Novel Pleiotropic Effects of Niacin

by

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ABSTRACT

**Introduction.** Obesity is associated with a chronic low-grade inflammation of the adipose tissue that has been linked to obesity-related comorbidities, such as insulin resistance and cardiovascular disease. Niacin exerts anti-inflammatory effects in certain tissues, including the lung, kidney, and retina; however, to date there have been no studies examining the anti-inflammatory properties of niacin in adipose tissue. **Objective.** Therefore, our objective was to determine the effects of niacin on high-fat diet-induced adipose tissue inflammation and to characterize the role of the niacin receptor (GPR109A) and post-receptor intermediates involved. **Methods.** Male C57BL/6 mice were placed on a control diet or high-fat diet and were maintained on such diets for the duration of the study. After 6 weeks on the control or high-fat diets, vehicle or niacin treatments were begun. Half of the mice from each group received vehicle (water) and the other half received niacin (200 mg/kg/day in drinking water) for 4 weeks. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. Identical studies were conducted concurrently in GPR109A-/- mice. **Results.** Niacin treatment attenuated high-fat diet-induced increases in adipose tissue expression of monocyte chemoattractant protein-1 (MCP-1) and IL-1β in the wild-type obese mice, indicating anti-inflammatory effects of niacin in the adipose tissue. MCP-1 is
involved in the recruitment of pro-inflammatory macrophages to the adipose
tissue in obesity. Niacin had no effect on total adipose tissue macrophage
content, as evidenced by unchanged CD68 expression, but reduced the
expression of the pro-inflammatory M1 macrophage marker CD11c in obese
wild-type mice. Niacin had varying effects on markers of M2, or anti-
inflammatory macrophages. Niacin increased serum concentrations of the anti-
inflammatory adipokine, adiponectin by 21% in obese wild-type mice, but had no
effect on lean wild-type or lean or obese GPR109A<sup>−/−</sup> mice. Niacin increased
adiponectin gene and protein expression in the wild-type mice on the high-fat
diet. This effect was lost in the GPR109A null mice. The increases in
adiponectin serum concentrations, gene and protein expression occurred
independently of changes in expression of PPARγ, C/EBPα, or SREBP-1c (key
transcription factors known to positively regulate adiponectin gene transcription)
in the adipose tissue. Further, niacin had no effect on adipose tissue expression
of ERp44, Ero1-Lα, or DsbA-L (key ER chaperones involved in adiponectin
production and secretion). In summary, short-term niacin treatment attenuates
obesity-induced adipose tissue inflammation through reduced pro-inflammatory
cytokine expression, increased anti-inflammatory cytokine expression and
reduced M1 macrophage content in the adipose tissue in a niacin receptor-
dependent manner. In additional studies, niacin decreased serum RBP4 (a
protein elevated in obesity and causally related to insulin resistance)
concentrations in obese wild-type mice by 22%. Niacin also tended to reduce
serum RBP4 in the lean wild-type mice, although not significantly, by 16%.
Interestingly, niacin significantly reduced serum RBP4 concentrations by 16% in the lean GPR109A−/− mice, but had no effect on obese GPR109A−/− mice. Adipose tissue and liver RBP4 gene and protein expression were unchanged in response to niacin treatment in any group. Since niacin was able to reduce serum RBP4 in wild-type and GPR109A null mice, but had no effect on RBP4 gene or protein expression in the liver or fat of any mice, niacin most likely reduces serum RBP4 concentrations in mice in a receptor-independent manner, possibly through increased clearance. Lastly, niacin attenuated high-fat diet-induced reductions in adipose tissue expression of GPR109A and GPR81, two members of a receptor subfamily involved in the metabolic sensing ability of the adipocyte. **Conclusions.** In summary, niacin produces novel pleiotropic actions on the adipose tissue including anti-inflammatory effects, decreased RBP4, and attenuation of high-fat diet-induced reduction in GPR109A and GPR81.
ACKNOWLEDGEMENTS

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<th>Full Form</th>
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<tr>
<td>AMPK</td>
<td>Adenosine monophosphate-activated protein kinase</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>C/EBP&lt;sub&gt;α&lt;/sub&gt;</td>
<td>CCAAT/enhancer binding protein &lt;i&gt;α&lt;/i&gt;</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DGAT2</td>
<td>Diacylglycerol acyltransferase 2</td>
</tr>
<tr>
<td>DsbA-L</td>
<td>Disulfide-bond oxidoreductase-like protein</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentanoic acid</td>
</tr>
<tr>
<td>Ero1-L&lt;sub&gt;α&lt;/sub&gt;</td>
<td>Endoplasmic reticulum oxidoreductase 1-L&lt;sub&gt;α&lt;/sub&gt;</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>ERp44</td>
<td>Endoplasmic reticulum chaperone of 44 kD</td>
</tr>
<tr>
<td>EWAT</td>
<td>Epididymal white adipose tissue</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HFD</td>
<td>High fat diet</td>
</tr>
<tr>
<td>HGP</td>
<td>Hepatic glucose production</td>
</tr>
<tr>
<td>HMW</td>
<td>High-molecular weight</td>
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<tr>
<td>HSL</td>
<td>Hormone sensitive lipase</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>LMW</td>
<td>Low-molecular weight</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td>MGL-2</td>
<td>Macrophage galactose N-acetyl-galactosamine specific lectin 2</td>
</tr>
<tr>
<td>MRC-1</td>
<td>Mannose receptor C, type 1</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NEFAs</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostaglandin D&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator activated receptor</td>
</tr>
<tr>
<td>PUMA-G</td>
<td>Protein upregulated in macrophages by interferon γ</td>
</tr>
<tr>
<td>RBP4</td>
<td>Retinol-binding protein 4</td>
</tr>
<tr>
<td>SIRT1</td>
<td>Sirtuin 1</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>TZD</td>
<td>Thiazolidinedione</td>
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CHAPTER I
REVIEW OF THE LITERATURE
INTRODUCTION

The incidence of obesity in the U.S. has reached epidemic proportions within the last 25 years. Obesity is associated with an increased risk of metabolic and cardiovascular disease (CVD), with CVD the leading cause of morbidity and mortality in the U.S. (179). Exaggerated adipose tissue lipolysis and increased serum non-esterified fatty acids (NEFAs) are characteristic features of obesity that often lead to the development of atherogenic dyslipidemia. Atherogenic dyslipidemia is a cluster of metabolic abnormalities characterized by moderately elevated LDL-C (130-159 mg/dL) and TGs (>150 mg/dL), small LDL particles and low HDL-C (<35 mg/dL) (87). Niacin is one of the most effective pharmacological interventions for the treatment of atherogenic dyslipidemia and has been shown to reduce CVD morbidity and mortality presumably by improving blood lipid and lipoprotein characteristics. Along with producing modest reductions in circulating very low-density lipoprotein (VLDL), LDL-C, TG and lipoprotein(a) concentrations, niacin is the most effective pharmacological tool for increasing circulating HDL-C concentrations.

In addition to its ability to improve blood lipid and lipoprotein characteristics, recent studies in a variety of tissues have demonstrated that niacin possesses
profound anti-inflammatory properties. More recent evidence from our laboratory suggests that niacin dramatically increases serum adiponectin concentrations (170; 171) and reduces serum RBP4 in obese men with the metabolic syndrome (MetS) (unpublished observations). Adiponectin, a cytokine secreted from adipose tissue, is one of the most promising biomarkers of the metabolic syndrome and CVD and may serve as an etiological link between these conditions. RBP4, a cytokine secreted from the liver and adipose tissue has been positively and causally associated with insulin resistance. Therefore, at least part of the health-related benefits of niacin may be attributed to its anti-inflammatory properties and ability to alter serum adipokine concentrations.

**NIACIN**

Niacin is a collective term used for nicotinic acid and nicotinamide (Figure 1). While both compounds are equally effective as nutrients or co-factors in glucose and lipid metabolic pathways, pharmacological doses of the acid form of niacin (nicotinic acid) have proven lipid-lowering qualities that do not exist with niacin in the amide form (66).

*Discovery of Niacin*

The pharmacological properties of nicotinic acid were initially discovered in 1955 by Rudolph Altschul and his colleague Abram Hoffer during their quests to identify pharmacological agents for the treatment of hypercholesterolemia and schizophrenia, respectively. Nicotinic acid and nicotinamide produced no beneficial effects on the symptoms of schizophrenia, but the authors did find that
Figure 1: Chemical Structures of Nicotinic Acid (left) and Nicotinamide (right).
pharmacological doses (3000 mg/day) of nicotinic acid decreased plasma cholesterol in normal and hypercholesterolemic subjects (11).

_Effects of Niacin on Lipids and CVD_

Prospective studies provide convincing evidence that niacin reduces CVD risk. The Coronary Drug Project (1; 39) was a nationwide, double-blind, placebo-controlled study designed to evaluate the long-term effects of niacin and other lipid-lowering agents on the primary endpoint of all-cause mortality in men with previous myocardial infarction. Niacin (3000 mg/day) reduced total cholesterol by 10% and TG by 26% during the six-year follow-up period. These changes were associated with reductions in non-fatal myocardial infarction and cerebrovascular incident by 26% and 24% compared to those treated with placebo. The combination of niacin and colestipol, a bile acid sequestrant, in patients with known CVD, increased HDL-C, decreased 2-year mortality rates (32; 44) and reduced the progression of atherosclerotic plaque formation in 61% of patients treated (23). In contrast, the recently completed AIM-HIGH study was terminated approximately 18 months earlier than planned because the addition of niacin produced no further benefits on cardiovascular events than statins alone (67). The results of these studies bring in to question the efficacy of niacin, but it is important to note that the majority of studies using niacin in high-risk populations impart reductions in overall CVD morbidity and mortality.
**Niacin Formulations**

Niacin is available in over-the-counter and prescription formulations. Over-the-counter forms of niacin are sold as immediate-release and extended-release. The pharmacokinetics of each of the niacin formulations are important because they determine how niacin is metabolized which in turn influences the adverse reactions associated with their use. Immediate-release niacin formulations generally reach peak absorption within one hour of ingestion, have a metabolic half-life of approximately one hour (143) and are marketed as “immediate-release”, “crystalline” or “plain” niacin. To date, Niacor is the only immediate-release niacin product approved by the FDA for its lipid-lowering properties.

The primary adverse effect of immediate-release formulations is severe cutaneous flushing. This has led to the development of extended release form of niacin. Extended-release formulations are produced by a variety of absorption-delaying techniques that increase dissolution times. Extended-release niacin is absorbed over the course of 8-12 hours, which makes it suitable for once-a-day dosing. Extended-release niacin is marketed as “controlled-release”, “no-flush” or “time-release”. Niaspan is the only extended-release formulation approved by the FDA for the treatment of dyslipidemia. While extended-release formulations have fewer flushing events, they experience a greater number of cases of hepatic toxicity due to slower absorption times. This is directly related to the dissolution rates of the extended-release formulation and the metabolic characteristics of niacin (40; 168). Hepatic metabolism of niacin occurs via two
pathways: the conjugative pathway and the amidation pathway (168; 169). The amidation pathway results in the formation of nicotinamide and pyrimidine metabolites, which have been associated with the hepatotoxic effects seen with some sustained-release niacin formulations (168). The diminished side effects with extended-release niacin make it the most prescribed form. However, the frequency and severity of cutaneous flushing experienced by patients taking each form of niacin has limited its use.

GPR109A: Receptor for Niacin

In 1962, Carlson et al. provided the first insight into the mechanism of action of niacin by demonstrating a reduction in adipose tissue lipolysis resulting in a rapid decrease in plasma free fatty acid concentrations (41; 43). Butcher et al. subsequently demonstrated that niacin reduces the accumulation of cAMP in isolated adipocytes (36). In 1980, Aktories et al. extended these findings by demonstrating that niacin inhibits adenylate cyclase activity in rat and hamster fat cell ghosts (8) and this inhibition of adenylate cyclase was abolished by pretreatment with pertussis toxin (9). In 2001, Lorenzen et al. demonstrated that nicotinic acid induced G protein activation in rat adipocytes and spleen, but not in other tissues (130). To identify the receptor for niacin, orphan receptors were selected based on their tissue expression profiles (228). Orphan receptors localized primarily to adipose tissue, spleen and macrophages were transfected in a mammalian cell line and GTPγS binding was measured in response to niacin treatment. Niacin-mediated stimulation of GTPγS binding was observed only in
membranes from cells co-transfected with the cDNA for the orphan receptor now known to be GPR109A and the G protein $G_{\alpha_{01}}$ (228). The niacin-induced stimulation was concentration-dependent and was eliminated by pretreatment with pertussis toxin (228). This led to the discovery of a G protein-coupled receptor (GPCR) for niacin in 2003 by three independent groups (200; 215; 228). The receptor for niacin couples to G proteins of the $G_i$ family and is known by multiple names: PUMA-G (protein up-regulated in macrophages by interferon-$\gamma$) in mice, HM74A in humans, GPR109A, NIACR1, and HCA2.

