METABOLISM CLINICAL AND EXPERIMENTAL XX (2015) XXX–XXX

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Metabolism

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Niacin inhibits fat accumulation, oxidative stress, and inflammatory cytokine IL-8 in cultured hepatocytes: Impact on non-alcoholic fatty liver disease☆,☆☆

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ARTICLE INFO

Article history:
Received 16 October 2014
Accepted 4 May 2015

Keywords:
Nicotinic acid
Steatosis
Hepatic inflammation
Non-alcoholic steatohepatitis

ABSTRACT

Objective. Non-alcoholic fatty liver disease (NAFLD) is a common disorder characterized by excessive hepatic fat accumulation, production of reactive oxygen species (ROS), inflammation and potentially resulting in non-alcoholic steatohepatitis (NASH), cirrhosis and end-stage liver disease. Recently, we have shown that niacin significantly prevented hepatic steatosis and regressed pre-existing steatosis in high-fat fed rat model of NAFLD. To gain further insight into the cellular mechanisms, this study investigated the effect of niacin on human hepatocyte fat accumulation, ROS production, and inflammatory mediator IL-8 secretion.

Materials and methods. Human hepatoblastoma cell line HepG2 or human primary hepatocytes were first stimulated with palmitic acid followed by treatment with niacin or control for 24 h.

Results. The data indicated that niacin (at 0.25 and 0.5 mmol/L doses) significantly inhibited palmitic acid-induced fat accumulation in human hepatocytes by 45–62%. This effect was associated with inhibition of diacylglycerol acyltransferase 2 (DGAT2) mRNA expression without affecting the mRNA expression of fatty acid synthase (FAS) and carnitine palmitoyltransferase 1 (CPT1). Niacin attenuated hepatocyte ROS production and it also inhibited NADPH oxidase activity. Niacin reduced palmitic acid-induced IL-8 levels.

Conclusions. These findings suggest that niacin, through inhibiting hepatocyte DGAT2 and NADPH oxidase activity, attenuates hepatic fat accumulation and ROS production respectively. Decreased ROS production, at least in part, may have contributed to the inhibition of pro-inflammatory IL-8 levels. These mechanistic studies may be useful for the clinical development of niacin and niacin-related compounds for the treatment of NAFLD/NASH and its complications.

Abbreviations: CPT 1, carnitine palmitoyltransferase 1; DCFDA, 6-carboxy-2-7 dichlorodihydroxyfluorescein diacetate; DGAT1, diacylglycerol acyltransferase 1; DGAT2, diacylglycerol acyltransferase 2; FAS, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; MPO, myeloperoxidase; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PKC, protein kinase C; ROS, reactive oxygen species.

☆ Disclosure: None.

☆☆ Disclosure: The authors do not have any potential conflicts of interest.

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http://dx.doi.org/10.1016/j.metabol.2015.05.002
0026-0495/

1. Introduction

NAFLD is the most common liver disease in the US and worldwide and significantly contributes to premature mortality [1,2]. It affects approximately 30% of the general adult population, and is one of the major pathophysiological components of patients with obesity, type 2 diabetes, and metabolic syndrome [1,2]. NAFLD is generally characterized by initial development of fatty liver with intracellular lipids exceeding 5% of the hepatic tissue (hepatic steatosis), sometimes associated with hepatic inflammation and varied degree of fibrosis termed non-alcoholic steatohepatitis (NASH). In addition to high risk for development of liver disease, NAFLD is associated with increased risk of cardiovascular disease [3]. Despite its severity and high prevalence, currently there is no pharmacological agent that is recommended and of proven benefit for the treatment of NAFLD and progressive NASH.

Although precise mechanisms involved in the progression of NAFLD are poorly understood, a "Two-Hit" hypothesis has been a widely accepted model to understand the pathogenesis of NAFLD [4]. The first hit involves an imbalance in fatty acid and triglyceride metabolism in hepatocytes resulting in hepatic steatosis [5–7]. The second hit comprises oxidative stress and lipid peroxidation resulting in progressive form NASH with hepatic inflammation, fibrosis, and cirrhosis.

