Interaction between metformin and leucine in reducing hyperlipidemia and hepatic lipid accumulation in diet-induced obese mice

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

Center for Obesity Reversal, Department of Biology, Georgia State University, 24 Peachtree Center Avenue, Atlanta, GA 30303, USA

NuSirt Biopharma Inc., 3835 Cleghorn Ave, Nashville, TN 37215, USA

Background. Leucine stimulates Sirt1 and AMPK signaling in vitro and in vivo. Since metformin converges on the same pathway, we have tested the ability of leucine to amplify the effects of metformin on AMPK-mediated hepatic lipid metabolism in diet-induced-obese insulin-resistant mice.

Methods. Mice were fed high leucine (24 g/kg diet) with or without sub-therapeutic levels of metformin (0.05–0.50 g/kg diet) or therapeutic levels of metformin (1.5 g/kg diet; ~300 mg/kg body weight).

Results. High-fat diet produced a 10-fold increase in inguinal fat pad weight and 25% increase in liver weight, histologically confirmed as steatosis. The leucine-metformin combinations reduced fat pad mass, normalized liver weight, liver and plasma lipids and inflammatory markers (interleukin 6, interleukin 1 beta, tumor necrosis factor alpha, monocyte chemotactic protein-1, C-reactive protein) comparable to the effects of therapeutic metformin. Moreover, the highest sub-therapeutic levels of metformin with leucine exerted significantly greater effects than therapeutic levels of metformin and fully reversed hepatic steatosis. These effects were mediated by upregulation of hepatic AMPK and associated changes in lipogenic gene expression (fatty acid synthase, stearoyl CoA desaturase, acetyl CoA carboxylase) in the liver.

Conclusion. A low-dose leucine-metformin combination exerts comparable effects on adiposity to therapeutic doses of metformin and fully reverses hepatic steatosis in diet-induced-obese mice.

© 2015 Elsevier Inc. All rights reserved.

Keywords: Diabetes, AMPK, Sirt1, Steatosis, Obesity

Abbreviations: ACC, acetyl CoA carboxylase; ALT, alanine aminotransferase; AMPK, 5’ adenosine monophosphate-activated protein kinase; AST, aspartate transaminase; CRP, C-reactive protein; FAS, fatty acid synthase; HFD, high-fat diet; HMB, β-hydroxy-β-methylbutyrate; IL6, interleukin 6; IL1β, interleukin 1 beta; Leu, leucine; LFD, low-fat diet; LKB1, liver kinase B1; MCP1, monocyte chemotactic protein 1; Met, metformin; NAD, nicotinamide adenine dinucleotide; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; Res, resveratrol; SCD1, Stearoyl-CoA desaturase; Sirt1, sirtuin 1; TNFa, tumor necrosis factor 1 alpha.

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

* Corresponding author at: Center for Obesity Reversal, Department of Biology, Georgia State University, 24 Peachtree Center Avenue, Atlanta, GA 30303, USA. Tel.: +1 404 413 5747.
E-mail address: bxue@gsu.edu (B. Xue).
1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disorders characterized by hepatic lipid accumulation and cellular degeneration in the absence of significant alcohol consumption which may progress to non-alcoholic steatohepatitis (NASH), cirrhosis or hepatocellular carcinoma [1]. The prevalence of NAFLD is 10–25% in the general population, and increases to ~75% in patients with obesity and diabetes [2]. The most widely accepted view of its pathogenesis is based on the “two-hit” or “multiple-hit” model with insulin resistance leading to hepatic lipid accumulation representing the first hit. Successive activation of multiple pathways resulting in oxidative and inflammatory stress could result in the second hit. However, despite the pivotal role of insulin resistance in the pathogenesis, insulin-sensitizing drugs have minimal effect on liver histology [3].

AMPK and Sirt1 are well known regulators of lipid and energy metabolism including hepatic lipid metabolism. High-fat diet (HFD) and excess energy intake have been shown to decrease Sirt1 and AMPK activity [4–6]. This in turn, may lead to mitochondrial loss or dysfunction, which plays a pivotal role in the development of metabolic diseases such as insulin resistance and diabetes. In contrast, both, Sirt1 and AMPK activation, prevents or attenuates glucose and lipid-induced increases in hepatic lipid accumulation, thus representing therapeutic targets [7–9].