In the years following its discovery, GPR109A remained an orphan receptor because an endogenous ligand for it had not been identified. Under physiological conditions, plasma nicotinic acid concentrations are too low to activate GPR109A, making it unlikely to be the endogenous ligand. In 2005, Taggart et al. demonstrated that $\beta$-hydroxybutyrate, a ketone body produced by the liver, is an endogenous ligand for GPR109A with an EC$_{50}$ of 767 ± 57 µM (206). $\beta$-hydroxybutyrate activates GPR109A and inhibits adipocyte lipolysis at concentrations seen during a 2-3 day fast (0.8 ± 0.06 mM) (206). $\beta$-hydroxybutyrate may represent a homeostatic mechanism for surviving starvation in which it acts in a negative feedback manner to inhibit lipolysis. Inhibition of lipolysis by elevated $\beta$-hydroxybutyrate concentrations may also be an attempt to regulate ketone body production by decreasing the serum level of fatty acid precursors available for hepatic ketogenesis (206).
GPR109A: Expression and Regulation

GPR109A is located on chromosome 12q24.31 in humans (228) and is predominantly expressed in adipocytes of white and brown adipose tissue, keratinocytes, and immune cells, including dermal dendritic cells, monocytes, macrophages and neutrophils (90; 136; 200; 209; 215; 228). Although a number of studies have reported that the receptor is not expressed in the liver (200; 215; 228), a more recent study demonstrated low basal levels of GPR109A in primary murine hepatocytes and human derived hepatocytes with expression being markedly inducible after exposure to inflammatory stimuli (122). A limited number of studies have investigated the role of various conditions or disease states in the regulation of GPR109A expression. A recent publication described an increase in GPR109A expression in diabetic mouse and human retina (77). Another study demonstrated reduced GPR109A expression in epididymal adipocytes and bone marrow adipocytes with aging (126). However, the regulation of GPR109A expression in the adipose tissue during obesity or diabetes is unknown.

GPR109A Function

Adipose Tissue

GPR109A activation in the adipocyte results in a Gᵢ-protein-mediated inhibition of adenylyl cyclase, which results in reduced concentrations of intracellular cAMP. Reduced cAMP concentrations in turn lead to decreased protein kinase A (PKA)-mediated activation of triglyceride lipases such as hormone sensitive lipase
(HSL). This causes a reduced hydrolysis of triglycerides leading to decreased free fatty acid release from adipose tissue (Figure 2). In 1962, Carlson and Oro showed that niacin lowered plasma concentrations of free fatty acids within minutes, followed by a rebound increase in free fatty acids within one hour (43). Consequently there are reduced levels of free fatty acids available for hepatic triglyceride synthesis. This will ultimately result in lower VLDL and LDL levels. This is one of the theories of the mechanism by which niacin reduces serum triglycerides, VLDL and LDL-C concentrations. However, some are skeptical about the contribution of niacin’s anti-lipolytic effects on blood lipids, considering there is generally a rebound in free fatty acids which negates the initial reduction in free fatty acids by niacin.

We have previously shown that niacin increases serum adiponectin concentrations in men with metabolic syndrome (170; 171). The ability of niacin to increase adiponectin secretion is highly dependent on the activation of GPR109A, as this effect is lost in GPR109A−/− mice (171). Importantly, the ability of GPR109A to stimulate adiponectin release from the adipocyte is very specific with no effect on other adipokines, including leptin and resistin. Many of the pleiotropic effects of niacin are the same effects that have been attributed to adiponectin. For example, Ouchi et al. demonstrated that adiponectin inhibits TNF-α-induced expression of endothelial adhesion molecules (157). They went on to show that adiponectin inhibits TNF-α-induced mRNA expression of monocyte adhesion molecules and adiponectin also suppresses TNF-α-induced
Figure 2: GPR109A Function: Adipocyte and Langerhans Cells/Keratinocytes. GPR109A is a receptor for endogenous (β-OHB) and exogenous (e.g. niacin) ligands. Activation of GPR109A (top panel above) in the adipocyte results in inhibition of adenylate cyclase activity and subsequent reduction in cAMP levels, and PKA, HSL/ATGL activity. This results in reduced hydrolysis of TG and subsequent suppression of FFA and glycerol release from the adipocyte. At the same time, adipocyte secretion of adiponectin is increased. The reduction of substrate availability to the liver limits TG and VLDL synthesis and subsequently reduces serum concentrations of TG and LDL-C and increases HDL-C. In epidermal Langerhans cells and keratinocytes (bottom panel above), GPR109A activation results in arachidonic acid-mediated prostaglandin synthesis, which will initiate a flushing response. ATP-adenosine triphosphate, cAMP-cyclic adenosine monophosphate, PKA-protein kinase A, HSL-hormone sensitive lipase, ATGL-adipose triglyceride lipase, TG-triglyceride, FFA-free fatty acid, VLDL-very low density lipoprotein, AcAc-acetoacetate, β-OHB-β-hydroxybutyrate, LDL-C-low density lipoprotein cholesterol, HDL-C-high density lipoprotein cholesterol, PLA2-phospholipase A2, COX-1-cyclooxygenase-1, PGE2-prostaglandin E2, PGD2-prostaglandin D2
IκB-α phosphorylation and subsequent NF-κB activation (159). These effects are very similar to the non-lipid-lowering effects of niacin and it is therefore possible that at least some of the beneficial effects of niacin can be credited to increased serum adiponectin concentrations.

Keratinocytes have been identified as being the primary mediators of GPR109A activation-induced rubor (or redness) that accompanies flushing. Using laser Doppler flowmetry to estimate the flux of red blood cells in the skin, Hanson et al. demonstrated that GPR109A in keratinocytes mediates the majority of rubor resulting from GPR109A activation (90). Through the use of global GPR109A knockout mice and mice solely expressing GPR109A on keratinocytes, they went on to show that while the Langerhans cells are responsible for an immediate short-lived redness upon GPR109A activation, GPR109A localized to the keratinocyte was responsible for a delayed, but more persistent rubor. They were unable to determine, however, if keratinocytes mediate any of the other side effects of niacin often accompanying redness including the itching, tingling or burning sensations.

Prior to development of methods to ease flushing, about one third of patients treated with niacin discontinue use due to the flushing side effect (2). In recent years, means to alleviate the flushing that accommodates niacin therapy have been developed. First, an extended-release niacin formula is now available which provides the same effects on blood lipids as niacin with a better tolerance
for flushing (147). Second, adding aspirin (~160 mg/day) to extended-release niacin may further improve tolerance for flushing (14). Third, laropiprant, a potent selective antagonist of prostaglandin D2 (PGD2) receptor subtype-1 (59; 202), effectively reduces the flush caused by niacin (117). This is why development of partial GPR109A agonists, drugs that block prostaglandin synthesis or release, prostaglandin receptor blockers, etc may be of importance to improve patient compliance on niacin.

More recently, Lukasova et al. have attributed the niacin-mediated inhibition of atherosclerosis progression to the activation of GPR109A in monocytes and macrophages (134). They demonstrated that niacin reduces the progression of atherosclerosis independent of its lipid-altering effects through the activation of GPR109A on immune cells (134). Further, they showed that macrophages localized to atherosclerotic lesions express GPR109A and that niacin-mediated GPR109A activation induces the expression of the cholesterol transporter ABCG1 and a subsequent increase in cholesterol efflux from macrophages to HDL3. These effects were not seen in the absence of GPR109A. In addition, Lukasova et al. demonstrated that niacin induces reduced M1-like (proinflammatory) macrophage differentiation via GPR109A.

Liver

Early studies shortly after GPR109A was discovered suggested that the receptor was not expressed in hepatocytes (79). More recent studies appear to
demonstrate that basal levels of the receptor are low (but present) and these levels are upregulated in response to inflammatory stimuli (122). Niacin can directly alter triglyceride synthesis in the liver, as well as suppress lipolysis in the adipocyte, resulting in subsequent suppression of substrate availability for triglyceride synthesis at the liver. Through repression of the activity of hepatic diacylglycerol acyltransferase 2 (DGAT2), the enzyme that catalyzes the final step in triglyceride synthesis, niacin reduces triglyceride synthesis, resulting in decreased VLDL secretion and eventually lower circulating LDL-C concentrations (79). Some researchers have hypothesized that this effect is not mediated through GPR109A, citing the original GPR109A paper which suggests that the receptor is not expressed in the hepatocytes (79). However, it cannot be definitively stated whether or not the receptor is involved in these direct effects of niacin in the liver, due to the conflicting reports of GPR109A expression in hepatocytes (122).

The mechanism by which niacin increases HDL cholesterol is not fully understood, although it is likely through lowering of the catabolic rate of apoA-I without altering apoA-I synthetic rates (24; 108; 183; 198). It has been proposed that niacin inhibits the removal of HDL-apo A-I at the level of the putative “HDL holoparticle (protein plus lipids) catabolism receptor” or pathways (107). Decreased HDL-apo A-I catabolism by niacin would increase HDL half-life and concentrations of Lp(A-I) HDL subtractions (107). Further, recent studies showed that niacin increases the expression of peroxisome proliferator-activated
receptor $\gamma$ (PPAR$\gamma$) (113; 182) and CD36 in monocytes and macrophages, and stimulates ABCA1 transporter, an important protein in the transport of cellular cholesterol to apoAI-containing HDL particles for the reverse cholesterol transport pathway (182). Elucidating the mechanism(s) by which niacin increases HDL-C has proven difficult due to the lack of animal models that respond with an increase in HDL-C in response to niacin, as is observed in humans treated with niacin. Van der Hoorn et al. have demonstrated that APOE*3Leiden transgenic mice expressing the human cholesteryl ester transfer protein (CETP) transgene (E3L.CETP mice) respond to niacin with an increase in HDL-C (217). They propose that ultimately, this is a GPR109A-dependent event, considering the HDL-C elevating effects of niacin stem from GPR109A-mediated inhibition of HSL in the adipose tissue. As previously mentioned, the inhibition of HSL in the adipocyte will reduce the availability of free fatty acids for the liver to produce TG and VLDL. Van der Hoorn et al. theorize that the subsequently reduced hepatic cholesterol content causes the reduced hepatic expression of CETP, as well as diminished release of CETP into the plasma, as evidenced by their E3L.CETP mice. In addition, they demonstrated that niacin inhibited plasma hepatic lipase activity, which may contribute to decreased HDL clearance due to reduced remodeling of HDL in the plasma (217). More recent data using GPR109A knockout mice support the concept that the HDL effect of niacin in wild-type mice is mediated through GPR109A in hepatocytes (122). However, these data are somewhat controversial since basal expression of GPR109A in the liver is low.
Vasculature

Niacin inhibits vascular inflammation by decreasing endothelial reactive oxygen species production, LDL oxidation, and subsequent vascular cell adhesion molecule–1 (VCAM-1) and MCP-1 expression and secretion, resulting in decreased monocyte and macrophage adhesion and accumulation, key events involved in atherogenesis (78; 107). Niacin also inhibits TNF-α-induced NF-κB activation (78). Further, a recent study demonstrated that niacin inhibits acute vascular inflammation and protects against endothelial dysfunction independent of changes in plasma lipid concentrations (230). These studies describe, for the first time, an anti-inflammatory role for niacin in decreasing atherosclerosis, separate from its conventional role as a lipid-regulating agent (107). Whether or not these effects of niacin are dependent upon activation of GPR109A are unknown.

Anti-inflammatory Properties of Niacin

Niacin is well-known for its beneficial lipid-altering effects. However, recent studies have shed light on anti-inflammatory properties of niacin in the kidney (49), lung (118), adipocytes (65), monocytes (64), vascular endothelial cells (229; 230), and retinal pigment epithelial cells (77). Niacin administration in rats with chronic kidney disease (CKD) reduces CKD-induced elevations in renal MCP-1 protein expression and NF-κB activation (49). Niacin treatment suppresses NF-κB activation and gene expression of the proinflammatory cytokines TNF-α and IL-6 in lung tissues of rats with endotoxemia (118).
Additionally, in cultured 3T3-L1 adipocytes, treatment with niacin reduces TNF-α-induced increases in the gene expression and secretion of the proinflammatory chemokines MCP-1, fractalkine and RANTES, as well as increases adiponectin mRNA (65). In isolated human monocytes activated by LPS, niacin treatment reduced the secretion of proinflammatory cytokines and chemokine TNF-α, IL-6 and MCP-1, mediated through suppression of the NF-κB signaling pathway (64). Gangi et al. demonstrated that niacin inhibits vascular inflammation by decreasing endothelial reactive oxygen species production and inflammatory cytokine production (78). Recently, it was reported that niacin suppressed TNF-α-induced increases in NF-κB activation, IL-6 and MCP-1 expression and secretion from retinal pigment epithelial cells (77). MCP-1 plays a major role in obesity-associated adipose tissue inflammation through recruitment of pro-inflammatory macrophages. Niacin also inhibits monocyte chemotaxis (65) and differentiation into the proinflammatory M1 macrophage state (134). Systemically, niacin reduces high-sensitivity C-reactive protein and TNF-α, systemic markers of inflammation (118; 120). Many of these anti-inflammatory properties of niacin have been linked to suppression of the NF-κB pathway (49; 64; 78; 118) and have been shown to be dependent on GPR109A activation (64; 65; 77; 134). In addition, evidence from our laboratory indicates that niacin dramatically increases serum concentrations of the anti-inflammatory adipokine, adiponectin, in obese men with the metabolic syndrome (170).
**GPR109A Agonist Development**

Numerous studies have clearly demonstrated that the antilipolytic effects of niacin are produced through the GPR109A receptor (42; 153). At the same time, more recent studies have demonstrated that GPR109A in epidermal Langerhans cells mediates nicotinic acid-induced flushing through the release of PGD$_2$ and PGE$_2$ (19; 47). The challenge has thus been to develop advanced GPR109A agonists which lower free fatty acids without significant vasodilation. These development studies have been complicated by the fact that nicotinic acid is a simple compound that is difficult to optimize by medicinal chemistry methods.

