ANNOUNCEMENT OF FINAL ORAL EXAMINATION
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

CANDIDATE: Kimberly Coughlan

DEPARTMENT OR PROGRAM: Pharmacology

TITLE OF DISSERTATION: “Inhibition of AMPK via Phosphorylation at Ser485/491: Multiple Mechanisms of Regulation”

DATE, TIME, AND PLACE: Thursday, June 25, 2015 at 2:00p.m.
Boston University School of Medicine
72 E. Concord Street (Room L 112)
Boston, MA 02118

EXAMINING COMMITTEE

FIRST READER: Dr. Neil Ruderman
SECOND READER: Dr. Asish Saha
THIRD READER:
CHAIRMAN OF THE EXAMINING COMMITTEE: Dr. Richard Wainford Email: rwainf@bu.edu
ADDITIONAL COMMITTEE MEMBERS: Dr. Susan Leeman
Dr. Barbara Kahn

Members of the committee are asked to confirm attendance by replying directly to the Chairman of the Examining Committee.

ALL MEMBERS OF THE SCHOOL OF MEDICINE FACULTY ARE INVITED TO ATTEND.
AMP-activated protein kinase (AMPK) is an energy-sensing enzyme that is activated when cellular energy is low and causes muscle and other cells to increase glucose uptake and fat oxidation, diminish lipid synthesis, and alter expression of various genes. AMPK activity is diminished in animals with the metabolic syndrome, though the mechanisms causing this reduction are unknown. To examine nutrient-induced changes in AMPK activity over time and factors that may regulate it, we compared rat muscle incubated with high glucose (HG) (30min-2h) and muscle of glucose-infused rats (3-8h) with appropriate controls. In addition to diminished AMPK activity (measured by the SAMS peptide assay) and phosphorylation of its activation loop at Thr\(^{172}\), we observed increased muscle glycogen, phosphorylation of AMPK’s \(\alpha1/\alpha2\) subunit at Ser\(^{485/491}\), and PP2A activity, and decreased SIRT1 expression, all of which have been shown to diminish AMPK activity. Dysregulation of one or more of these factors could contribute to pathophysiological changes leading to metabolic syndrome associated disorders.

Since recent studies suggest phosphorylation at Ser\(^{485/491}\) may play an important role in AMPK inhibition, we sought to determine how phosphorylation of this site is regulated. We investigated whether insulin or diacylglycerol (DAG) signaling pathways may be involved, since both are increased in at least one of the HG models. Akt and Protein Kinase (PK)D1
phosphorylated AMPK at Ser\textsuperscript{485/491} and diminished its activity in C2C12 myotubes, downstream of insulin and the DAG-mimetic PMA, respectively. Additionally, p-AMPK Ser\textsuperscript{485/491} was increased in muscle and liver of fed versus fasted mice and liver of diabetic mice. Our results suggest that Akt- and PKD1-mediated inhibition of AMPK via Ser\textsuperscript{485/491} phosphorylation may inhibit energy-metabolizing processes, while favoring energy-storing processes. Our results highlight the fact that phosphorylation of Ser\textsuperscript{485/491} can inhibit AMPK activity independent of changes in p-AMPK Thr\textsuperscript{172}, a measure which is often used as a readout of AMPK activity. We hypothesize that Akt-mediated inhibition of AMPK is an acute, physiological response to insulin, whereas PKD1-mediated inhibition may be associated with more chronic pathophysiological changes. Thus, PKD1 inhibition or prevention of Ser\textsuperscript{485/491} phosphorylation may represent new strategies for therapeutic AMPK activation as treatment for the metabolic syndrome.