We have previously demonstrated that the branched-chain amino acid leucine (Leu) has a unique role as an activator of the AMPK/Sirt1 pathway and thereby modulates lipid and energy metabolism. Leucine activates Sirt1, at least in part, by lowering the activation energy for NAD+ and consequently also co-activates and amplifies the effects of other sirtuin activators [10]. For example, the combination of low dose resveratrol with leucine increased insulin sensitivity, muscle glucose utilization and palmitate oxidation in vitro and in vivo [11]. Moreover, Sirt1 activation leads to deacetylation of the protein kinase LKB1 which promotes AMPK phosphorylation [9]. Consistent with this concept, leucine, but not other amino acids such as alanine or valine, activated AMPK in a Sirt1-dependent manner [12].

Metformin (Met) is the first-line drug for treating type 2 diabetes, especially in overweight and obese patients [13]. Because of its insulin-sensitizing effects, it was also suggested as a treatment option for NAFLD/NASH. Although some studies have shown some beneficial effects on clinical markers such as improvement of alanine aminotransferase (ALT) and aspartate transaminase (AST) levels, it does not confer a consistent beneficial effect on liver histology [3] and is not recommended as a treatment for NAFLD or NASH [3,14]. However, since metformin also converges on the AMPK/Sirt1 pathway, and heterozygous Sirt1 knockout mice develop severe NASH [15], we tested the ability of leucine to amplify the effects of metformin on lipid metabolism and hepatic steatosis in vitro and in vivo in an obese mouse model of insulin resistance. Since we previously demonstrated synergy between leucine and resveratrol (Res) [11], we also sought to determine whether adding resveratrol to the Leu + Met combination would confer further benefit.

2. Material and Methods

2.1. Animals and Diets:

Six to eight weeks old male C57BL/6J mice were purchased from Jackson Laboratories. Obesity and insulin resistance were induced via an 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.

2.1.1. For Study 1 (Treatment Groups Without Resveratrol)

1) Control (low-fat diet (LFD), standard diet (LabDiet 5001), 2) HFD (Research Diets, 60% fat), 3) HFD + leucine (24 g/kg diet; Sigma Aldrich, St. Louis, MO) + metformin 0.15 g/kg diet (Sigma Aldrich, St. Louis, MO) (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/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 body weight. This concentration was used to compare the full therapeutic effects of the standard dose to the effects of the combinations of leucine with the lower doses of metformin, which are sub-therapeutic doses and previously found to exert no effect [16].

2.1.2. For Study 2 (Treatment Groups With Resveratrol)

1) LFD, 2) HFD control, 3) HFD + leucine (24 g/kg diet), 4) HFD + leucine + resveratrol (12.5 mg/kg/diet; Sigma Aldrich, St. Louis, MO) (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).

Animals were housed in polypropylene cages at a room temperature of 22 °C and regime of 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. At the end of the treatment period (6 weeks) all animals were humanely euthanized with CO2 inhalation. Blood was collected via trunk bleed and tissues were collected for further experiments as described below.

2.2. Liver Histology

Liver tissues were fixed in 10% neutral formalin, embedded in paraffin and cut into 5 μm sections. Sections were processed for hematoxylin and eosin (H&E) staining and histological images were recorded using Nikon Eclipse E800 Microscopy with Zeiss AxioCam camera.

2.3. Gene Expression

Total RNA from liver was extracted using the Tri-Reagent kit (Molecular Research Center, Cincinnati, OH) and gene expression was assessed by quantitative reverse transcription (RT)-PCR (ABI Universal PCR Master Mix, Applied Biosystems, Foster City, CA) using a Stratagene Mx3000p thermocycler (Stratagene, La Jolla, CA). Cyclophilin was used to normalize the gene expression data. The primer and probe sets used in the assays were

for hematoxylin and eosin (H&E) staining and histological images were recorded using Nikon Eclipse E800 Microscopy with Zeiss AxioCam camera.
purchased from Applied Biosystems/Life Technologies (Grand Island, NY).

2.4. Western Blot

The P-AMPK and AMPK antibody were obtained from Cell Signaling (Danvers, MA). β-actin antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, Dallas, TX). Protein levels of cell extracts were measured by bicinchoninic acid assay (BCA) kit (Thermo Fisher Scientific, Waltham, MA). For Western blot, 10–50 μg protein was resolved on 4–15% gradient polyacrylamide gels (Criterion precast gel, Bio-Rad Laboratories, Hercules, CA), transferred to nitrocellulose membranes, incubated in blocking buffer (5% non-fat dry milk in TBS) and then incubated with primary antibody (1:1000 dilution), washed and incubated with horseradish peroxidase- or fluorescence-conjugated secondary antibody (1:10,000 dilution). Visualization was conducted using Li-COR Odyssey Fc Imaging system (Li-COR Biosciences, Lincoln, NB) and band intensity was assessed using Quantity One (Bio-Rad Laboratories, Hercules, CA), with correction for background and loading controls.