From 2005 to 2008, close to 40 patent publications on GPR109A agonists have appeared (197). GlaxoSmithKline has published a number of patent applications focusing on xanthine analogs (197). Schering Plough has published a paper looking at barbituric acid derivatives as GPR109A agonists (197). Merck has been working on anthranilic acid derivative as potent GPR109A agonist (190). Hoffmann-La Roche has taken the strategy to look at pyrido pyrimidinones as selective agonists of GPR109A (165). Most recently Incyte has also developed a series of tricyclic compounds derived from the corresponding xanthenes (197). A common feature of these advanced GPR109A agonists is an acidic moiety in the form of a carboxylic acid, tetrazole or N-H that is capable of interacting with the basic GPR109A Arg111 residue at transmembrane helix 3 of the receptor through salt bridge formation. This interaction is believed to be a key anchoring
element for the agonist to bind to receptor according to modeling studies (62; 216).

Several GPR109A agonists have progressed to clinical trials. MK-0354 and MK-1903, GPR109A agonists produced by Arena Pharmaceuticals and developed by Merck, made it through Phase 2 clinical trials that evaluated patients with dyslipidemia (119). Unfortunately, treatment with MK-0354 or MK-1903 produced little to no changes in HDL, LDL or cholesterol and development of these drugs was discontinued by Merck (25). Incyte recently announced the progression of INCB-19602 (structure undisclosed) into clinical development. In Phase 1 trials, INCB-19602 lowered FFAs in normal volunteers without rebound effects. This suggested the possibility that INCB-19602 might be a useful therapeutic agent in type 2 diabetes and Phase 2 trials were initiated to address this question. Unfortunately, the ability of INCB-19602 to lower fasting plasma glucose levels in type 2 diabetes was inconclusive and the clinical trial was terminated before completion. GlaxoSmithKline announced that the compound GSK-256073 has moved into Phase I clinical trials in late 2005. Though the structure was undisclosed, it is likely that this candidate belongs to either the anthranilide or xanthine series. Positive results from these studies led to Phase 2 clinical trials in 2009. These studies compared GSK-256073 (197) to Niaspan and were completed February 2010. The results have yet to be released. Additionally, SCH 900271, a potent GPR109A agonist, has been demonstrated to inhibit lipolysis and reduce serum triglycerides without adverse flushing effects
in vivo and progressed to clinical trials. While these clinical trials were completed in 2010, results have not yet been made public (160).

**GPR109B and GPR81**

GPR109A has recently been identified as a member of a subfamily of metabolite GPCRs which includes GPR81 and GPR109B (4). All three receptors are localized on the human chromosome 12q24 and all are activated by hydroxyl-carboxylic acid intermediates of energy metabolism. Further, all three receptors are predominantly expressed in adipose tissue (228) and mediate antilipolytic effects through coupling to G_\text{i}-type G proteins (80).

While GPR109A is the high-affinity receptor for niacin, GPR109B (HM74; found only in higher primates) is known as a low-affinity niacin receptor (228). GPR109A and GPR109B are highly homologous, differing only by 15 amino acids and a shortened C-terminal tail for GPR109A (228). One endogenous ligand for GPR109B is the $\beta$-oxidation intermediate 3-OH-octanoic acid (3). Much like $\beta$-hydroxybutyrate and GPR109A, 3-OH-octanoic acid reaches plasma concentrations sufficient to activate GPR109B in times of increased $\beta$-oxidation rates, like in diabetic ketoacidosis, under a ketogenic diet or starvation and appears to mediate a negative feedback regulation of adipocyte lipolysis to counteract prolipolytic influences (3).
GPR81 is coupled to \( \text{G} \) \text{-type G proteins} (80) and is activated by lactate (38; 125). GPR81 shares a 52% amino acid sequence identity in humans to GPR109A (200; 215; 228). Like \( \beta \)-hydroxybutyrate, infusion of lactate reduces lipolysis in vivo (28; 82; 97) as does treatment of adipocytes in vitro (22; 58). The effect of lactate to reduce lipolysis is mediated through activation of GPR81 (38; 125), although the receptor has recently been shown to not be involved in the regulation of lipolysis during intensive exercise when lactate concentrations are elevated (5).

It is important to note that with the recent deorphanization of these receptors, there has been a recommendation to the International Union of Basic and Clinical Pharmacology (IUPHAR) to rename this group of receptors (GPR81, GPR109A, GPR109B) as hydroxy-carboxylic acid (HCA) receptors, whereby GPR81 is HCA\(_1\), GPR109A is HCA\(_2\), and GPR109B is HCA\(_3\) (154). Studies have revealed that all three receptors bind hydroxy-carboxylic acids as their endogenous ligands, leading to the proposed nomenclature for this receptor family.

**ADIPONECTIN**

Although adipose tissue is a well-known storage depot for excess energy, its role as an endocrine organ was not recognized until the discoveries of leptin and adiponectin in the mid-1990s. Since then, hundreds of molecules have been identified as being secreted from the adipose tissue and many of them play a role
in regulation of metabolism. These adipocyte-derived factors are collectively referred to as “adipokines”. Some of the most well studied adipokines include adiponectin, leptin, resistin, retinol-binding protein 4 (RBP4), TNF-α, and MCP1. Each of these adipokines appears to play a role in whole body energy homeostasis, yet some roles are better defined than others.

Adiponectin was initially identified in 1995 (189). Of over 100 adipokines identified, adiponectin is the most abundantly secreted protein from adipose tissue, accounting for up to 0.05% of total plasma proteins (189). Adiponectin is constitutively expressed as a 30 kDa monomer and undergoes posttranslational modification to form higher order structures including: 1) homotrimers of 90 kDa 2) hexamers of 180 kDa [low-molecular weight (LMW)] and 3) 12-18mers of greater than 300 kDa [high-molecular weight (HMW)] (214). The expression and circulating concentrations of adiponectin are reduced in obesity (98; 236) and the reduction in adiponectin appears to precede the development of insulin resistance (96). The most compelling evidence for the role of adiponectin in the development of insulin resistance was made in mice with a targeted deletion of adiponectin. Adiponectin null mice had delayed clearance of free fatty acids in the plasma, low mRNA levels of fatty acid transport protein in skeletal muscle and elevated adipose tissue and circulating TNF-α concentrations (137). Despite having normal glucose tolerance on a chow diet, adiponectin null mice experienced more severe high-fat diet-induced obesity and glucose intolerance.
than wild-type mice, which was reversed with adenoviral-mediated expression of adiponectin (137).

Regulation of Adiponectin Gene Expression and Secretion

Adiponectin production and secretion is regulated transcriptionally and post-translationally. The key factors involved in adiponectin production and secretion are Sir2uin 1, the transcription factors peroxisome proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)), C/EBP\( \alpha \), and SREBP-1c, and the endoplasmic reticulum (ER) chaperones ERp44, Ero1-L\( \alpha \), and DsbA-L.

Sirtuin 1 (SIRT1), the mammalian ortholog of yeast Sir2, is a NAD\(^+\)-dependent protein deacetylase that acts as a master metabolic regulator of immunity and metabolism at the liver and adipose tissue (191). SIRT1 has recently been proposed to be at the center of a regulatory loop involving (PPARs) that controls the metabolic response of tissues, including adipose tissue, to nutrient and physiological signals (205). Within the adipocyte, SIRT1 binds and represses PPAR\( \gamma \) (Figure 3) (167). SIRT1 overexpression in 3T3-L1 adipocytes reduces PPAR\( \gamma \) gene and protein expression, while knockdown of SIRT1 increases PPAR\( \gamma \) expression (167). Further, suppression of SIRT1 stimulates secretion of HMW adiponectin from mature adipocytes (Figure 3) (175).

The nuclear receptor and transcription factor, PPAR\( \gamma \), when activated, increases adiponectin gene expression, production and secretion (Figure 3) (138). Others
have shown that *in vitro*, niacin increases PPAR\(\gamma\) mRNA expression (233; 243), induces nuclear expression of PPAR\(\gamma\) protein and enhances PPAR\(\gamma\) transcriptional activity (113). Thiazolidinediones (TZDs), potent PPAR\(\gamma\) agonists, have been demonstrated to improve insulin sensitivity and significantly increase circulating and adipose tissue adiponectin concentrations (138). The transcription factor C/EBP\(\alpha\) has also been shown to regulate genes involved in lipid and glucose metabolism in adipose tissue, including adiponectin (156). It has been demonstrated that chronic niacin administration increases C/EBP\(\alpha\) mRNA *in vivo* (124). The binding of SREBP-1c to the sterol regulatory element promotes transcription of enzymes involved in lipid metabolism (199). The adiponectin promoter is transactivated by SREBP-1c (127). Overexpression of SREBP-1c in 3T3-L1 adipocytes increases adiponectin gene and protein expression (127).

While studies have shown that PPAR\(\gamma\) agonists increase adiponectin by promoting adiponectin transcription (138), others have shown that PPAR\(\gamma\) agonists increase adiponectin through changes at the post-transcriptional or post-translational level, without changes to adiponectin gene expression (27; 178). Adiponectin undergoes a series of post-translational modifications in the ER of the adipocyte, including glycosylation, hydroxylation and oligomerization. Several ER chaperones are involved in the oligomerization of adiponectin to form trimeric, hexameric and HMW oligomeric complexes (*Figure 3*). Three key ER chaperones involved in the oligomerization and secretion of adiponectin are
Figure 3: Regulation of Adiponectin Gene Expression and Secretion (222). Note that the PPARγ agonists have been shown to increase adiponectin production at the transcriptional as well as the post-translational levels. In particular, activation of PPARγ induces expression of Ero1-Lα, which in turn binds to ERp44 as a preferred partner, leading to the release of HMW adiponectin trapped by thiol-mediated retention. Nutritional changes can modulate the biosynthesis of adiponectin through SIRT1. Figure from (222).
ERp44, Ero1-Lα and DsbA-L. While adiponectin is undergoing its extensive post-translational modifications, it is retained in the ER through disulfide bond formation (at Cys36 in humans and Cys39 in mice) with ERp44 (223). When ERp44 is overexpressed, adiponectin is retained inside the cell (223). This thiol-mediated retention may be imperative for adiponectin to undergo its post-translational modifications, including oligomerization into the most biologically active HMW form (222). On the other hand, Ero1-Lα, an ER chaperone known to preferentially bind to ERp44, may serve as a means for adiponectin to be released from the ER. When ERp44 is overexpressed, and is blocking the release of adiponectin, Ero1-Lα overexpression then results in increased secretion of adiponectin. Ero1-Lα overexpression increases adiponectin secretion from adipocytes (175), while transcriptional repression of ERp44 also increases adiponectin secretion (129). Additionally, co-immunoprecipitation studies demonstrated that overexpression of Ero1-Lα results in reduced ERp44-adiponectin associations and increased ERp44-Ero1-Lα associations and increased adiponectin secretion (223). DsbA-L is another ER chaperone highly expressed in the adipose tissue, and its expression is upregulated by treatment with TZDs and downregulated by obesity and treatment with inflammatory stimuli, like TNF-α (128). Overexpression of DsbA-L increases cellular and secreted levels of HMW adiponectin (128). These increases are dependent upon disulfide bond formation between DsbA-L and adiponectin (128).
Physiologic Effects of Adiponectin

Studies have demonstrated that the physiologic effects of adiponectin are produced primarily by binding to one of two adiponectin receptors, appropriately named AdipoR1 or AdipoR2. Adiponectin lowers blood glucose acutely through a reduction in hepatic glucose production (HGP) with little or no effect on glucose disposal (20; 54; 174). AdipoR1 increases AMPK activation, while AdipoR2 increases PPAR-α target genes by adiponectin in the liver (237). AdipoR1 plays more of a role with glucose production, while AdipoR2 is involved with glucose uptake (237). Reductions in circulating adiponectin impairs glucose tolerance and increases HGP providing further support for the role of adiponectin in normal hepatic metabolism (151). The glucose-lowering action of adiponectin in the liver has been attributed to AMPK activation, providing a mechanistic link to a signal transduction pathway already established as an antagonist of HGP and lipogenesis (218).