In numerous studies, hepatic oxidative stress and lipid peroxidation have been shown to occur in patients with NAFLD [8–11]. Once hepatic steatosis is present, increased oxidative stress can trigger pathobiological processes involved in lipid peroxidation and subsequent hepatic inflammation, fibrosis, and necrosis leading to the development of advanced NASH and cirrhosis. Lipid peroxidation products can activate transcription factors NF-kB and AP-1 in patients with NAFLD [12], key transcription factors that regulate the expression of several pro-inflammatory cytokines and hepatic inflammation. The Two Hit hypothesis, in principle, explains pathophysiological processes involved in the development of advanced steatohepatitis in many but not in all patients. It has been thought that in the latter, the second hit oxidative stress insult may be mild and may not be sufficient to induce hepatic inflammation and fibrosis.

Niacin has long been used for the treatment of dyslipidemia associated with atherosclerotic cardiovascular disease (reviewed in [13]). During the past several years, our research has been focused on defining the pharmacological role of niacin in decreasing plasma triglycerides, low-density lipoproteins, and increasing high-density lipoproteins related to atherosclerosis (reviewed in [14]). Previously, we have also reported that niacin decreases vascular oxidative stress by increasing cellular redox potential in human aortic endothelial cells [15]. In order to extend the use of niacin for other indications, we recently showed that niacin prevented the accumulation of fat in liver and regressed pre-existing hepatic steatosis in high-fat fed rat model of NAFLD [16]. In support of our study in experimental NAFLD model, Hu et al in a small uncontrolled study in 39 patients recently showed that niacin therapy (2 g/day) significantly reduced liver fat content in Chinese patients with hypertriglyceridemia [17]. However, hepatocellular mechanisms of action of niacin on pathophysiological events involved in NAFLD are not known. In an effort to understand cellular mechanisms, this study investigated the effect of niacin on human hepatocyte fat accumulation, DGAT2 enzyme, ROS production, and inflammatory mediator IL-8, that are key hepatic pathophysiological events involved in the pathogenesis of NAFLD.

2. Methods

2.1. Materials

Tissue culture supplies, media, fetal bovine serum (FBS), palmitic acid, BSA, and nicotinic acid (Niacin) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). 6-Carboxy-2-7 dichlorodihydroxyfluorescein diacetate (DCFDA) was obtained from Molecular Probes. IL-8 ELISA kit was procured from Life Science Technology.

2.2. Cell Culture and Treatment

Normal human primary hepatocytes and human hepatoblastoma cell line HepG2 cells were obtained from Lonza Biologics and American Type Culture Collection respectively. Normal human primary hepatocytes were grown according to the instructions provided by Lonza. In brief, cells were plated in tissue culture dishes coated with type 1 collagen (at a density of 200,000 cells/cm^2) in hepatocyte basal growth medium (Lonza) supplemented with growth factors and 2% FBS. The plates were incubated at 37 °C in a tissue culture incubator (5% CO_2) for 4–5 h. The medium was changed to hepatocyte maintenance media during the experiment. HepG2 cells were grown in DMEM containing 10% FBS, 2 mmol/L glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. For experiments, normal human primary hepatocytes or HepG2 cells were first incubated with pathophysiologically relevant concentrations of palmitic acid (0.5 mmol/L, complexed with BSA) for 24 h to induce fat accumulation as described previously [18]. Cells were then incubated in the absence or presence of niacin (0.25–0.5 mmol/L) for 24 h.

2.3. Cell Viability

To assess the cell viability under the experimental conditions as noted above, cellular cytotoxicity assays were performed using the commercially available lactate dehydrogenase (LDH) cytotoxicity assay kit according to the instructions provided by the vendor (Cayman Chemical Company).

2.4. Measurement of Fat Accumulation

To determine the effect of niacin on neutral lipid accumulation in intact cells, cells were stained with 0.1 μg/mL Nile Red O for 5 min. Cells were collected and the fluorescence intensity was measured using Novostar Fluorimeter at the excitation/emission of 480/620 nm as described previously [19].

2.5. mRNA Expression of Diacylglycerol Acyltransferase 2 (DGAT2), Fatty Acid Synthase (FAS), and Carnitine Palmitoyltransferase 1 (CPT1)

Total RNA was isolated by TRIzol method (Invitrogen). One microgram of RNA was subjected to DNase I digestion, and cDNA

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Table 1 – Niacin inhibits fat accumulation in hepatocytes.