2.5. Plasma Biochemistry

Blood was collected from fed mice, and plasma free fatty acid and triglyceride levels were measured using enzymatic colorimetric methods according to manufacturer’s instructions (Wako, Richmond, VA). Plasma C-reactive protein (CRP) levels were measured in fed mice using a mouse CRP ELISA kit (Life Diagnostics, West Chester, PA).

2.6. Liver Triglyceride Measurements

Liver lipid extraction was conducted as previously described with minor modifications [17,18]. Briefly, ~100 mg of liver was thawed, minced and weighted in glass tube. Lipids were extracted in 2:1 CHCl₃/methanol at room temperature overnight. The lipid portion was then dried down under N₂ and redissolved in a measured volume of 2:1 CHCl₃/methanol. Diluted H₂SO₄ was added to the sample, which was then vortexed and centrifuged to split the phases. The aqueous upper phase was aspirated and discarded, and an aliquot of the bottom phase was dried down and dissolved in 2% Triton X-100. The triglyceride content was then measured using TG kit/L-Type TG M (Wako Chemicals, Richmond, VA) and normalized to liver weight.

2.7. Cell Culture

Human hepatoma HepG2 cells (ATCC, Manassas, VA) were grown in Dulbecco’s modified Eagle’s medium (DMEM, 5.5 mM glucose) containing 10% fetal bovine serum (FBS) and antibiotics (1% penicillin-streptomycin) at 37 °C in 5% CO₂ in air. Media was replaced with fresh medium every 2–3 days. Cells were split at a 1:4 ratio at 70–80% confluency.

2.8. Fatty Acid Oxidation

Cellular oxygen consumption was measured using a Seahorse Bioscience XF24 analyzer (Seahorse Bioscience, Billerica, MA) in 24-well plates at 37 °C. HepG2 cells were seeded at 40,000 cells per well. Lipid accumulation was induced by 48 h incubation with 25 mM glucose. Cells were treated for 24 hours with the indicated treatments, washed twice with non-buffered carbonate-free pH 7.4 low glucose (2.5 mM) DMEM containing carnitine (0.5 mM), equilibrated with 550 μL of the same media in a non-CO₂ incubator for 30 minutes, and then inserted into the instrument for 15 minutes of further equilibration. O₂ consumption was measured in three successive baseline measurements at eight-minute intervals prior to injection of palmitate (200 μM final concentration). Post-palmitate-injection measurements were taken over a 3-hour period with cycles consisting of 10 min break and three successive measurements of O₂ consumption.

2.9. Oil Red Staining

HepG2 cells were grown in 6-well plate. Lipid accumulation was induced by incubation in 25 mM glucose plus 100 nM insulin for 48 h. Treatment (metformin 0.1 mM, leucine 0.5 mM or combination) was added for further 24 h. Cells were washed twice with PBS, then incubated with 10% formalin for 1 hour. Then cells were washed twice with ddH₂O, then with 60%

---

Fig. 1 – In vitro data in HepG2 cells. HepG2 were incubated with 25 mM glucose for 48 h to induce lipid accumulation, then treated with metformin (0.1 mM), leucine (0.5 mM) or the combination (Leu + Met) for 24 h. a: Oxygen consumption rate (OCR) after 200 μM palmitate injection was measured and the area under the curve (AUC) was calculated. Data are represented as mean ± SEM (n = 5–10). b: Triglyceride accumulation normalized to protein amount. Data are represented as mean ± SEM (n = 3–4).
isopropanol for 5 minutes. Cells were dried completely, then incubated with oil red O working solution for 25 min. Cells were washed 4 times with ddH2O, then 100% isopropanol was added to elute the oil red O dye and absorbance was measured at 500 nm. Absorbance was normalized to isopropanol absorbance of unstained cells (blank).

2.10. Statistical Analysis

All data are expressed as mean ± SEM. Data were analyzed by one-way ANOVA, and significantly different group means (p < 0.05) were separated by the least significant difference test using GraphPad Prism version 6 (GraphPad Software, La Jolla CA, www.graphpad.com).