Adiponectin has also been shown to be actively engaged in the control of hepatic and serum TGs and has been shown to prevent alcoholic and non-alcoholic steatosis in the liver (234; 236). The TG-lowering effects of adiponectin appear to be mediated through activation of the AdipoR1 and AdipoR2 receptors which activate AMPK and PPARα, respectively, which are both well known to increase fatty acid oxidation (237). Targeted disruption of both receptors abolishes adiponectin binding, resulting in increased tissue TG content, inflammation and marked glucose intolerance (237).
In addition to peripheral effects, adiponectin has been shown to activate AMPK in the arcuate nucleus of the hypothalamus via activation of the AdipoR1 receptor (116). Receptor activation elicits an increase in food intake and decrease in energy expenditure. Other studies have demonstrated that intracerebroventricular infusion of adiponectin produced no effect on food intake but increased energy expenditure (174).

Adiponectin directly improves endothelial dysfunction by stimulating production of nitric oxide (61), inhibiting proliferation of vascular smooth muscle cells (12) and inhibiting the conversion of macrophages to foam cells (158). Additionally, adiponectin treatment inhibits TNF-α-induced monocyte adhesion to endothelial cells and expression of adhesion molecules in endothelial cells (157). Adiponectin also directly inhibits pro-inflammatory signaling in macrophages (72).

**Regulation of Adiponectin in Disease States**

Circulating and tissue expression of adiponectin are altered in many disease states. Although adipose tissue is the source of circulating adiponectin, serum adiponectin concentrations are inversely related to body fat mass (13). Adipose tissue adiponectin gene expression and circulating adiponectin concentrations are reduced in obesity (13; 138) and increase with weight loss (95). In addition, adiponectin levels are reduced with increased total and LDL-C (235), nonalcoholic hepatic steatosis (210), insulin resistance and type 2 diabetes (95), conditions often associated with obesity. Further, adiponectin concentrations are
reduced in coronary artery disease and atherosclerosis (69; 157), hypertension (103), and states of inflammation (201). On the other hand, circulating adiponectin concentrations are positively associated with HDL-C (141), type 1 diabetes (185; 201), and anorexia (161). Interestingly, serum adiponectin concentrations are elevated in states of impaired kidney function (201). In addition, circulating adiponectin concentrations are typically higher in females than males (235) and are positively associated with age (53).

**RETINOL-BINDING PROTEIN 4**

Retinol-binding protein 4 (RBP4) is a 21 kDa protein secreted primarily by liver and adipose tissue, which serves as the principal transporter of retinol (vitamin A). The RBP4-retinol complex interacts with transthyretin, a protein that assists in the transport of thyroxin and retinol in the blood (241). Yang et al. first linked RBP4 with insulin resistance in 2005 (238). Until then, the only known function of RBP4 was to deliver retinol to tissues (177). Since the discovery of RBP4 as a novel adipo-/hepatokine involved in the pathogenesis of insulin resistance, over 450 papers have been published with “RBP4” in the title or abstract.

*Regulation of RBP4 Gene Expression and Secretion*

The RBP4-retinol complex interacts with transthyretin, which prevents its loss by filtration through the renal glomeruli. It has been demonstrated that increased transthyretin or alterations in RBP4-transthyretin binding may contribute to insulin resistance by increasing circulating RBP4 concentrations (146). In fact,
compounds that interfere with RBP4 binding to transthyretin, such as the synthetic retinoid fenretinide, profoundly reduce circulating RBP4 concentrations, resulting in improved insulin sensitivity (21; 238).

As a transporter of retinol, secretion of RBP4 is highly affected by retinol status. A deficiency of retinol blocks secretion of RBP4 and results in defective delivery and supply of retinol to the epidermal cells. Further, iron deficiency lowers serum retinol and iron-deficient rodents exhibit reduced circulating RBP (180). RBP4 release from adipose tissue is enhanced in response to iron supplementation, possibly through increased RBP4 transcription (71).

An important modulator of RBP4 gene expression in vivo is the high mobility group A1 gene (HMGA1) (48). HMGA1 is a protein that binds to the adenine-thymine rich regions of DNA and transactivates promoters, supporting gene activation (35). HMGA1<sup>−/−</sup> mice have severely decreased insulin receptor expression and insulin signaling and exhibit a type 2 diabetic-like phenotype (73). HMGA1 is necessary for basal and cAMP-induced RBP4 gene and protein expression (48).

**Physiologic Effects of RBP4**

Elevated RBP4 concentrations contribute significantly to the insulin resistance observed in obesity and type 2 diabetes in both rodents and humans (86; 238). A role for RBP4 in obesity and insulin resistance was initially suggested from
DNA arrays in adipose-specific GLUT4<sup>−/−</sup> mice, which developed insulin resistance in skeletal muscle and liver (238). Since then, a number of studies have demonstrated that circulating RBP4 concentrations are increased in several mouse models of obesity and insulin resistance and in obese humans with MetS (51; 123; 173; 238). Mice with a heterozygous or homozygous deletion of RBP4 have improved insulin sensitivity while transgenic overexpression of human RBP4 or injection of recombinant RBP4 causes insulin resistance (238).

In 2005, the original paper describing the role of RBP4 in insulin resistance clearly demonstrated a causal positive association between serum and adipose tissue concentrations of RBP4 and insulin resistance (238). Since then, others have indicated a positive correlation between serum RBP4 concentrations and insulin resistance or obesity (50; 86; 111). However, recently, the association between circulating and tissue concentrations of RBP4 with obesity and/or insulin resistance has been challenged. Some studies have indicated that serum RBP4 is not increased in obesity or insulin resistance (30; 83; 240). Further, the association between RBP4 gene expression in adipose tissue and liver with insulin resistance is also controversial. It was initially reported that RBP4 mRNA was increased in adipose tissue of insulin resistant mice and obese humans (111; 238), indicating a positive association of adipose tissue RBP4 gene expression and insulin resistance. However, others have reported reduced adipose tissue RBP4 gene expression in obesity (29; 105). Although the liver is
the major source of circulating RBP4, hepatic RBP4 gene expression appears to be unaltered in differing metabolic states (238).

A recently identified functional polymorphism of the human RBP4 promoter causes increased RBP4 expression in adipocytes and is associated with increased plasma RBP4 concentrations and risk for type 2 diabetes (115; 149). Further studies suggest that elevated circulating RBP4 concentrations are involved in the development of dyslipidemia and cardiovascular disease (50; 86; 111). Lowering of serum RBP4 concentrations by treatment with fenretinide results in normalization of serum RBP4 levels and improvements in insulin resistance in obese mice (238). In humans, RBP4 appears to be a good biomarker for insulin resistance and the metabolic syndrome, but the regulation of RBP4 is unclear.

**Regulation of RBP4 in Disease States**

As mentioned above, circulating and tissue RBP4 concentrations may or may not be altered in conditions of obesity, insulin resistance or type 2 diabetes. However regulation of RBP4 in other disease states has been reported. In chronic liver diseases, circulating RBP4 concentrations are inversely correlated with the degree of hepatocellular injury and reflect the efficacy of antiviral treatment for viral hepatitis (10; 102). Additionally, serum RBP4 concentrations are increased in humans with polycystic ovary syndrome (144), alopecia areata (6), dilated inflammatory cardiomyopathy (26), cerebral infarction (187), and
chronic kidney disease (52). In addition, epicardial adipose tissue expression of RBP4 is increased in coronary artery disease (184). On the other hand, serum RBP4 levels are significantly lower in patients with psoriasis (81), ovarian cancer (131), preeclampsia (195), cholesterol gallstone disease (221), and diffuse cutaneous systemic sclerosis (213) when compared to controls.

REGULATION OF ADIPONECTIN AND RBP4 BY LIPID-ALTERING DRUGS
Pharmacological agents used to treat primary and combined hyperlipidemia reduce cardiovascular disease morbidity and mortality. Risk reduction has been attributed to improvements in blood lipid and lipoprotein characteristics. However, each class of available lipid-lowering drugs (statins, fibrates, omega-3 fatty acids and niacin) has been shown to exhibit pleiotropic effects that broaden their anticipated actions. Indeed, the results of a growing number of available studies demonstrate an increase in circulating concentrations of adiponectin in response to lipid-altering drugs. Each lipid-lowering drug consistently increases circulating adiponectin concentrations, with the greatest effects produced by niacin. The results of the available studies suggest that a strong relationship exists between pharmacological alterations in blood lipids and adiponectin that is not obvious for other adipokines.

The mechanism(s) by which these lipid-altering drugs increase adiponectin is largely unknown. It has been reported that statins activate PPARγ via extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase activation (239). PPARγ activation using TZDs produces well-known
increases in adiponectin gene expression and secretion in vitro as well as serum adiponectin concentrations in vivo, suggesting that statins may increase adiponectin expression in a similar fashion. In light of recent studies showing that TZDs acutely increase the release of adiponectin in rodents, it will be interesting to see if statins perform similarly and to determine if these effects are modulated in humans to a similar extent. Fibrates bind to the nuclear receptor PPAR\(\alpha\), but studies suggest that fibrates may increase adiponectin directly through increased adiponectin production, independent of PPAR\(\alpha\) activation (148; 150).

While many studies have demonstrated the ability of omega-3 fatty acids to increase plasma adiponectin concentrations, the mechanism by which this occurs remains unclear. Studies have suggested that the increase in adiponectin following omega-3 fatty acid administration appears to be due to PPAR\(\gamma\) activation (152). Another mechanism explored to explain the ability of omega-3 fatty acids to increase serum adiponectin concentrations involves the inflammatory macrophages often found in adipose tissue of obese subjects. Adiponectin mRNA expression and secretion are reduced when adipocytes are cocultured with macrophages (203). Itoh et al. discovered that treatment with the omega-3 fatty acid eicosapentaenoic acid (EPA) reversed the coculture-induced decrease in adiponectin secretion without altering adiponectin mRNA expression (100). TNF-\(\alpha\) treatment of adipocytes reduces adiponectin secretion and the authors found that EPA treatment suppressed the coculture-induced increase in
TNF-α expression, suggesting a possible mechanism by which EPA increases serum adiponectin concentrations.

While most lipid-altering drugs (statins, fibrates, niacin and omega-3 fatty acids) generally increase adiponectin, very little is known regarding the impact of these drugs on RBP4 concentrations. One study examined the effect of simvastatin (40 mg/d for 3 months) on plasma RBP4 concentrations in nondiabetic patients with metabolic syndrome at increased risk for cardiovascular complications (166). In this study, circulating RBP4 levels were unchanged in response to simvastatin treatment (166). On the other hand, rosvastatin (2.5 mg/d for 12 weeks) decreased serum RBP4 in a subgroup of patients with type 2 diabetes, hyperlipidemia and poor glycemic control (208). However, these data are complicated by the fact that there was no control group and rosvastatin treatment had no effect on the group of patients with type 2 diabetes and hyperlipidemia as a whole.

The PPARα agonist fenofibrate is one of the principal lipid-lowering agents used for the treatment of hypertriglyceridemia (74). Fenofibrate reduces the mRNA expression levels of RPB4 in 3T3-L1 adipocytes (231). Further, fenofibrate treatment reduces RBP4 mRNA levels in adipose tissue, but not in the liver of obese rats, which correlates with decreased serum RBP4 concentrations and increased insulin sensitivity (231). In addition, in men with insulin resistance and dyslipidemia, eight weeks of fenofibrate treatment resulted in a 30% reduction in
serum RBP4 concentrations, which correlated with reduced body weight and increased insulin sensitivity (231). Taken together, these results suggest that fenofibrate may improve insulin sensitivity through a reduction in serum RBP4 concentrations.

Niacin’s primary effects are to lower serum triglycerides and raise HDL-C. In fact, niacin is the most effective lipid-lowering agent to increase HDL-C (219) and is the only agent known to reduce the pro-atherogenic lipoprotein, Lp(a). In agreement with the significant improvement in tissue and circulating lipid metabolism produced by niacin, our group and others have shown that niacin produces remarkable changes in circulating adiponectin (124; 170; 171; 225-227) (Table 1).

