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Leucine amplifies the effects of metformin on insulin sensitivity and glycemic control in diet-induced obese mice☆

Lizhi Fu[a], *Antje Bruckbauer*[b], *Fenfen Li*[a], *Qiang Cao*[a], *Xin Cui*[a], *Rui Wu*[a], *Hang Shi*[a], *Michael B. Zemel*[b], *Bingzhong Xue*[a,*]

[a] *Center for Obesity Reversal, Department of Biology, Georgia State University, Atlanta, GA*
[b] *NuSirt Biopharma Inc., Knoxville, TN*

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ABSTRACT

Background and objective. The Sirt1/AMPK signaling pathway is a key sensor of energy status and regulates glucose and lipid metabolism. Leucine (Leu) activates Sirt1 by lowering its Km for NAD^+ and potentiates other sirtuin/AMPK-activators, resulting in improvement of insulin sensitivity. Since metformin (Met) converges on this pathway, we hypothesized that leucine would amplify its gluco-regulatory effects.

Materials and methods. The effects of Leu (24 g/kg diet) + Met (0.05–0.5 g/kg diet) combinations were compared to standard therapeutic Met (1.5 g/kg diet; ~300 mg/kg BW) on glycemic control in high fat diet induced insulin resistant mice for 6 weeks. The effects of Leu on Met stimulation of Sirt1 and AMPK activities were further evaluated in adipocytes.

Results. Sub-therapeutic levels of Met combined with Leu resulted in increases in Sirt1 activity and in tissue P-AMPK/AMPK ratio and corresponding dose-responsive improvements in fasting and post-prandial glucose, in glucose response to an insulin tolerance test and in the area under the curve in glucose tolerance tests. Changes were evident within 7 days of treatment and sustained throughout the 6-week study duration. The Leu + Met (0.25 g/kg)-combinations produced a comparable effect to a standard therapeutic Met dose, while the Leu + Met (0.5 g/kg diet) resulted in greater improvements. Since resveratrol also synergizes with leucine to augment sirtuin signaling and insulin sensitivity, we tested the addition of resveratrol to Leu–Met and found no additional benefit.

Conclusion. These data demonstrate that adding Leu to Met enables a dose reduction of 66% with improved efficacy and of 83% with comparable efficacy to standard metformin in diet-induced obese mice, and addition of resveratrol does not provide further benefit.

Abbreviations: ACC, acetyl CoA carboxylase; AMP, adenosine monophosphate; AMPK, 5' adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; BCAA, branched-chain amino acid; BCKD, branched-chain α-ketoacid dehydrogenase; BW, body weight; DIO, diet-induced obese; GTT, glucose tolerance test; HFD, high-fat-diet; $HOMA_{IR}$, homeostasis model assessment of insulin resistance; HMB, β-hydroxy-β-methyl-butyrate; Leu, leucine; ITT, insulin tolerance test; LFD, low-fat-diet; Met, metformin; NAD, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; OCR, oxygen consumption rate; Res, resveratrol; Sirt1, sirtuin 1.

☆ All authors have read and agree to the publication of the manuscript.

* *Corresponding author at:* 24 Peachtree Center Avenue, Atlanta, GA 30303. Tel.: +1 404 413 5747; fax: +1 404 413 5301.
E-mail address: bxue@gsu.edu (B. Xue).

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1. Introduction

Metformin is considered the initial drug of choice for treating type 2 diabetes, as it is highly efficacious, exhibits an excellent safety profile, does not promote weight gain, does not increase the risk for hypoglycemia and has been shown to reduce the risk of diabetes-related comorbidities and death [1]. However, despite these advantages, its therapeutic utility is often limited by the occurrence of dose-related gastrointestinal adverse effects (especially at the full therapeutic dose of 1500 to 2000 mg/day), which often lead to dose reduction and/or compliance issues in 30% of patients and drug discontinuation in up to 10% of patients [2,3]. Moreover, metformin monotherapy often fails to achieve optimal glycemic control due to inter-individual variability in response to drug initiation and maintenance [4].

The blood glucose lowering effect of metformin is caused by suppression of hepatic glucose production as well as increased peripheral glucose disposal [4–7]. Most of these effects are dependent on activation of the 5' adenosine monophosphate-activated protein kinase/sirtuin1 (AMPK/Sirt1) pathway. Despite some controversy regarding whether metformin activates AMPK as a consequence of a mild inhibition of the mitochondrial respiratory chain complex 1 [8], or due to an inhibition of AMP-deaminase [9], both mechanisms result in increased cellular adenosine monophosphate (AMP) and activation of AMPK, either with or without inhibition of mitochondrial energy production. AMPK activation, in turn, increases the ratio of oxidized to reduced nicotinamide adenosine dinucleotide (NAD^+/NADH ratio) resulting in increased Sirt1 activity [10]. There is also evidence supporting an AMPK-independent mechanism of action, as hepatic gluconeogenesis has been shown to be reduced in mice lacking hepatic AMPK, suggesting adenosine triphosphate (ATP) depletion rather than direct inhibition of gluconeogenic gene expression as a contributing mechanism for inhibited hepatic glucose production [11].