Treatment conditions	Fat accumulation (fluorescence units/mg protein)
Human primary hepatocytes	
Control	2500 ± 600
PA (0.5 mmol/L)	31750 ± 1797
PA (0.5 mmol/L) + Niacin 0.25 mmol/L	18600 ± 2096 ($P < 0.05$)
PA (0.5 mmol/L) + Niacin 0.5 mmol/L	11600 ± 1198 ($P < 0.05$)
HepG2 cells	
Control	14574 ± 963
PA (0.5 mmol/L)	41688 ± 3083
PA (0.5 mmol/L) + Niacin 0.25 mmol/L	26350 ± 3157 ($P < 0.05$)
PA (0.5 mmol/L) + Niacin 0.5 mmol/L	31398 ± 2787 ($P < 0.05$)

Normal human primary hepatocytes or HepG2 cells were incubated with palmitic acid (0.5 mmol/L, complexed with BSA) for 24 h to induce fat accumulation. Cells were then treated in the absence (control) or presence of niacin (0.25–0.5 mmol/L). For 24 h, intracellular fat accumulation was assessed by staining cells with Nile Red O and measuring fluorescence as described in Methods section. PA, palmitic acid. Data are expressed as mean ± SE of 3 independent experiments. P-values shown are comparisons to the palmitic acid treated cells. Comparisons between control vs. palmitic acid and control vs. palmitic acid + niacin (0.25 mmol/L and 0.5 mmol/L) were statistically significant at $P < 0.05$.

was synthesized using iScript cDNA synthesis kit (Bio-Rad). Cellular mRNA expressions of FAS, DGAT2, and CPT1 were performed by Real-time PCR (iCycler real-time PCR detection system, Bio-Rad). PCR conditions used were as follows: 95 °C for 15 seconds (denaturation), 55 °C for 30 seconds (annealing), 72 °C for 30 seconds (elongation). A total of 40 cycles were used. Specific primer sequences used were as follows. FAS: forward-TACGTACTGGCCTACACCCAGA, reverse-TGAACTGCTGCACGAAGAAGCATAT; CPT-1: forward-CCAGAGCAGCACCCCAAT, reverse-CTGCAATCATGTAGGAAA CTCCATAG; DGAT2: forward-GCTACAGGTCATCTCAGTGCTC, reverse-GTGAAGTAGAGCACAGCATGAG. mRNA expression levels were calculated by dCT method using β-actin as an internal control.

2.6. *Cellular ROS Production*

ROS generation in hepatocytes was measured using DCFDA (which mainly detects H_2O_2) fluorescence method as described previously [15]. After appropriate treatment, cells were incubated with DCFDA (10 μmol/L) for 30 min. Cells were collected and lysed in PBS containing Triton X-100 (0.5%). The fluorescence intensity in the cell lysate was measured using BMG Lab tech instrument with excitation and emission wavelength of 488 and 520 respectively.

2.7. *Measurement of NADPH Oxidase Activity*

NADPH oxidase activity was measured by lucigenin assay according to the previously described procedure [20]. In brief, NADPH oxidase activity was measured in a 50 mmol/L phosphate buffer (pH 7.0) containing 5 μmol/L lucigenin and 100 μmol/L NADPH. Cell lysate was added to start the reaction. Photon emission was measured every 15 seconds for 10 minutes in a luminometer.

2.8. *Measurement of IL-8*

IL-8 levels in the supernatant were measured by using an ELISA kit (Invitrogen Corporation). IL-8 levels were expressed as pg/mL of the supernatant.

2.9. *Statistical Analysis*

Data presented are the mean ± SE of 3 separate experiments. Statistical significance was calculated by using the unpaired Student's t test, and a value of $P < 0.05$ was considered significant.

3. Results

Studies were performed in the human hepatoblastoma cell line HepG2 and key findings confirmed in human primary hepatocytes. HepG2 cells retain biochemical and morphological properties characteristic of hepatocytes. Several investigators have used HepG2 cells for studies related to fatty acid and triglyceride metabolism, steatosis, hepatic oxidative stress, and hepatic inflammation involved in the development of NAFLD [18,21–27]. Fatty acid-induced fat accumulation and hepatic inflammation (IL-8 production involved in hepatic neutrophil chemoattraction) were similar in HepG2 cells and primary human hepatocytes suggesting the suitability of HepG2 cells as an in-vitro model to investigate NAFLD [18,21,22]. Previously, it was shown that pathophysiologically relevant concentrations of palmitic acid in the range of 0.3–0.5 mmol/L significantly induced fat accumulation and IL-8 expression in HepG2 cells [18]. Thus, we used 0.5 mmol/L palmitic acid in all our studies.