In our initial studies, we demonstrated that treating primary rat adipocytes and 3T3-L1 adipocytes stably expressing the niacin receptor with niacin resulted in a significant increase in adiponectin secretion (171). Additional studies showed that a single dose of niacin acutely elevates serum adiponectin concentrations in rats (171). Westphal and colleagues reported that serum total adiponectin concentrations increased by 54% in humans after four weeks of extended-release niacin (1000 mg/day) and by 94% after six weeks (1500 mg/day) (225). Additional studies reported that six weeks of extended-release niacin (1500 mg/day) increased serum total adiponectin concentrations in men with metabolic syndrome (170; 226). Niacin treatment significantly increases serum low and medium-molecular weight adiponectin, but produces a dramatic (63% - 88%)
Table 1. **Effects of Niacin on Adiponectin.**  

<table>
<thead>
<tr>
<th>Ref*</th>
<th>Subjects</th>
<th>Treatment</th>
<th>Effects on Adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>171</td>
<td>Male Sprague-Dawley rats</td>
<td>Single dose IR(^a) niacin (30 mg/kg)</td>
<td>Adiponectin increased by 37% at 10 min, peaked at 1 hr and remained elevated for 24 hr.</td>
</tr>
<tr>
<td>170</td>
<td>Men with MetS(^b)</td>
<td>6 weeks of ER(^c) niacin (1500 mg/day)</td>
<td>Adiponectin increased by 46% from 5.7±0.5 to 8.4±0.7 µg/mL.</td>
</tr>
<tr>
<td>226</td>
<td>Men with MetS</td>
<td>6 weeks of ER niacin (1500 mg/day)</td>
<td>Adiponectin increased by 56% from 6.1±2.3 to 10.1±4.0 µg/mL.</td>
</tr>
<tr>
<td>227</td>
<td>Men with MetS</td>
<td>6 weeks of ER niacin (1500 mg/day)</td>
<td>LMW(^d) and MMW(^e) adiponectin increased by 35% and 33%, respectively, but HMW(^f) adiponectin increased by 88%.</td>
</tr>
<tr>
<td>225</td>
<td>Humans</td>
<td>6 weeks of ER niacin (1500 mg/day)</td>
<td>Adiponectin increased by 94% from 4.83±2.39 to 9.35±6.06 µg/mL.</td>
</tr>
<tr>
<td>225</td>
<td>Humans</td>
<td>4 weeks of ER niacin (1000 mg/day)</td>
<td>Adiponectin increased by 54% from 4.83±2.39 to 7.45±5.71 µg/mL.</td>
</tr>
<tr>
<td>124</td>
<td>Humans with IGT(^g) and low HDL-C</td>
<td>6 months of extended release niacin (1000 mg/day)</td>
<td>Serum adiponectin increased from 3.7±0.2 to 5.1±0.4 µg/mL. Adiponectin mRNA increased 4 fold in abdominal subcutaneous adipose tissue.</td>
</tr>
</tbody>
</table>

\(^{a}\)Immediate-release  \(^{b}\)Metabolic Syndrome, \(^{c}\)Extended-release  \(^{d}\)Low molecular weight, \(^{e}\)Middle molecular weight, \(^{f}\)High molecular weight, \(^{g}\)Impaired glucose tolerance, *Reference
increase in the most biologically active HMW form of adiponectin in men with metabolic syndrome (170; 227).

Interestingly, beneficial effects of niacin on adipocyte biology have also been demonstrated (124). For example, adipocytes isolated from humans treated with niacin had reduced mean and maximal diameter and volume compared to control subjects. Further, insulin sensitivity was also improved in these adipocytes. These findings support the idea of pleiotropic effects of niacin to improve metabolic parameters in its target tissues, namely the adipocyte.

The mechanism by which niacin increases adiponectin remains unclear, but results from our laboratory show that niacin has direct effects on adipose tissue secretion of adiponectin that appear to be dependent on the GPR109A receptor. Results from our laboratory using G-protein uncoupling agents and GPR109A loss-of-function studies in 3T3-L1 adipocytes show that the increase in adiponectin is dependent on receptor activation (171). Although the exact mechanisms are poorly understood, it is possible that niacin increases adiponectin secretion by reducing endoplasmic retention time in a manner reminiscent to that produced by PPARγ agonists (223). Although niacin can acutely (within 10 minutes) increase circulating adiponectin in rodents (171), long-term niacin treatment in patients with impaired glucose tolerance increased adiponectin mRNA expression by four-fold in adipose tissue and was associated with an increased circulating adiponectin (124). In support of these findings,
direct treatment of 3T3-L1 adipocytes with niacin also increases adiponectin gene expression (65). Additionally, the transcription factors PPARγ and C/EBPα, which positively regulate adiponectin transcription, are also up-regulated in response to niacin treatment (124). Taken together, the data suggest that niacin produces short-term effects that increase the vesicular secretion of adiponectin into the circulation along with long-term effects that appear to modulate adiponectin gene expression. To date, no studies have been published examining the effects of niacin on RBP4.

The studies examining the effect of chronic niacin administration on serum adiponectin were conducted in humans with overweight or obesity and/or metabolic syndrome (124; 170; 226) or patients with HIV and antiretroviral therapy-related dyslipidemia (16), suggesting that niacin's pleiotropic effects are evident in subjects with metabolic dysfunction due to general improvements in the metabolic milieu.

Of the studies that demonstrated that chronic niacin administration increases serum adiponectin, only one tried to determine possible mechanisms by which this occurs (124). Linke et al. showed that six months of extended-release niacin increased serum adiponectin concentrations by 35%, and this was accompanied by a 4-fold increase in adiponectin mRNA expression in subcutaneous adipose tissue of humans with impaired glucose tolerance (124). Further, PPARγ and
C/EBPα mRNA expression were also increased 1.6- and 1.5-fold, respectively in the niacin-treated group.

REGULATION OF ADIPONECTIN AND RBP4 BY ADIPOSE TISSUE INFLAMMATION

Obesity is a disorder of surplus adipose tissue. Excess triglyceride being stored within the adipocyte results in adipocyte hypertrophy and a dysregulation of adipokine secretion, including reduced production of anti-inflammatory adipokines like adiponectin (13), and increased production of pro-inflammatory chemokines and cytokines including MCP-1 (186) and TNF-α (93). Obesity is also characterized by a marked infiltration of M1 (pro-inflammatory) macrophages into the adipose tissue (75; 224), which also produce inflammatory cytokines that propagate adipose tissue inflammation.

Adipose tissue of obese humans and rodents contains a higher number of macrophages than what is found in adipose tissue of lean subjects (224). These adipose tissue macrophages (ATM) participate in inflammatory pathways that are activated in the fat of obese individuals (224). Since the initial discovery of increased adipose tissue infiltration by macrophages during obesity, two distinct populations of ATM have been identified: M1 (“classically activated”; pro-inflammatory macrophages) which have been recruited to the adipose tissue as a result of obesity and M2 (“alternatively activated”; resident or anti-inflammatory macrophages) that reside in the adipose tissue of lean individuals. Macrophages
polarize to either the M1 or M2 phenotype depending on the surrounding tissue microenvironment and the subset of circulating monocyte precursors from which they are derived (57). Obesity induces a phenotypic switch in adipose tissue macrophage population from an anti-inflammatory M2 state to a pro-inflammatory M1 state (135). M1 macrophages present in the adipose tissue of obese individuals are F4/80(+)CD11c(+) and highly express genes encoding TNF-α and iNOS, while M2 macrophages present in adipose tissue of lean subjects are typically CD11c(-) and highly express Ym1, arginase-1 and IL-10, genes generally associated with anti-inflammatory macrophages (135).

The classification of ATM into two distinct populations is a simplification of the macrophage population present in the adipose tissue during obesity. Macrophages are most likely activated along a continuum between these two polarization states, with M1 and M2 representing the extremes of the continuum. In fact, ATM are also known as M1, M2a, M2b and M2c, with each subset expressing particular cytokines, chemokines and surface receptors. The heterogeneity among ATM is likely to reflect the plasticity of these cells in response to exposure to the adipose tissue microenvironment (139). M1 macrophages are typically activated by interferon γ (IFNγ) and lipopolysaccharide (LPS), M2a by IL-4 or IL-13, M2b by immune complexes in combination with IL-1β or LPS, and M2c by IL-10, transforming growth factor-β (TGF-β) or glucocorticoids (140).
Zeyda et al. reported in 2007 that human ATM are of an anti-inflammatory phenotype, but are capable of excessive production of pro-inflammatory mediators (242). Specifically, ATM produce the anti-inflammatory cytokines IL-10 and IL-1Ra, but also produced large amounts of TNF-α, IL-1β, MCP-1, IL-6 and MIP-1α, cytokines that are generally produced in low amounts from M2 macrophages (242). Further supporting this notion of M2 macrophages with M1-like cytokine production, Shaul et al. identified CD11c(-) ATM that express elevated levels of hallmark M1 transcripts, coincident with reduced expression of some M2 transcripts, including CD163, and increased expression of M2 genes with anti-inflammatory functions such as IL-10 in response to 8 weeks of HFD. They also describe CD11c(+) ATM that after 8 weeks of HFD expressed increased transcripts typically associated with an M2 phenotype, including arginase-1 (196).

The mechanisms underlying HFD-induced reduction of adiponectin production include altered hormonal milieu (60; 89; 142), oxidative stress (76), and inflammation. One of the major mechanisms by which HFD-induced adipose tissue inflammation decreases adipose tissue and serum concentrations of adiponectin is through the up-regulation of inflammatory cytokines, including TNF-α and IL-6. TNF-α has inhibitory effects on adiponectin expression through suppressing its promoter activity (138), and IL-6 has also been shown to inhibit adiponectin expression in adipocytes (33). Serum RBP4 concentrations are positively correlated with not only obesity, but also inflammation (15).
Interestingly, elevated circulating RBP4 concentrations are positively associated with systemic inflammation, despite maintaining low RBP4 expression in the adipose tissue (17).

CONCLUSIONS AND OBJECTIVES

With the increased occurrence of obesity comes a greater incidence of cardiovascular and metabolic diseases, often accompanied by hyperlipidemia. The use of niacin is critical in the treatment of hyperlipidemia, but it may also exhibit pleiotropic effects that could benefit health. Niacin increases circulating adiponectin concentrations, primarily the biologically active HMW multimers, both acutely and chronically, yet the mechanism by which this occurs remains to be elucidated. Additionally, niacin may exhibit anti-inflammatory properties in the adipose tissue directly, or through increased adiponectin production. Further, circulating RBP4 concentrations are altered in many disease states and appear to be causally associated with insulin resistance, yet no studies have examined the effects of niacin on RBP4. Lastly, we aimed to determine whether high fat diet or niacin treatment alters expression of GPR81 and GPR109A, receptors that are crucial to the metabolic sensing ability of the adipocyte. The purpose of these studies is to determine the pleiotropic effects of niacin on adipokine production, adipose tissue inflammation and receptor expression.
CHAPTER II
NOVEL ANTI-INFLAMMATORY EFFECTS OF NIACIN
IN THE ADIPOSE TISSUE

ABSTRACT

Introduction. Obesity is associated with a chronic low-grade inflammation of the adipose tissue that has been linked to obesity-related comorbidities, such as insulin resistance. It has been demonstrated that niacin exerts anti-inflammatory effects in vascular endothelial cells, monocytes, 3T3-L1 adipocytes, retinal pigment epithelial cells the lung and kidney. However, to date there have been no studies examining anti-inflammatory properties of niacin in the adipose tissue.

Objective. Therefore, our objective was to determine the effects of niacin on high-fat diet (HFD)-induced adipose tissue inflammation and to characterize the role of the niacin receptor (GPR109A) and post-receptor intermediates involved.

Methods. Male C57BL/6 mice were placed on a control diet or HFD and were maintained on such diets for the duration of the study. After 6 weeks on the control or high-fat diets, vehicle or niacin treatments were begun. Half of the mice from each group received vehicle (water) and the other half received niacin (200 mg/kg/day in drinking water) for 4 weeks. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. Identical studies were conducted concurrently in GPR109A⁻/⁻ mice.

Results. Niacin treatment
attenuated HFD-induced increases in adipose tissue gene expression of monocyte chemoattractant protein-1 (MCP-1) and IL-1β in the wild-type obese mice, indicating anti-inflammatory effects of niacin in the adipose tissue. Niacin had no effect on total adipose tissue macrophage content, as evidenced by unchanged CD68 expression, but reduced the expression of the pro-inflammatory M1 macrophage marker CD11c in obese wild-type mice. Niacin either had no effect on or reduced adipose tissue expression of markers of M2, or anti-inflammatory macrophages. Niacin increased serum concentrations of the anti-inflammatory adipokine, adiponectin by 21% in obese wild-type mice, but had no effect on lean wild-type or lean or obese GPR109A−/− mice. Niacin increased adiponectin gene and protein expression in the wild-type mice on the high-fat diet. This effect was lost in the GPR109A null mice. The increases in adiponectin serum concentrations, gene and protein expression occurred independently of changes in expression of PPARγ, C/EBPα, or SREBP-1c (key transcription factors known to positively regulate adiponectin gene transcription) in the adipose tissue. Further, niacin had no effect on adipose tissue expression of ERp44, Ero1-Lα, or DsbA-L (key ER chaperones involved in adiponectin production and secretion). Conclusions. In summary, niacin treatment attenuates obesity-induced adipose tissue inflammation through reduced pro-inflammatory cytokine expression, increased anti-inflammatory cytokine expression, and reduced M1 macrophage content in the adipose tissue in a niacin receptor-dependent manner.
INTRODUCTION

The incidence of obesity in the U.S. has reached epidemic proportions within the last 25 years. Obesity is associated with an increased risk of metabolic and cardiovascular disease (CVD), with CVD one of the leading causes of morbidity and mortality in the U.S. (179). Exaggerated adipose tissue lipolysis and increased serum non-esterified fatty acids (NEFAs) are characteristic features of obesity that often lead to the development of atherogenic dyslipidemia. Atherogenic dyslipidemia is a cluster of metabolic abnormalities characterized by moderately elevated LDL-C (130-159 mg/dL) and TGs (>150 mg/dL), small LDL particles and low HDL-C (<35 mg/dL) (87). Niacin is one of the most effective pharmacological interventions for the treatment of atherogenic dyslipidemia and has been shown to reduce CVD morbidity and mortality presumably by improving blood lipid and lipoprotein characteristics. Along with producing modest reductions in circulating very low-density lipoprotein (VLDL), LDL-C, TG and lipoprotein(a) concentrations, niacin is the most effective pharmacological tool for increasing circulating HDL-C concentrations.