3. Results

Based on our in vitro experiments showing a significant stimulation of palmitate-induced fat oxidation (Fig. 1A) and an associated 56% reduction of the high glucose induced lipid accumulation after 24 h treatment with Met-Leu in HepG2 liver cells (Fig. 1B), we evaluated the effects of different Met-Leu combinations on liver lipid metabolism and histology in DIO-mice. HFD markedly increased lipogenic gene expression, while adding Leu + Met for 6 weeks to the HFD resulted in significant dose-dependent reduction in acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD) 1 and fatty acid synthase (FAS) (Fig. 2 A, B and C) and a concomitant

Fig. 2 – Lipogenic gene expression and AMPK protein expression in liver of DIO mice. DIO mice were fed an HFD with indicated treatments for 6 weeks. At the end of the treatment period, gene expression of the lipogenic genes FAS (a), SCD1 (b) and ACC (c) in the liver of the DIO mice was determined. d and e: protein expression of P-AMPK and AMPK in liver, expressed as the ratio of P-AMPK/AMPK (d) and the corresponding Western blots (e). Data are represented as mean ± SEM (n = 4–6).
dose-dependent increase in P-AMPK/AMPK ratio in the liver (Fig. 2D). While there was no difference in total body weight between the treatment groups and the HFD (as described in [19]), there was a significant reduction of fat pad mass (31%) and liver weight (27%) at the end of treatment duration (Tables 1 and 2), as well as a reversal of the HFD induced hyperlipidemia to normal levels (measured by fasting free fatty acids and plasma triglycerides, Table 2). Consistent with these changes, the Leu + Met combinations prevented completely the HFD induced hepatic lipid accumulation, as hepatic triglycerides were completely returned to LFD concentrations (4-fold reduction compared to HFD, Fig. 3D), and liver histology samples show no difference between the LFD and the Leu + Met groups (Fig. 3B). In addition, the inflammatory serum marker C-reactive protein (CRP) as well as interleukin (IL)-6, IL-1 beta, tumor necrosis factor (TNF)-alpha and monocyte chemo tactic protein (MCP)-1 gene expression in liver was significantly reduced by the Leu + Met combinations (Fig. 4). For all of these parameters, metformin concentrations of 0.25 and 0.5 g/kg diet combined with leucine achieved comparable or better effects than full-dose metformin (1.5 g/kg diet). Addition of resveratrol in study 2 did not further augment any of these effects.

4. Discussion

These data indicate that the combination of leucine and metformin stimulates hepatic fat oxidation in vitro, and inhibits lipid storage and reverses the HFD-induced lipid accumulation in obese and insulin-resistant mice.

Obesity, diabetes, dyslipidemia and metabolic syndrome are risk factors for the development of NAFLD, and insulin resistance is the most predictive factor for the progression to NASH [14,20]. Impaired hepatic insulin signaling leads to increased de novo lipogenesis, decreased fat oxidation and impaired lipid export. This may contribute to lipotoxicity and hepatocyte injury secondary to the generation of lipotoxic metabolites (e.g. ceramides, diacylglycerols) and reactive oxygen species [21], especially in the presence of a high fat diet and/or excessive caloric intake. Peripheral insulin resistance in muscle and adipose tissue further increases the production of free fatty acids due to increased adipocyte lipolysis and decreased muscle fat oxidation [1,22]. On the other hand, NAFLD may also worsen systemic insulin resistance since it increases hyperglycemia due to increased hepatic gluconeogenesis, thus contributing to a vicious cycle [20].

HFD feeding in mice has been demonstrated to induce obesity, insulin resistance and NAFLD [23,24]. Accordingly in this study, the HFD caused significant obesity (10-fold increase in subcutaneous fat pad weights (Tables 1 and 2) and 36% increase in body weight, as well as significant insulin resistance (data presented in [19]). Furthermore, hepatic insulin resistance was indicated by the increase in lipogenic genes in the liver and the suppression of the P-AMPK/AMPK ratio (Fig. 2). This was associated with a 25% increase in liver weight and excessive hepatic lipid accumulation (Tables 1 and 2, Fig. 3). The Leu + Met combination decreased de novo lipogenesis and increased hepatocyte fat oxidation in the in vitro studies (Fig. 1) and decreased hepatic lipogenic gene expression in DIO mice (Fig. 2),
leading to overall reductions in hepatic fat accumulation (Table 2 and Fig. 3), which was also associated with improved peripheral insulin resistance and increased fat oxidation in muscle (data presented in [19]) and normalization of free fatty acids and plasma triglycerides (Table 2).

