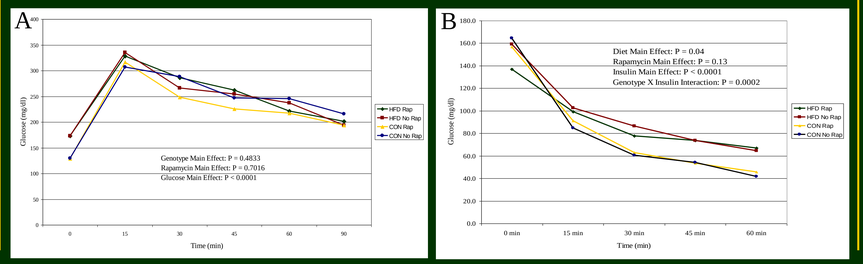


Figure 5. The effect of acute rapamycin treatment on intraperitoneal glucose tolerance (A) and insulin tolerance (B) in high fat fed (n = 8) and control (n = 8) mice.

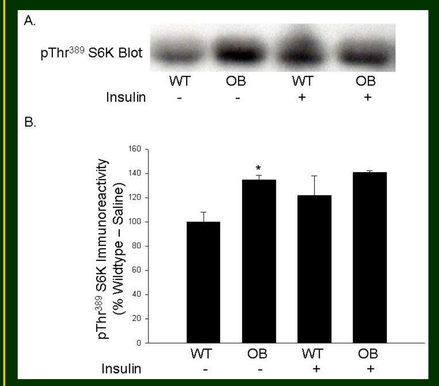


Figure 6. Phosphorylation of S6K on Thr389 in quadriceps muscles of Ob/Ob and wildtype mice. * P < 0.05, WT vs. OB in the absence of insulin. Muscles were rapidly removed from mice 15 minutes following insulin injection and frozen in liquid nitrogen.

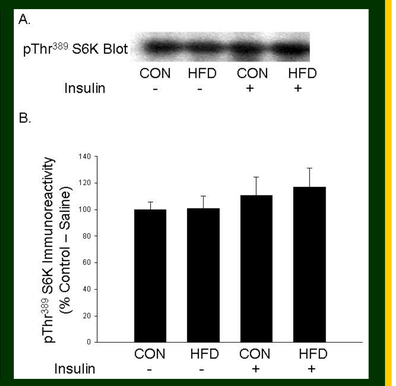


Figure 7. Phosphorylation of S6K on Thr389 in quadriceps muscles of mice fed a HFD and standard diet. Muscles were rapidly removed from mice 15 minutes following insulin injection and frozen in liquid nitrogen.

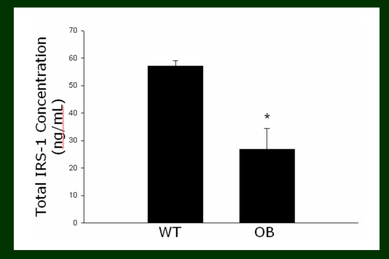


Figure 8. Total abundance of IRS-1 in quadriceps muscles of Ob/Ob and wildtype mice. * P < 0.05, WT vs. OB.

Conclusion

- Acute inhibition of mTOR with rapamycin does not improve insulin action in OB mice or mice fed a HFD.
- In the basal state, mTOR activity in skeletal muscle of OB mice is greater than WT mice.
- In the insulin stimulated state, mTOR activity in skeletal muscle of OB mice is similar to WT mice.
- Total IRS-1 levels in skeletal muscle are significantly lower in OB mice compared to wildtype mice, indicating that overactive mTOR signaling may promote IRS-1 degradation.

References

1. **Takano, A., I. Usui, T. Haruta, J. Kawahara, T. Uno, M. Iwata, and M. Kobayashi**. 2001. Mammalian Target of Rapamycin Pathway Regulates Insulin Signaling via Subcellular Redistribution of Insulin Receptor Substrate 1 and Integrates Nutritional Signals and Metabolic Signals of Insulin. *Molecular and Cellular Biology*. 21: 5050-5062.

2. **Tremblay, F., A. Gagnon, A. Veilleux, A. Sorisky, and A. Marette**. 2005. Activation of the Mammalian Target of Rapamycin Pathway Acutely inhibits Insulin Signaling to Akt and Glucose Transport in 3T3-L1 and Human Adipocytes. *Endocrinology*. 146: 1328-1337.

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