Although niacin’s most well recognized action is to improve blood lipid and lipoprotein characteristics, it is becoming increasingly clear that niacin possesses pleiotropic benefits in addition to its known therapeutic effects. Evidence from our laboratory and others has demonstrated that niacin dramatically increases serum concentrations of the adipokine, adiponectin, in obese men with the metabolic syndrome (170; 226). Additionally, a single dose of niacin given orally or through
intraperitoneal injection acutely increases serum adiponectin concentrations in rats and mice within minutes, and this effect is dependent upon activation of the niacin receptor (171). Others have demonstrated that niacin treatment results in increased adiponectin mRNA (65; 124). Some of the health-related benefits of niacin may be attributed to increased serum adiponectin concentrations. Adiponectin is one of the most promising biomarkers of the metabolic syndrome and CVD and adiponectin possesses insulin-sensitizing, anti-atherosclerotic and anti-inflammatory properties.

More recently, studies have demonstrated that niacin has anti-inflammatory effects in a number of tissues. Recent studies have demonstrated anti-inflammatory effects of niacin in the kidney (49), lung (118), 3T3-L1 adipocytes (65), monocytes (64), retinal pigment epithelial cells (77), and vascular endothelial cells (229; 230). Niacin administration in rats with chronic kidney disease (CKD) reduces CKD-induced elevations in renal MCP-1 protein expression and NF-κB activation (49). Niacin treatment suppresses NF-κB activation and gene expression of the proinflammatory cytokines TNF-α and IL-6 in lung tissues of rats with endotoxemia (118). Additionally, in cultured 3T3-L1 adipocytes, treatment with niacin reduces TNF-α-induced increases in the gene expression and secretion of the proinflammatory chemokines MCP-1, fractalkine and RANTES, as well as increases adiponectin mRNA (65). In isolated human monocytes activated by LPS, niacin treatment reduced the secretion of proinflammatory cytokines and chemokine TNF-α, IL-6 and MCP-1, mediated
through suppression of the NF-κB signaling pathway (64). Recently, it was reported that niacin suppressed TNF-α-induced increases in NF-κB activation, IL-6 and MCP-1 expression and secretion from retinal pigment epithelial cells. Gangi et al. demonstrated that niacin inhibits vascular inflammation by decreasing endothelial reactive oxygen species production and inflammatory cytokine production (78). Systemically, niacin reduces high-sensitivity C-reactive protein and TNF-α, systemic markers of inflammation (118; 120). Niacin also inhibits monocyte chemotaxis (65). Many of these anti-inflammatory properties of niacin have been linked to activation of the niacin receptor (64; 77).

Obesity is associated with a chronic low-grade inflammation characterized by increased adipose tissue expression of pro-inflammatory chemokines and cytokines like MCP-1 (186) and TNF-α (93), as well as infiltration of M1 (pro-inflammatory) macrophages (75; 224). The objective of the current investigation was to determine the effects of niacin administration on obesity-induced adipose tissue inflammation through alteration in cytokine profile and macrophage infiltration of the fat, with a special emphasis on the role of adiponectin.

MATERIALS AND METHODS

Materials

Niacin (nicotinic acid) was purchased from Sigma-Aldrich (St. Louis, MO). Rabbit polyclonal adiponectin antibody was from Abcam, Inc. (Cambridge, MA). Mouse monoclonal actin antibody was from Millipore (Temecula, CA).
Animal Studies

Thirty-two male C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were housed in groups of four in filter-top cages and were acclimated for three days. After three days of acclimatization, mice were either placed on a high fat diet (HFD; 60% kcal as fat; n=16) or a control diet (10% kcal as fat; n=16) obtained from Research Diets (New Brunswick, NJ) and were maintained on such diets for the duration of the study.

After six weeks on the control or high-fat diets, vehicle or niacin treatments were begun. Half of the mice from the control and high-fat diet groups received vehicle (water) and the other half received niacin (200 mg/kg/day) dissolved in drinking water for four weeks. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. After five weeks of vehicle or niacin treatments, mice were fasted overnight and sacrificed. Whole blood was collected and processed for serum and tissues were flash frozen in liquid nitrogen and stored at -80°C until analysis.

The same studies were conducted concurrently in global GPR109A−/− mice. Thirteen GPR109A−/− mice were placed on the control diet (7 received vehicle; 6 received niacin), and nine GPR109A−/− mice were placed on the high-fat diet (4 received vehicle; 5 received niacin). Initial GPR109A−/− mice breeding pairs were a generous gift from Dr. Stefan Offermanns (Bad Nauheim, Germany). All
animal studies were approved by the Auburn University Institutional Animal Care and Use Committee prior to initiation.

Liver Lipid Analysis

Frozen liver samples (~100 mg) were homogenized with a hand-held homogenizer and sonicated for 30 sec in 1.0 mL SET buffer (sucrose 250 mM, EDTA 2 mM and Tris-HCl 10 mM, pH 7.4). Complete cell destruction was achieved by one freeze-thaw cycle and two 15 sec cycles of sonication. Liver TG were analyzed using a TG assay kit from Cayman Chemicals (Ann Arbor, MI). Protein content was measured using the Lowry Protein Assay method (Bio-Rad; Hercules, CA), and expressed as µMol/mg protein.

Serum Analysis

Serum total adiponectin and insulin concentrations were measured using ELISA kits from Millipore (Temecula, CA). Serum high molecular weight (HMW) adiponectin concentrations were measured using an ELISA kit from Alpco Diagnostics (Salem, NH). Glucose and triglyceride concentrations were measured using kits from Cayman Chemicals (Ann Arbor, MI). Serum NEFAs were measured using a kit from Wako Chemicals (Richmond, VA).

Real-time PCR

RNA was isolated from epididymal white adipose tissue (EWAT) using Qiagen RNeasy Lipid Tissue Mini Kit (Valencia, CA). RNA (0.5 or 1 µg) was reverse
transcribed into cDNA using iScript cDNA Synthesis Kit from Bio-Rad. PCR primers used in the real-time-PCR analysis are listed in Table 2.

Analyses were performed on a Bio-Rad iCycler iQ thermocycler. Samples were analyzed in 30 μl reactions using SYBR Green PCR Master Mix (Bio-Rad). All expression levels were normalized to the corresponding 36B4 mRNA levels.

Immunoblot Analysis

EWAT pads (~100 mg) were homogenized with a handheld homogenizer in RIPA buffer (NP40, sodium deoxycholate, SDS, NaCl, Tris pH 6.8, EDTA, protease and phosphatase inhibitors) and the protein fractions of the epididymal fat and were isolated. A DC protein assay (Bio-Rad) was then conducted on the samples to determine the protein concentration for each sample. Proteins were then separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Membranes were blocked in LI-COR Odyssey blocking buffer (Lincoln, NE) for 1 h and incubated overnight with primary antibodies (1:1,000). Membranes were then washed three times with PBS-0.1% Tween-20 and incubated with infrared-conjugated secondary antibodies (1:10,000) for 1 h. Following three more washes, blots were scanned with a LI-COR Odyssey infrared scanner.

Statistical Analysis

Data were analyzed using a T-test or one-way ANOVA. When differences were
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer 5' - 3'</th>
<th>Reverse Primer 5' - 3'</th>
</tr>
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<td>ATGTGGTAAGAGAAGTAGTAGAGTC</td>
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<td>SIRT1</td>
<td>TGGCAGTAACAGTGACAGTG</td>
<td>CCAGATCCTCCAGACACATTC</td>
</tr>
<tr>
<td>PPARγ</td>
<td>TTATGGAGCCTAAGTGGAGTGGAGTGGTGGAGTGGTGGAGTC</td>
<td>AGCAGGGTTGCTTTGGATGTC</td>
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<tr>
<td>C/EBPα</td>
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<tr>
<td>CD11c</td>
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<td>GAGCGATGTCCTGCTTTAG</td>
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<tr>
<td>IL-1β</td>
<td>GCAGCGACACATCAACAAG</td>
<td>GTTCATCTCGGAGCCTGTAG</td>
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<td>TNF-α</td>
<td>CGTGGGAACTGGCAGAGAGG</td>
<td>GTAGACAGAAGAAGCGTTG</td>
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<tr>
<td>IL-6</td>
<td>AGCCAGAGTCCTTCAGAGAG</td>
<td>GATGGTCTTTGGTCCTTCAGCC</td>
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<td>CD163</td>
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<td>Arginase-1</td>
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<td>MRC-1</td>
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<td>YM-1</td>
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<td>MGL-2</td>
<td>TCTGGGACAGCACACTGAGAG</td>
<td>TCGAGGAGCTGCTGCTTCAG</td>
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<tr>
<td>36B4</td>
<td>CACTGCTGAACATGTGGAAC</td>
<td>CCACAGACAATGCCAGGAC</td>
</tr>
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</table>

Table 2. Primers used for real-time RT-PCR. **SIRT1** Sirtuin 1, **PPARγ** Peroxisome proliferator-activated receptor γ, **C/EBPα** CCAAT/enhancer-binding protein α, **SREBP-1c** Sterol regulatory element-binding protein-1c, **ERp44** Endoplasmic reticulum protein 44, **Ero1-Lα** Endoplasmic oxidoreductin-1-like protein, **DsbA-L** Disulfide-bond A oxidoreductase-like protein, **MCP-1** monocyte chemoattractant protein-1, **IL-1β** Interleukin-1β, **TNF-α** Tumor necrosis factor-α, **IL-6** Interleukin-6, **MRC-1** Macrophage mannose receptor C type 1, **MGL-2** Macrophage galactose-type C-type lectin 2.
observed, a Bonferroni post hoc test was conducted to determine where differences occurred. Data were analyzed using GraphPad Prism software version 4.0 (GraphPad Software, La Jolla, CA). Significance was accepted at the $P < 0.05$ level.

RESULTS

*Effects of diet and niacin on metabolic parameters in mice*

HFD caused significant increases in body weight, epididymal fat pad weight, serum glucose and insulin concentrations in both wild-type and GPR109A$^{-/-}$ mice. Niacin had no effect on epididymal fat pad weight, serum glucose or insulin concentrations (Table 3). Interestingly, niacin tended to increase serum glucose concentrations in lean wild-type and GPR109A$^{-/-}$ mice, although this did not reach statistical significance. Niacin is known to suppress the release of free fatty acids from the adipocyte, resulting in a transient reduction in circulating NEFAs (41; 43). In the current investigation, niacin reduced serum NEFA concentrations in the wild-type lean mice, but not any other group of mice (Table 3).

*Niacin reduces hepatic lipid content in obese mice in a receptor-dependent manner*

Fatty liver disease is the most common hepatic irregularity observed in clinical practice. Non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of liver fat $>$5% per liver weight in the presence of $<$10 g of daily alcohol consumption and is associated with central obesity and insulin resistance (37).
<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>GPR109A&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Diet Vehicle</td>
<td>Niacin</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>32.1 ± 1.1</td>
<td>33.3 ± 1.1</td>
</tr>
<tr>
<td>Epididymal Fat Pad Wt (g)</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>149.5 ± 9.8</td>
<td>192.9 ± 16.5</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.78 ± 0.3</td>
<td>0.81 ± 0.1</td>
</tr>
<tr>
<td>Serum Triglycerides (mg/dl)</td>
<td>77.4 ± 5.3</td>
<td>83.4 ± 2.6</td>
</tr>
<tr>
<td>NEFAs (mMol)</td>
<td>1.2 ± 0.1</td>
<td>0.84 ± 0.1&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>14.4 ± 1.0</td>
<td>14.4 ± 0.6</td>
</tr>
<tr>
<td>HMW Adiponectin (µg/ml)</td>
<td>4.2 ± 0.5</td>
<td>3.5 ± 0.4</td>
</tr>
</tbody>
</table>

Table 3. Effects of HFD and niacin on metabolic parameters in mice. *P<0.05, †P<0.01, ‡P<0.001 vs mice of the same genetic makeup on the control diet; §P<0.05 vs mice of the same genetic makeup and on the same diet receiving vehicle.
HFD resulted in significantly elevated hepatic triglyceride content in wild-type mice (759 ± 120 vs 1395 ± 183 µMol/mg protein; P<0.01), but not in the GPR109A⁻/⁻ mice (305 ± 104 vs 566 ± 138 µMol/mg protein; P>0.05). Increased hepatic triglyceride content is associated with reduced circulating adiponectin concentrations (34); and furthermore, high circulating adiponectin is protective against the development of fatty liver disease. Interestingly, the GPR109A⁻/⁻ mice exhibited significantly lower liver triglyceride content than the wild-type mice (Figure 4). Further, niacin reduced liver triglyceride content in the obese wild-type mice (1395 ± 183 vs 821 ± 183 µMol/mg protein; P<0.05), but had no effect in the GPR109A⁻/⁻ mice (Figure 4). Niacin did not alter serum triglyceride concentrations in any group of mice (Table 3).