Inflammation often represents the “second hit” and potentiates the risk for progression from simple steatohepatosis to NASH and cirrhosis [20]. Obesity is associated with a generalized chronic inflammatory state and pro-inflammatory cytokines such as TNF-α, IL-6 and C-reactive protein (CRP) are elevated in

Table 2 – Inguinal fat pad mass, liver weight and lipids (study 1).

<table>
<thead>
<tr>
<th></th>
<th>LFD</th>
<th>HFD</th>
<th>Leu + Met 0.15</th>
<th>Leu + Met 0.25</th>
<th>Leu + Met 0.5</th>
<th>Met 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal fat pad mass (gram)</td>
<td>0.2168 ± 0.012 a</td>
<td>1.738 ± 0.24 b</td>
<td>1.768 ± 0.36 bc</td>
<td>1.348 ± 0.21 bc</td>
<td>1.159 ± 0.06 c</td>
<td>1.193 ± 0.16 c</td>
</tr>
<tr>
<td>Liver weight (gram)</td>
<td>1.432 ± 0.03 a</td>
<td>1.794 ± 0.15 b</td>
<td>1.547 ± 0.12 ab</td>
<td>1.437 ± 0.08 a</td>
<td>1.305 ± 0.03 a</td>
<td>1.420 ± 0.08 a</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>0.9126 ± 0.05 a</td>
<td>1.110 ± 0.07 b</td>
<td>1.155 ± 0.06 b</td>
<td>0.8816 ± 0.06 a</td>
<td>0.8945 ± 0.05 a</td>
<td>0.9206 ± 0.04 a</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dL)</td>
<td>98.19 ± 10.81 a</td>
<td>153.8 ± 25.94 b</td>
<td>114.0 ± 18.48 ab</td>
<td>66.51 ± 6.63 a</td>
<td>66.46 ± 7.7 a</td>
<td>99.47 ± 10.65 a</td>
</tr>
</tbody>
</table>

DIO mice were fed an LFD or HFD with or without Leu + Met combinations for 6 weeks. At the end of the treatment period, inguinal fat pad mass, liver weight, plasma and liver lipids, and triglyceride content of liver sections were measured. Data are represented as mean ± SEM (n = 4–10). Non-matching superscripts denote significant differences between treatment groups (p < 0.05).

Fig. 3 – Liver histology and liver triglycerides. DIO mice were fed an HFD with indicated treatments for 6 weeks. At the end of the treatment period, liver sections were fixated and stained with H&E to visualize fat accumulation. Representative figures are shown for Leu + Met combination with resveratrol (study 2) (a), and Leu + Met combination without resveratrol (study 1) (b). Quantitative analysis of liver triglycerides was performed and normalized to liver weight for study 2 (c) and study 1 (d). Data are represented as mean ± SEM (n = 6–9).
obese patients [25]. Pro-inflammatory cytokines have been suggested by some as noninvasive biomarkers to distinguish hepatosteatosis from NASH [26]; although the clinical utility of these markers in predicting severity of NASH and fibrosis is unclear, most studies have shown a positive correlation with higher inflammatory marker levels in NASH [26–29]. In this study, the HFD-induced up-regulation of all inflammatory markers in liver (IL6, IL-1 beta, TNF alpha and MCP-1) and in plasma (CRP) was significantly blunted or fully reversed by the Leu + Met treatment combinations.

AMPK and Sirt1 are key regulators of energy metabolism in multiple tissues including the liver with overlapping metabolic