3.1. *Niacin Inhibits Fat Accumulation in Human Primary Hepatocytes and HepG2 Cells*

In this study, we examined whether niacin would have similar effect on fat accumulation in both human primary hepatocytes and HepG2 cells. As shown in Table 1, niacin (at 0.25 mmol/L and 0.5 mmol/L doses) significantly inhibited palmitic acid-induced fat accumulation by about 45–62% in human primary hepatocytes. Niacin, at 0.125 mmol/L dose, had minimal non-significant reduction in fat accumulation (data not shown). In HepG2 cells, niacin treatment (at 0.25 mmol/L and 0.5 mmol/L doses) also significantly reduced palmitic acid-induced fat deposition by 47–51% (Table 1).

Because of similar effect of niacin on fat accumulation both in HepG2 cells and normal human primary hepatocytes

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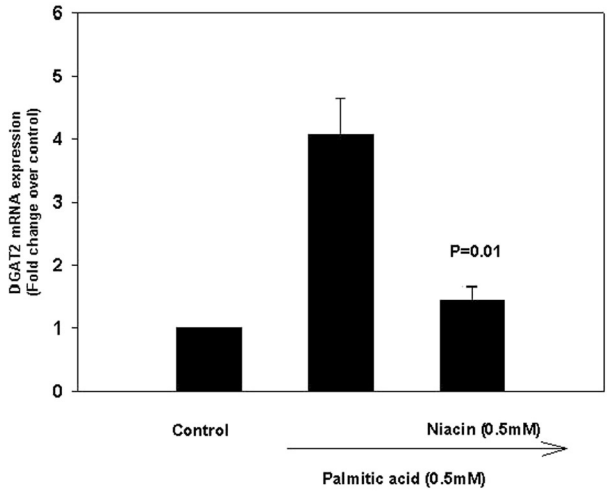

Fig. 1 – Effect of niacin on DGAT2 mRNA expression in HepG2 cells. HepG2 cells were first stimulated with palmitic acid for 24 h. These cells were then treated with niacin or control for 24 h. Cellular mRNA expression of DGAT2 was measured by real-time PCR as described in Methods section. Data are expressed as mean ± SE of 3 separate experiments, and statistical comparisons were between palmitic acid and palmitic acid + niacin treated groups. Comparisons between control and palmitic acid were statistically significant ($P < 0.05$). Statistical comparison between control and palmitic acid + niacin was not statistically significant.

and because of ease of growing HepG2 cells, we used HepG2 cells for further studies assessing mechanisms of action of niacin on fat accumulation. We examined the effect of niacin on mRNA expression of DGAT2, FAS, and CPT-1 that are major enzymes involved in triglyceride synthesis, fatty acid synthesis, and fatty acid oxidation respectively.

The findings indicated that palmitic acid robustly induced DGAT2 mRNA expression (Fig. 1). Niacin almost blocked palmitic acid-induced DGAT2 mRNA expression (Fig. 1). Niacin had no significant effect on the mRNA expression of FAS and CPT-1. FAS and CPT-1 mRNA expressions (fold change over control) are as follows: FAS: control = 1.03 ± 0.24, palmitic acid = 0.85 ± 0.11, palmitic acid ± niacin = 0.75 ± 0.10; CPT-1: control = 1.0 ± 0.04, palmitic acid = 1.05 ± 0.1, palmitic acid ± niacin = 1.0 ± 0.15.

3.2. *Niacin Did Not Affect Cell Viability*

We used LDH cytotoxicity assay to determine the effect of niacin on cellular viability under the experimental conditions used in HepG2 cells. Incubation of cells with palmitic acid for 24 h had no significant effect on cell viability as compared to controls. Additionally, niacin did not influence cell viability in cells treated with palmitic acid. Cell viability (LDH release, mU/mL) data are as follows: control = 1.96 ± 0.62, palmitic acid = 1.90 ± 0.49, palmitic acid ± 0.25 mmol/L niacin = 1.91 ± 0.76, palmitic acid ± 0.5 mmol/L niacin = 1.97 ± 0.71.

3.3. *Niacin Inhibits ROS Production in Hepatocytes*

Stimulation of human primary hepatocytes with palmitic acid robustly augmented ROS production (Fig. 2A). Niacin (at 0.25 mm and 0.5 mmol/L doses) significantly reduced palmitic acid-induced ROS levels in human primary hepatocytes (Fig. 2A). Comparative studies conducted in HepG2 cells also showed that niacin (0.25 and 0.5 mmol/L doses) significantly inhibited palmitic acid-induced ROS production by 51–64% (Fig. 2B).