* Niacin attenuates HFD-induced adipose tissue inflammation in a receptor-dependent manner

Anti-inflammatory effects of niacin have been demonstrated in kidney (49), lung (118), adipocyte cell culture lines (65), monocytes (64), and vascular endothelial cells (229; 230). However, to date there have been no studies examining the anti-inflammatory properties of niacin in adipose tissue *in vivo*. We therefore examined the effects of niacin on macrophage infiltration of the adipose tissue. As expected, HFD significantly increased MCP-1 gene expression in the adipose tissue of wild-type and GPR109A⁻/⁻ mice (Figure 5A & B). Niacin reduced MCP-1 expression in the adipose tissue of obese wild-type mice, but not any other group of mice (Figure 5A & B), demonstrating novel anti-inflammatory effects of niacin in the adipose tissue.
Figure 4. Effects of HFD and niacin on hepatic lipid content. (A) Liver triglyceride content quantified from wild-type and GPR109A⁻/⁻ mice treated with or without niacin. aP<0.01 compared to wild-type mice on control diet; bP<0.05 compared to wild-type mice on control diet; cP<0.01 compared to wild-type mice on HFD; *P<0.05 compared to wild-type mice on HFD receiving vehicle. (B) Hematoxylin and eosin stain of liver slices. (C) Oil red O stain of liver slices.
Figure 5. Effects of HFD and niacin on markers of adipose tissue inflammation. MCP-1 gene expression in EWAT of wild-type mice (A) or GPR109A\(^{-/-}\) mice (B), CD68 gene expression in EWAT of wild-type mice (C) or GPR109A\(^{-/-}\) mice (D), AND CD11c gene expression in EWAT of wild-type mice (E) or GPR109A\(^{-/-}\) mice (F). All values were normalized to 36B4; *P<0.05, **P<0.01, ***P<0.001
Since MCP-1 plays a major role in the recruitment of monocytes and macrophages to the adipose tissue, we examined the expression of the general monocyte and macrophage marker, CD68. As expected, HFD significantly increased CD68 expression in the adipose tissue, indicating increased presence of macrophages in the EWAT (Figure 5C & D). However, niacin had no effect on CD68 expression. It appears that rather than altering the amount of macrophages present in the adipose tissue, niacin may be promoting a shift in macrophage polarization away from the M1, or pro-inflammatory, macrophage phenotype to the M2, or anti-inflammatory, phenotype. To address this, we examined adipose tissue expression of CD11c, a known marker of M1 macrophages. High-fat diet significantly increased adipose tissue expression of CD11c, indicating the presence of inflammatory macrophages, and niacin treatment significantly reduced CD11c expression in the fat of obese wild-type mice (Figure 5E & F).

Upon further examination of cytokine expression in the fat, we demonstrated that HFD increased gene expression of the pro-inflammatory cytokines IL-1β and TNF-α in the EWAT of both wild-type and GPR109A<sup>−/−</sup> mice. Niacin significantly reduced expression of IL-1β in the fat of obese wild-type mice (Figure 6A & B). This is important because IL-1β is linked to the pathogenesis of insulin resistance (104). IL-1β is also highly expressed in inflammatory macrophages.
Figure 6. Effects of HFD and niacin on markers of adipose tissue inflammation. IL-1β gene expression in EWAT of wild-type mice (A) or GPR109A^{−/−} mice (B), TNF-α gene expression in EWAT of wild-type mice (C) or GPR109A^{−/−} mice (D), IL-6 gene expression in EWAT of wild-type mice (E) or GPR109A^{−/−} mice (F), and IL-10 gene expression in EWAT of wild-type mice (G) or PUMA-G^{−/−} mice (H). All values were normalized to 36B4; *P<0.05, **P<0.01
The reduction in IL-1β could be indicative of niacin’s shift in polarization away from the M1 towards the M2 phenotype. There was no effect of niacin on TNF-α expression in wild-type or GPR109A−/− mice (Figure 6C & D), and there was no effect of HFD or niacin on IL-6 expression (Figure 6E & F). IL-10 is an anti-inflammatory cytokine associated with macrophages of the M2 spectrum. Its expression was significantly increased in the EWAT of wild-type and GPR109A−/− mice, and niacin had no effect on IL-10 expression in either group of mice (Figure 6G & H).

To determine if niacin’s anti-inflammatory effects in the adipose tissue are mediated through reduced presence of M1 and increased presence of M2 macrophages, we examined the expression of M2 macrophage markers in the fat. HFD increased adipose tissue expression of Arginase-1, MRC-1 and YM-1, genes that are highly expressed in M2 macrophages (Figure 7C-H), and to our surprise, niacin treatment reduced Arginase-1 and MRC-1 gene expression in the EWAT of obese wild-type mice. CD163 and MGL2, M2 macrophage markers were unaffected by diet or niacin treatment in any group of mice, and YM-1 was unaffected by niacin (Figure 7A, B, G, H & I).
Figure 7. Effects of HFD and niacin on M2 macrophage markers. CD163 gene expression in EWAT of wild-type mice (A) or GPR109A^-/- mice (B), Arginase-1 gene expression in EWAT of wild-type mice (C) or GPR109A^-/- mice (D), MRC-1 gene expression in EWAT of wild-type mice (E) or GPR109A^-/- mice (F), YM-1 gene expression in EWAT of wild-type mice (G) or GPR109A^-/- mice (H), and MGL-2 gene expression in EWAT of wild-type mice (I) or GPR109A^-/- mice (J). All values were normalized to 36B4; *P<0.05, **P<0.01
Niacin increases the anti-inflammatory adipokine, adiponectin

We and others have previously demonstrated that niacin treatment increases serum adiponectin concentrations (170; 225). Adiponectin is an adipokine secreted predominantly from the adipose tissue with anti-inflammatory, insulin-sensitizing, and cardioprotective effects. In the current investigation, we found that niacin treatment increased serum adiponectin concentrations in wild-type mice on the HFD by 21%, but had no effect in wild-type mice on the control diet (Figure 8A & Table 3) or the GPR109A−/− mice (Figure 8B).

We then examined local production of adiponectin in the adipose tissue and found that both wild-type and GPR109A−/− mice on the HFD had significantly reduced adiponectin gene expression (Figure 9A & B). Niacin increased adiponectin gene expression in the wild-type mice on the HFD by 124%, but not GPR109A−/− mice (Figure 9A & B). This could explain the increase in serum
Figure 8. Effects of HFD and niacin on serum adiponectin. Serum adiponectin concentrations in wild-type mice (A) or GPR109A−/− mice (B). *P<0.05 compared to mice receiving vehicle
**Figure 9.** Effects of HFD and niacin on adiponectin gene expression. Adiponectin gene expression in EWAT of wild-type mice (A) or GPR109A\(^{-/-}\) mice (B). \(^aP<0.001\) compared to mice on control diet vehicle; \(^bP<0.05\) compared to mice on high fat diet receiving vehicle. Adiponectin values were normalized to 36B4.
adiponectin in response to niacin in the wild-type mice on the HFD, but not any other treatment group.

Like the gene expression, adiponectin protein expression in the epididymal fat pads was dramatically reduced in wild-type mice on the HFD (Figure 10A). Niacin increased adiponectin protein expression in the wild-type mice on the HFD by 65% (Figure 10C), but had no effect in mice on the control diet (Figure 10B). This reflects the ability of niacin to increase serum adiponectin concentrations in the wild-type mice on the HFD, but not control diet. Interestingly, the GPR109A−/− mice appeared to be protected from the HFD-induced reduction in EWAT adiponectin protein expression (Figure 11A). Further, niacin had no effect on adiponectin protein expression in the EWAT of GPR109A−/− mice on control (Figure 11B) or high fat diets (Figure 11C).

_Niacin-mediated increases in adiponectin occur independently of changes in adipose tissue gene expression of SIRT1, transcription factors or ER chaperones involved in adiponectin production_

Based on previous reports of the involvement of SIRT1 in adiponectin production and secretion (175; 176), we examined the role of SIRT1 in niacin-mediated increases in adiponectin production and secretion. SIRT1 gene expression in EWAT was unaffected by diet or niacin in wild-type or GPR109A−/− mice (Figure 12A & B).
Figure 10. Effects of HFD and niacin on adiponectin protein expression in wild-type mice. Adiponectin protein expression in EWAT of wild-type mice and their corresponding quantified band densities; *P<0.05; **P<0.01. Adiponectin values were normalized to actin.
Figure 11. Effects of HFD and niacin on adiponectin protein expression in PUMA-G<sup>−/−</sup> mice. Adiponectin protein expression in EWAT of GPR109A<sup>−/−</sup> mice and their corresponding quantified band densities. Adiponectin values were normalized to actin.
Figure 12. Effects of HFD and niacin on SIRT1 gene expression in EWAT of wild-type mice (A) or GPR109A−/− mice (B).
Cellular and secreted adiponectin levels can be regulated at the level of transcription. The adiponectin promoter contains binding sites for several transcription factors that have been shown to positively regulate adiponectin gene transcription including peroxisome proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) (101), CCAAT/enhancer-binding protein \( \alpha \) (C/EBP\( \alpha \)) (162), and sterol regulatory element-binding protein-1c (SREBP-1c) (63; 194). Others have demonstrated that niacin increases PPAR\( \gamma \) activity and expression (113) and increases adipose tissue expression of C/EBP\( \alpha \) (124). Since we demonstrated that niacin increases adiponectin gene expression in the epididymal fat of obese wild-type mice, we examined the effects of niacin on these transcription factors.

PPAR\( \gamma \) activation results in increased adiponectin gene expression and serum concentrations (138). We therefore examined PPAR\( \gamma \) gene expression in the epididymal fat pads of our mice. PPAR\( \gamma \) gene expression was reduced in the epididymal fat of both wild-type and GPR109A\(^{-/-}\) mice on the high fat diet. However, niacin had no effect on PPAR\( \gamma \) gene expression in the wild-type mice (Figure 13A). Interestingly, niacin increased PPAR\( \gamma \) gene expression in the lean GPR109A\(^{-/-}\) mice by 39% (Figure 13B).

Others have shown that chronic niacin administration increases adipose tissue gene expression of C/EBP\( \alpha \), a transcription factor involved in regulation of genes involved in lipid and glucose metabolism in adipose tissue, including adiponectin (124). We measured gene expression of C/EBP\( \alpha \) in the EWAT of wild-type and
Figure 13. Effects of HFD and niacin on gene expression of transcription factors. PPARγ gene expression in EWAT of wild-type mice (A) or GPR109A−/− mice (B). C/EBPα gene expression in EWAT of wild-type mice (C) or GPR109A−/− mice (D). SREBP-1c gene expression in EWAT of wild-type mice (E) or GPR109A−/− mice (F); aP<0.05, bP<0.01 compared to mice on control diet receiving vehicle. All values were normalized to 36B4.
GPR109A−/− mice and found that C/EBPα gene expression was significantly reduced in the EWAT of obese wild-type mice (Figure 13C). However, the GPR109A−/− mice were protected from obesity-induced reductions in C/EBPα gene expression (Figure 13D). Further niacin had no effect on C/EBPα gene expression in any group of mice (Figure 13C & D).

The binding of SREBP-1c to the sterol regulatory element promotes transcription of enzymes involved in lipid metabolism (199). The adiponectin promoter is transactivated by SREBP-1c (127). Overexpression of SREBP-1c in 3T3-L1 adipocytes increases adiponectin gene and protein expression (127). Adipose tissue gene expression of SREBP-1c was unaffected by diet or niacin in all groups of mice (Figure 13E & F).

The ER chaperones ERp44, Ero1-Lα, and DsbA-L are known to be involved in adiponectin production and secretion (223). In order to address an additional mechanism by which niacin could be increasing serum adiponectin concentrations, we examined ERp44, Ero1-Lα and DsbA-L gene expression in the epididymal adipose tissue. ERp44 and Ero1-Lα were not significantly affected by diet or niacin treatment in wild-type or GPR109A−/− mice (Figure 14). DsbA-L expression in the adipose tissue of wild-type mice was significantly reduced by HFD, and niacin tended to increase its expression in this group of mice, but not significantly (Figure 14).
**Figure 14.** Effects of HFD and niacin on gene expression of ER chaperones. ERp44 gene expression in EWAT of wild-type mice (A) or GPR109A<sup>−/−</sup> mice (B), Ero1-Lα gene expression in EWAT of wild-type mice (C) or GPR109A<sup>−/−</sup> mice (D), and DsbA-L gene expression in EWAT of wild-type mice (E) or GPR109A<sup>−/−</sup> mice (F). All values were normalized to 36B4. **P<0.01
DISCUSSION

Recent studies have generated interest in the beneficial effects of niacin, independent of its lipid-modifying capabilities. Niacin is now recognized to possess vascular anti-oxidant and anti-inflammatory properties that can contribute to improvements in cardiovascular outcomes, independent of alterations in blood lipid profile. Ganji and colleagues demonstrated that niacin directly reduces endothelial reactive oxygen species production and subsequent LDL oxidation in cultured human aortic endothelial cells (78). Others have shown that niacin reduces expression or release of pro-inflammatory mediators such as CRP, TNF-α and MCP-1 (64; 65; 120). Lukasova et al. demonstrated that niacin inhibited atherosclerotic disease progression without altering total or HDL-cholesterol levels (134). They also demonstrated that niacin inhibited MCP-1-induced recruitment of macrophages to atherosclerotic plaques. We and others have demonstrated the ability of niacin to increase adipocyte production and circulating concentrations of the anti-inflammatory cytokine, adiponectin (65; 170; 171; 225). These anti-inflammatory, lipid-independent effects of niacin may explain some of the health-related benefits of niacin in the face of unaltered blood lipid profile.