Insulin resistance characterizes type 2 diabetes, resulting in impaired glucose and lipid metabolism in muscle, adipose tissue and liver. AMPK and Sirt1 are key regulators of energy metabolism and, due to their bidirectional interaction and cross-activation, they are targets of common activators and produce similar metabolic outcomes [12,13]. There is large body of evidence showing an association between decreased activity of the AMPK/Sirt1 axis in obesity, insulin resistance and diabetes, while activation of either one prevents and improves hyperglycemia and insulin resistance [14–18].

Our previous work on the branched-chain amino acid (BCAA) leucine has demonstrated that leucine, as well as its metabolite β-hydroxy-β-methyl-butyrate (HMB), activates Sirt1 by lowering the activation energy for NAD^+[19], enabling Sirt1 activation at a lower NAD^+/NADH ratio characteristic of energy replete states. Additionally, leucine and HMB coactivate and amplify the effects of other compounds that converge on the AMPK/Sirt1 axis, thus enabling significant dosage reduction [19]. This synergistic effect has been demonstrated for resveratrol and other polyphenols as well as for metformin [19–21]. Thus, a combination of leucine with a lose dose of metformin could substantially reduce the development of metformin's adverse effects without interfering with its efficacy on improving insulin sensitivity. Consistent with this notion, the short-term efficacy of a combination of HMB and resveratrol with low dose metformin in improving insulin tolerance in *db/db* mice was recently demonstrated [20]. The present study was designed to more comprehensively evaluate the long-term efficacy of leucine in augmenting the effects of metformin on insulin sensitivity in a mouse model of diet-induced obesity (DIO) and insulin resistance and to determine whether resveratrol can further enhance the response.

2. Research design and methods

2.1. Animals and diets

Six to eight week old male C57BL/6 mice were purchased from Jackson Laboratories. Obesity and insulin resistance were induced via a high-fat diet (HFD) for 6 weeks. The animals were then randomized into one of the following groups with 10 animals/group and kept on their diet for 6 weeks.

For study 1 (treatment groups without resveratrol): 1) Control (low-fat diet (LFD), standard diet (LabDiet 5001) 2) High-fat diet (HFD, Research Diets, 60% fat), 3) HFD + Leucine (24 g/kg diet) + metformin (0.15 g/kg diet) (Leu + Met 0.15), 4) HFD + Leucine + metformin (0.25 g/kg diet) (Leu + Met 0.25), 5) HFD + Leucine + metformin (0.5 g/kg diet) (Leu + Met 0.5), 6) HFD + metformin control (1.5 g metformin/kg diet) (Met 1.5). The 1.5 g metformin/kg diet concentration was designed as a standard therapeutic dose to achieve a final dosing of ~300 mg/kg BW, and the lower doses were selected as sub-therapeutic doses that we previously found to exert no effect [20].

For study 2 (treatment groups with resveratrol): 1) LFD, 2) HFD Control, 3) HFD + Leucine (24 g/kg diet) (Leu), 4) HFD + Leucine + resveratrol (12.5 mg/kg/diet) (Leu + Res), 5) HFD + Leucine + resveratrol + metformin (0.25 g/kg diet) (Leu + Res + Met 0.25), 6) HFD + Leucine + resveratrol + metformin (0.15 g/kg diet) (Leu + Res + Met 0.15), 7) HFD + Leucine + resveratrol + metformin (0.05 g/kg diet) (Leu + Res + Met 0.05), 8) HFD + metformin control (1.5 g/kg diet) (Met 1.5).

For study 3 (treatment groups with HMB): 1) LFD control, 2) HFD control, 3) HFD + HMB (Ca-HMB, 2 g/kg diet) + metformin (0.15 g/kg diet) (HMB + Met 0.15), 4) HFD + HMB + metformin (0.25 g/kg diet) (HMB + Met 0.25), 5) HFD + HMB + metformin (0.5 g/kg diet) (HMB + Met 0.5), 6) HFD + metformin control (1.5 g metformin/kg diet) (Met 1.5).

Animals were housed in polypropylene cages at a room temperature of 22 °C with a 12 h light/dark cycle. The animals had free access to water and their experimental food throughout the experiment. Body weight was measured every week. Blood glucose was measured in the fed or fasted state using an OneTouch Ultra Glucose meter (Lifescan, Milpitas, CA). At the end of the treatment period (6 weeks) all animals were humanely euthanized with CO_2 inhalation. Blood and tissues were collected for further experiments as described below.

This study and all animal procedures were performed under the auspices of an Institutional Animal Care and Use Committee-approved protocol of the Georgia State University