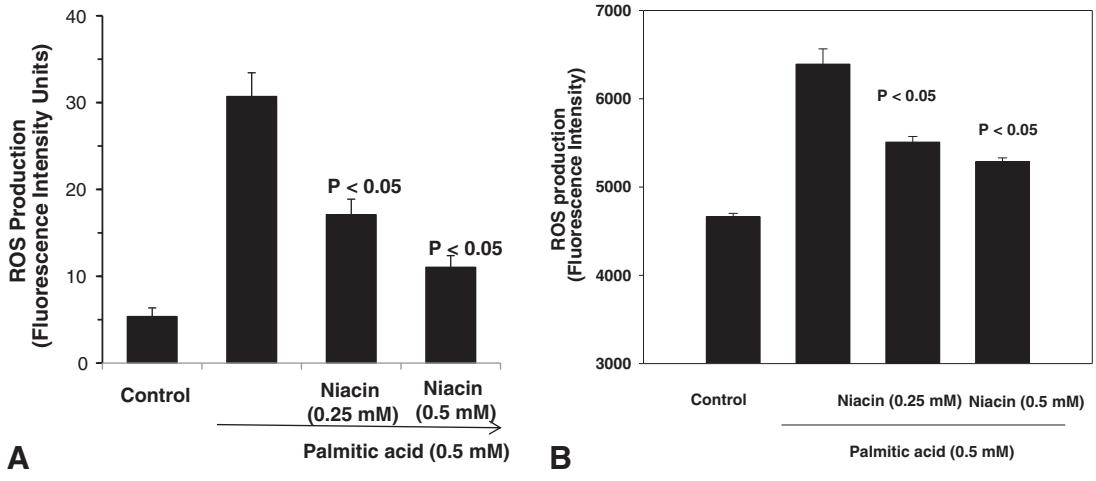

Fig. 2 – Niacin inhibits cellular ROS production in hepatocytes. Normal human primary hepatocytes (A) or HepG2 cells (B) were initially stimulated with palmitic acid for 24 h. Cells were then incubated in the absence or presence of niacin for 24 h. Cellular ROS production was measured by DCFDA fluorescence method as described in Methods section. Data are expressed as mean ± SE of 3 independent experiments. P-values shown are comparisons to the palmitic acid treated cells. Comparisons between control vs. palmitic acid and control vs. palmitic acid + niacin (0.25 mmol/L and 0.5 mmol/L) were statistically significant at $P < 0.05$.

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3.4. Niacin Decreased NADPH Oxidase Activity in HepG2 Cells

In order to understand niacin-mediated reduction in ROS production, we further examined the effect of niacin on NADPH oxidase, a major enzyme involved in ROS production. The findings showed that palmitic acid strongly increased NADPH oxidase activity in HepG2 cells (Fig. 3). Niacin treatment significantly decreased palmitic acid-induced NADPH oxidase activity in HepG2 cells (Fig. 3).

3.5. Niacin Decreased IL-8 Levels in HepG2 Cells

To gain some initial insight into the hepatocyte inflammation, we examined the effect of niacin on IL-8, one of the major inflammatory cytokines implicated in hepatic inflammation. Stimulation of HepG2 cells with palmitic acid markedly increased IL-8 secretion in the media (Fig. 4). Additional studies showed that niacin, in a dose-dependent manner significantly, but only partially, reduced IL-8 secretion induced by palmitic acid (Fig. 4).

4. Discussion

Excessive accumulation of triglycerides in hepatocytes plays an important pathophysiological role in the pathogenesis of hepatic steatosis and NAFLD. Diacylglycerol acyltransferases (DGAT1 and DGAT2) are important rate-limiting enzymes in the esterification of fatty acids to triglycerides and catalyzes the final step of triglyceride synthesis. With special reference to hepatic steatosis, overexpression of liver-specific DGAT2 in mice resulted in increased hepatic steatosis [28]. Additionally, knockdown of DGAT2 but not DGAT1 with antisense oligonucleotides in rats and mice fed high-fat diet markedly decreased hepatic lipids and reversed hepatic steatosis and insulin resistance [29,30]. In our studies, we showed that niacin inhibited fat accumulation induced by palmitic acid in human hepatocytes. Niacin had no effect on the mRNA expression of FAS and CPT-1, the key enzymes involved in fatty acid synthesis and oxidation respectively. However, niacin treatment significantly inhibited mRNA expression of DGAT2. These findings suggest that niacin, by directly affecting palmitic acid-mediated transcription of DGAT2, decreases hepatocyte triglyceride synthesis. We have previously shown that niacin, in a non-stimulated HepG2 cells, non-competitively inhibited microsomal DGAT2 activity [14].