Fig. 4 – Inflammatory marker in liver and plasma. DIO mice were fed an HFD with indicated treatments for 6 weeks. At the end of the treatment period gene expression of the inflammatory markers IL6 (a), IL-1 beta (b), TNF alpha (c) and MCP1 (d) in liver tissue as well as CRP in plasma (e) was measured. Data are represented as mean ± SEM (n = 4–10).
methyl-butyrate (HMB), has been demonstrated as a direct Sirt1 expression (SREBP-1c, ACC, FAS, SCD-1), serum lipids and fat results in AMPK activation [7], decreases lipogenic gene MCP-1[15,36,37]. Leucine, a swel la si t sm e t a b o l i t e multiple inflammatory cytokines such as TNF- secretion and glucose utilization in muscle, and by reducing insulin resistance by increasing pancreatic beta-cell insulin secretion and glucose utilization in muscle, and by reducing multiple inflammatory cytokines such as TNF-α, NF-κB, and MCP-1[15,36,37]. Leucine, as well as its metabolite β-hydroxy-β-methyl-butyrate (HMB), has been demonstrated as a direct Sirt1 activator, reducing the activation energy for NAD+. This enables Sirt1 activation at lower NAD+ concentrations that are characteristic of energy replete states. In addition, leucine and HMB amplify the effects of other compounds such as polyphenols and metformin that also merge on the AMPK/Sirt1 axis which allows substantial dose reduction of the individual compounds [10,11,16]. This synergistic activation of the AMPK/Sirt1 axis resulted in beneficial effects on insulin resistance by increasing muscle and glucose fat oxidation, and muscle glucose utilization [11,16]. In this study we demonstrate that the Leu + Met combination also synergistically activated hepatic AMPK leading to beneficial effects on hepatic lipid metabolism with a reduction of lipogenesis and lipid accumulation. However, although hepatic P-AMPK/AMPK was higher in the animals on the Leu + Met combinations than in those on the therapeutic dose of metformin, this difference did not achieve statistical significance. Nonetheless, the Leu + Met combination elicited a significantly greater suppression of the hepatic acyl CoA carboxylase, indicating a correspondingly greater suppression in hepatic lipogenesis. Consistent with this, the Leu + Met combination fully reversed the HFD-induced increase in hepatic triglycerides. This together with the effects on peripheral insulin resistance (as presented in [19]) and inflammation may prevent or reverse NAFLD.

NAFLD is the most common cause of liver disease and most common indication for liver transplantation, and is associated with increased risk for cardiovascular and other metabolic complications, contributing to increased overall mortality [39]. However, at present there are no specific treatment options for NAFLD/NASH available, and therapeutic approaches mainly focus on improving insulin resistance and preventing risk factors. Lifestyle modifications such as weight loss, exercise and diet are highly recommended but compliance is challenging. Drug therapy such as anti-diabetic drugs (e.g. metformin, pioglitazone), lipid-lowering drugs (e.g. statins, fibrates), anti-obesity medication (e.g. orlistat), and antioxidants (e.g. vitamin E) have been demonstrated to improve some metabolic parameters but either have minimal effects on liver histology or have other adverse effects limiting their use [3,14,40]. The Leu + Met combinations demonstrated beneficial effects on multiple risk factors for NAFLD such as insulin resistance, hyperlipidemia and inflammation with the same or better efficacy than full-dose metformin. In addition, this combination allowed substantial dose reduction of metformin, which reduces the risk of potential adverse effects. Moreover, it reversed completely the HFD-induced hepatic lipid accumulation which was not accomplished by full dose metformin. Thus, this combination may be useful in the treatment or prevention of NAFLD or NASH.

5. Conclusion

We demonstrate in this study the beneficial effects of a Leu + Met combination on hepatic lipid metabolism in DIO-insulin resistant mice. The combination enabled a dose reduction of up to 80% of metformin without loss of efficacy (compared to therapeutic dose of metformin) on metabolic parameters such as plasma lipids, inflammatory markers and insulin resistance. In addition, the Leu + Met 0.5 combination reversed the HFD-induced hepatic lipid accumulation completely, suggesting that these Leu + Met combinations may be a promising therapeutic approach to treat or prevent NAFLD.

Authors’ Contribution

AB was responsible for design and execution of the cell experiments. LZ was responsible for execution of the animal experiments. FL, QC, XC and RW were responsible in the assistance of the completion of animal experiments. BZ, HS and MBZ were responsible for experimental design of the animal studies, data analysis and interpretation. BZ and HS supervised the animal studies and sample analysis. AB, BZ, HS and MBZ were responsible for writing and editing the manuscript.

Funding

Financial support for this study was provided by NuSirt Biopharma, Nashville, TN.

Submission Declaration and Verification

Data were presented as a poster presentation at the ADA 2014 conference, San Francisco (Diabetes 2014; 63 (Suppl 1): A463).

Disclosures

The authors Antje Bruckbauer and Michael B. Zemel are employees and stockholders of NuSirt Biopharma. All other authors declare that they have no conflict of interest.
References