Our findings on increased hepatocyte DGAT2 mRNA expression by palmitic acid is in line with other previously reported studies in high-fat loaded hepatocytes (by treating with fatty acids) as well as in livers of rats and mice fed high-fat diet [31,32]. However, the regulatory mechanisms of fatty acid/high fat diet-mediated alterations in hepatocyte DGAT2 mRNA transcription and posttranscriptional processes have not been studied in detail. Using gene knockdown and overexpression approaches, recent studies show that the transcription factor CAAT/enhancer-binding protein-β (C/EBPβ, one of the regulators of adipogenesis) plays an important role in regulating DGAT2 expression and hepatic steatosis [33–35]. In HepG2 cells, activation of protein kinase C (PKC) has been shown to markedly increase C/EBPβ mRNA expression [36]. Based on these studies, it is likely that the stimulation of hepatocytes with palmitic acid as well as feeding high-fat diet to animals that activate hepatic PKC [18,37] may upregulate C/EBPβ resulting in increased DGAT2 expression and steatosis.

Metabolic imbalances associated with impaired oxidation of NADH to NAD^+ and increased ratio of NADH/NAD^+ are shown to regulate numerous signaling pathways including

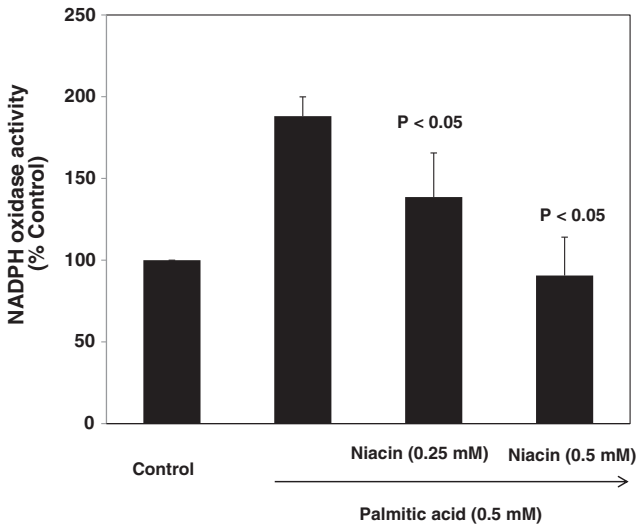

Fig. 3 – Niacin inhibits NADPH oxidase activity in HepG2 cells. Cells were first stimulated with palmitic acid for 24 h, and followed by treatment with niacin or control for 24 h. Cellular NADPH oxidase activity was measured according to the procedure described in Methods section. Data are expressed as mean ± SE of 3 independent experiments, and P-values indicated are comparisons to the palmitic acid treated cells. Comparisons between control and palmitic acid were statistically significant (P < 0.05). Statistical comparison between control and palmitic acid + niacin (0.25 mmol/L and 0.5 mmol/L) was not significant.

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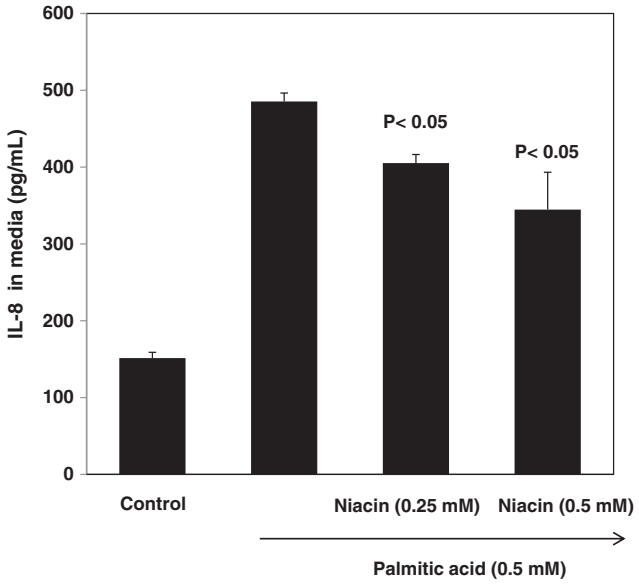

Fig. 4 – Effect of niacin on palmitic acid-induced IL-8 levels in HepG2 cells. Cells were stimulated with palmitic acid for 24 h. Cells were then treated with niacin or control for 24 h. IL-8 secreted in the medium was assayed by ELISA as described in Methods section. Data are expressed as mean ± SE of 3 separate experiments, and P-values shown are comparisons to the palmitic acid treated cells. Comparisons between control vs. palmitic acid and control vs. palmitic acid + niacin (0.25 mmol/L and 0.5 mmol/L) were statistically significant at P < 0.05.

PKC [38], and increased cytosolic NADH/NAD^+ ratio stimulate PKC activity [39]. It is possible that niacin, as a precursor for the synthesis of NAD^+, increases cellular concentrations of NAD^+. We propose that niacin by increasing the formation of NAD^+ decreases NADH/NAD^+ ratio and subsequently inhibits PKC activity in hepatocytes. Niacin-mediated inhibition of PKC activity may decrease the expression of C/EBPβ which may in turn attenuates DGAT2 mRNA transcription. However, additional studies are needed to investigate the possible above indicated mechanisms by which niacin participates in PKC-C/EBPβ signaling events for its effect on reducing DGAT2 mRNA expression and hepatocyte fat content.

In addition to inhibition of hepatocyte fat content, our studies showed that niacin decreased hepatocyte production of ROS, a second-hit insult involved in the pathogenesis of NAFLD. Niacin-mediated reduction in ROS was associated with significant inhibition of NADPH oxidase, a key enzyme involved in ROS production. Although NADPH oxidase is primarily located in hepatic Kupffer cells, hepatocytes have also been shown to exhibit NADPH activity and may participate in increased ROS production in hepatocytes [40–43]. Our findings suggest that niacin, at least in part, through inhibiting NADPH oxidase may decrease ROS generation in hepatocytes. It is also possible that other enzymatic processes may also participate in niacin-mediated reduction in ROS generation in hepatocytes. Additionally, our data indicate that niacin significantly, but only partially, decreased hepatocyte IL-8 secretion.

IL-8 is one of the major pro-inflammatory cytokines, involved in inflammatory response for chemoattraction of neutrophils, basophils, and T cells [44]. Patients with NASH have increased circulating IL-8 compared to healthy individuals, suggesting that this cytokine may be one of the major mediators involved in the development of hepatic inflammation and NASH [45,46]. It has been proposed that increased hepatocyte production of IL-8 may stimulate recruitment of T cells and neutrophil activation and transmigration with subsequent release of toxic factors such as ROS and proteases, which may increase hepatic inflammatory responses and liver injury [18]. Although detailed studies are warranted to identify mechanisms by which niacin inhibits hepatocyte IL-8, it is likely that the ability of niacin to decrease hepatocyte IL-8 may be beneficial in reducing heightened hepatic inflammation associated with the pathogenesis of NAFLD.

Increased hepatic migration and accumulation of inflammatory cells (e.g., neutrophils) in patients with NASH can generate excessive levels of myeloperoxidase (MPO), a potent oxidant generating neutrophilic enzyme involved in inflammation. It has been shown that plasma MPO levels as well as hepatic MPO-derived oxidation products are elevated in patients with NASH, suggesting the potential contribution of MPO in NASH [47]. Recently, we have shown that niacin significantly decreases MPO release and activity in human neutrophils [48]. Thus, niacin could mitigate the deleterious effect of IL-8 on not only neutrophil chemotaxis but also decreasing MPO release by neutrophils.

Increasing evidence suggests that the steatotic liver caused by the accumulation of fat in hepatocytes is susceptible to secondary hits such as oxidative stress and subsequent hepatic inflammation and fibrosis [4,49]. Similarly, our observations in this study suggest that palmitic acid-mediated fat accumulation caused an induction of ROS in hepatocytes. Numerous studies indicated that the glucose-6-phosphate dehydrogenase (G6PD), plays an important role in the production of NADPH and restoring intracellular redox state in the setting of increased oxidant stress [50,51]. Further mechanistic studies are required to investigate the role of G6PD-NADPH-GSH pathways in niacin-mediated effects on NADPH oxidase and ROS production.

Although these in-vitro findings addressed the effect of niacin on hepatocyte fat accumulation and ROS production, future clinical studies are needed to establish the beneficial effects of niacin on steatosis, oxidative stress, and hepatic inflammation in patients with NAFLD. The concentrations of niacin (0.25–0.5 mmol/L) used in our in-vitro studies are clinically relevant and comparable to the niacin concentrations observed in human plasma and probably in the liver after oral administration of niacin in clinical doses. In humans, plasma level of niacin was found to be about 0.3 mmol/L after oral ingestion of 2 g of niacin [52], and high plasma concentrations of niacin are also corroborated by a recent study [53]. Also because of the first pass effect and transport through the portal venous system, niacin concentrations in liver tissue will be much higher than in plasma levels after oral administration of 1–3 g of niacin. For this reason, in clinical trials for niacin for treatment of NAFLD/NASH, lower doses than currently used may be needed.

Although these data support the potential benefits of niacin in the treatment of fatty liver disease and NASH, caution should be taken with respect to the previously

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reported adverse and toxic effects in patients taking higher doses of niacin. It is important to point out that the formulation of niacin bears an important relationship to its adverse effects. Whereas immediate-release niacin produces more flushing, hepatotoxicity is increased with the use of very slow-release preparation in the past [53]. Recently available agents have been developed as extended-release preparations with an optimized intermediate-release rate so that flushing and hepatotoxicity are minimized [52,54].

The most significant side effect is the niacin flush, a prostaglandin D2-mediated vaso-cutaneous reaction (reviewed in [55]). In majority of the patients niacin flush is significantly abated with the co-administration of aspirin, and this side effect is considerably diminished after several days of continuous treatment with niacin [55]. Niacin has also been shown to impair glucose metabolism, increase insulin resistance and plasma glucose levels [56]. However, several studies have shown that modest increase in glucose level in niacin treated patients could be easily counteracted by adjusting the diet, exercise, and antidiabetic medications (reviewed in [54,57]). Hepatotoxicity, as assessed by elevations in transaminases, in some patients is another adverse event seen in patients taking niacin [53,58,59]. Although increased levels of alanine aminotransferase and/or aspartate aminotransferase were observed in some patients taking higher concentrations of niacin as compared to placebo, the levels of these enzymes were not greater than 3 times the upper limit in majority of the patients [57,60]. In fact, in a recent uncontrolled study in dyslipidemic patients with steatosis, niacin administration (2 g/day for 16 weeks) improved liver enzymes including alanine aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase [17]. These initial clinical observations are consistent with the potentially beneficial effect of niacin on fat accumulation and anti-oxidant effects as suggested in this and our original report in a rat NAFLD model [16]. However, cellular mechanistic studies as described in this and future studies are needed to fully understand the beneficial and adverse effects of niacin for its use in the treatment of NAFLD.

In conclusion, our in-vitro findings show that niacin inhibits hepatocyte fat accumulation, ROS production, and pro-inflammatory IL-8 levels, the key pathophysiological mediators of hepatic first and second hits involved in the pathogenesis of NAFLD and progressive NASH. Clinical development of niacin and niacin-related compounds for the treatment of NAFLD and NASH would offer a cost effective option in addressing the unmet need of developing therapeutic agent(s) for NAFLD. Taken together with studies in the in-vivo rat model, and a preliminary report of an uncontrolled clinical trial, these in-vitro mechanistic data lay the foundation for a future randomized clinical trial to assess a potential new use indication of niacin or its formulations in the treatment of NAFLD and NASH for which there is no currently approved treatment.

Authors' Contributions

Shobha H. Ganji: Dr. Ganji contributed in the experimental design, conduct of the study, data collection and analysis.

Moti L. Kashyap: Dr. Kashyap contributed in the design of the study, data interpretation, and manuscript writing.

Vaijinath S. Kamanna: Dr. Kamanna contributed in the design of the study, data analysis and interpretation, and manuscript writing.

Acknowledgments

We thank Neil Hoa for technical assistance in fluorescent measurements of cellular fat and ROS levels. This study was supported in part by a grant from the Southern California Institute for Research and Education.

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Please cite this article as: Ganji SH, et al, Niacin inhibits fat accumulation, oxidative stress, and inflammatory cytokine IL-8 in cultured hepatocytes: Impact on non-alcoholic fatty liver disease, Metabolism (2015), http://dx.doi.org/10.1016/j.metabol.2015.05.002

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