In the current investigation, we showed for the first time that niacin administration improves the inflammatory state of the adipose tissue through alterations in adipose tissue cytokine and macrophage content. Niacin attenuated HFD-induced increases in MCP-1, IL-1β and CD11c, genes associated with
inflammatory M1 macrophages. We hypothesize that since adipose tissue expression of the general macrophage marker CD68 was unaltered by niacin in the face of reduced CD11c expression, that niacin treatment was promoting a shift in macrophage polarization away from the M1 towards the M2 phenotype. The macrophage profile of the adipose tissue is important, as increased M1 macrophage presence in the adipose tissue has been linked to obesity-associated insulin resistance (163).

M2 macrophages exist along a continuum and express different markers based on exposure to the microenvironment of the adipose tissue. In response to HFD feeding, while there is a clear increase in M1 macrophage markers, in many cases there are increases in M2 markers as well (75; 196), as we demonstrated with IL-10, arginase-1, MRC-1 and Ym1. Interestingly, niacin did not further increase the expression of M2 markers as we had hypothesized. Others have shown that HFD increases adipose tissue expression of the M2 markers arginase-1, Ym1 and IL-10 and the insulin-sensitizing omega-3 fatty acid DHA further increases these M2 markers (212). The DHA-mediated increases in arginase-1, Ym1 and IL-10 occurred in the stromal vascular fraction and not the adipocyte fraction of the adipose tissue (212). While we also showed HFD-induced increases in the M2 markers arginase-1, Ym1 and IL-10, we found that niacin reduced arginase-1 expression and had no effect on adipose tissue expression of Ym1 and IL-10. Since many of the cytokines we examined are produced by both adipocytes and macrophages (MCP-1, TNF-α, IL-10, IL-1β),
etc), it will be important in the future to separate the stromal vascular fraction from the adipocyte fraction to determine the effects of niacin in each of these populations. It’s also important to note that the two main sites of expression of the niacin receptor are on the adipocyte and the macrophage, further demonstrating the importance of examining possible differential effects of niacin in these two cell types highly present in the adipose tissue.

Initially, we were surprised that niacin reduced expression of markers associated with both pro-inflammatory M1 (CD11c, MCP-1, IL-1β) and anti-inflammatory M2 macrophages (arginase-1 and MRC-1). When considering the factors that promote macrophage infiltration of the adipose tissue, (1) increased adipocyte production of MCP-1, (2) free fatty acids, (3) dead and dying adipocytes, (4) hypoxia, (5) oxidative stress, and (6) ER stress, we found a possible explanation (204). Free fatty acids are known to drive both M1 and M2 macrophages into the adipose tissue. Increased local and systemic free fatty acids resulting from adipocyte lipolysis during weight loss induces the recruitment of M2 macrophages to the adipose tissue (114). Niacin is a known inhibitor of adipocyte lipolysis. The niacin-mediated reduction in release of free fatty acids from the adipocyte will potentially inhibit the recruitment of M1 and M2 macrophages to the adipose tissue.

As previously mentioned, the main sites of expression of the niacin receptor are the adipocytes and the immune cells, including macrophages. Interestingly,
GPR109A expression on macrophages increases upon treatment with pro-inflammatory stimuli like TNF-α, interferon γ or LPS (188). It is unknown whether GPR109A is expressed in the resident, anti-inflammatory M2 macrophages present in the adipose tissue of lean individuals. In the current investigation, we demonstrated anti-inflammatory effects of niacin in the obese wild-type mice only, which exhibit increased presence of pro-inflammatory M1 macrophages in the adipose tissue. It is possible that the reason we did not see anti-inflammatory effects of niacin in the lean mice is that niacin is exerting its anti-inflammatory effects through directly binding to its receptor on the adipocytes and M1 macrophages that are polarized to an inflammatory state.

During obesity, the adipocyte produces and secretes a number of adipokines that contribute both positively and negatively to the systemic inflammatory process. In adipose tissue of lean individuals, the adipocytes maintain a generally non-inflammatory cytokine profile. However, with obesity, the adipocyte hypertrophies to store excess triglyceride, and with this adipocyte hypertrophy comes a dysregulation of adipokine secretion (142). The adipocyte produces less of the anti-inflammatory adipokines like adiponectin and will produce more pro-inflammatory cytokines such as TNF-α, contributing to the chronic inflammation seen with obesity (13; 94). The mechanisms underlying HFD-induced reduction of adiponectin production include altered hormonal milieu (60; 89; 142) oxidative stress (76), and inflammation. One of the major mechanisms by which HFD-induced adipose tissue inflammation decreases adipose tissue
and serum concentrations of adiponectin is through the up-regulation of inflammatory cytokines. Adipose tissue inflammation is characterized by increased expression of TNF-α and IL-6 in the adipose tissue. TNF-α has inhibitory effects on adiponectin expression through suppressing its promoter activity (138), and IL-6 has also been shown to inhibit adiponectin expression in adipocytes (33).

SIRT1, the mammalian ortholog of yeast Sir2, is a NAD⁺-dependent protein deacetylase that acts as a master metabolic regulator of immunity and metabolism at the liver and adipose tissue (191). SIRT1 has recently been proposed to be at the center of a regulatory loop involving PPARs that controls the metabolic response of tissues, including adipose tissue, to nutrient and physiological signals (205). Within the adipocyte, SIRT1 binds and represses PPARγ (167). SIRT1 overexpression in 3T3-L1 adipocytes reduces PPARγ gene and protein expression, while knockdown of SIRT1 increases PPARγ expression (167). Further, others have shown that suppression of SIRT1 stimulates secretion of HMW adiponectin from mature adipocytes (175). Interestingly, Qiao et al. reported diminished SIRT1 protein expression (176), concomitant with reduced adiponectin gene expression in the fat of obese animals. Additionally, Qiao et al. demonstrated that overexpression of SIRT1 or knocking down endogenous SIRT1 increased or decreased adiponectin gene expression in adipocytes, respectively (176). We therefore sought to determine if niacin affected SIRT1 gene expression in the adipose tissue of our animals.
While we demonstrated that SIRT1 gene expression tended to be reduced in the fat of obese wild-type and GPR109A<sup>−/−</sup> mice, niacin had no effect on SIRT1 expression.

Of the studies that demonstrated that chronic niacin administration increases serum adiponectin, only one elucidated possible mechanisms by which this occurs (124). Linke et al. showed that six months of extended-release niacin increased serum adiponectin concentrations by 35%, and this was accompanied by a 4-fold increase in adiponectin mRNA expression in subcutaneous adipose tissue of humans with impaired glucose tolerance (124). In addition, Linke et al. demonstrated that PPAR<sub>γ</sub> and C/EBP<sub>α</sub> mRNA expression were also increased 1.6- and 1.5-fold, respectively in the niacin-treated group. We showed no effect of niacin on PPAR<sub>γ</sub> or C/EBP<sub>α</sub> gene expression in the epididymal fat of wild-type mice. However, this does not rule out the involvement of PPAR<sub>γ</sub> in niacin's ability to increase adiponectin. Examination of PPAR<sub>γ</sub> activity or translocation to the nucleus may be of importance in order to further address the role of PPAR<sub>γ</sub> in the mechanism of action of niacin. While we showed that the increase in adiponectin mRNA and serum concentrations were not a result of increased PPAR<sub>γ</sub> gene expression, others have shown that in vitro, niacin increases PPAR<sub>γ</sub> mRNA expression (233; 243), induces nuclear expression of PPAR<sub>γ</sub> protein and enhances PPAR<sub>γ</sub> transcriptional activity (113).
In addition to the effects on adiponectin gene expression, PPARγ agonists can increase adiponectin production and/or secretion through alteration of ER chaperone expression (128; 129; 175). ER chaperones including ERp44 and DsbA-L are important in adiponectin multimerization (223), while Ero-1Lα promotes the release of adiponectin from the adipocyte (175). We therefore examined whether ER chaperone expression was altered by short term niacin administration and found that ERp44, Ero1-Lα and DsbA-L expressions were unaffected by niacin treatment. It does not appear that niacin increases adiponectin production through any of the mechanisms currently known to regulate adiponectin that we examined.

The ability of niacin to increase adiponectin may be just one of the multiple anti-inflammatory effects of niacin in the adipose tissue, or it may be a contributing factor to niacin’s anti-inflammatory properties. Adiponectin possesses anti-inflammatory properties including promoting macrophage polarization from an M1 towards and M2 phenotype (155). Adiponectin treatment increases gene and protein expression of arginase-1, Mgl-1 and IL-10 and reduces expression of TNF-α and MCP-1 in isolated murine peritoneal macrophages (155). Further, adiponectin suppresses the activity of NF-κB, a nuclear transcription factor involved in obesity-induced adipose tissue inflammation (7).

Interestingly, beneficial effects of niacin on adipocyte biology have been demonstrated (124). For example, adipocytes isolated from humans treated with
niacin had reduced mean and maximal diameter and volume compared to control subjects (124). Further, insulin sensitivity was also improved in these adipocytes. While we did not demonstrate improvements in insulin sensitivity in our mice, there is evidence that niacin treatment improves insulin sensitivity in the adipocyte, and our findings suggest this could be due to reduced adipose tissue inflammation.

Regarding the more conventional, intended pharmacologic effects of niacin, we examined the effect of niacin on triglyceride accumulation in the liver. Fatty liver disease is the most common hepatic irregularity observed in clinical practice. Non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of liver fat >5% per liver weight in the presence of <10 g of daily alcohol consumption and is associated with central obesity and insulin resistance (37). In our mice, HFD resulted in significantly elevated hepatic triglyceride content in wild-type mice, but not in the GPR109A−/− mice. Strangely, the GPR109A−/− mice exhibited significantly lower hepatic triglyceride content than the wild-type mice. Further, niacin reduced liver triglyceride content in the obese wild-type mice, but had no effect in the GPR109A−/− mice. Increased hepatic triglyceride content is associated with reduced circulating adiponectin concentrations (34); and furthermore high circulating adiponectin is protective against the development of fatty liver disease. Adiponectin protects against fatty liver through activation of the adiponectin receptor (AdipoR2) on hepatocytes, leading to PPARα activation, resulting in increased β-oxidation and reduced fatty acid synthesis (234; 237).
Niacin increased circulating adiponectin concentrations and reduced hepatic triglyceride content in our wild-type obese mice. While we have not yet examined the downstream targets of adiponectin signaling, the increased adiponectin seen in this group of mice suggests a possible mechanism for the niacin-mediated reduction in hepatic lipid content.

In summary, the findings of the current investigation indicate that niacin reduces adipose tissue inflammation through reduced pro-inflammatory cytokine, chemokine and macrophage content and through increased serum and adipose tissue adiponectin concentrations. The increases in adiponectin occurred independently of changes in expression of key transcription factors and ER chaperones involved in adiponectin production. These findings support the idea of pleiotropic effects of niacin to improve metabolic parameters in its target tissues, namely the adipose tissue. Further, all of these anti-inflammatory effects of niacin were lost in the niacin receptor knockout mice. This is consistent with previous reports that the niacin receptor is necessary to mediate anti-inflammatory properties of niacin (64; 65; 134). In addition, it is important to note that we did not see the beneficial effects of niacin in the lean wild-type mice that were seen in the obese wild-type mice. There was very little expression of inflammatory cytokines and M1 macrophages in the EWAT of the lean mice. This suggests that the pleiotropic actions of niacin are greatest when facing metabolic dysfunction.
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CHAPTER III

NIACIN DECREASES SERUM RETINOL-BINDING PROTEIN 4 (RBP4) IN A RECEPTOR-INDEPENDENT MANNER

ABSTRACT

Introduction. Recent unpublished studies from our laboratory demonstrated that niacin reduces serum retinol-binding protein 4 (RBP4) concentrations in men with metabolic syndrome. However, little is known about the effects of chronic niacin administration on RBP4 in lean and obese rodents or the mechanism(s) by which niacin decreases RBP4. Objective. Therefore, our objective was to characterize the role of GPR109A, the receptor for niacin, in the mechanism by which chronic niacin administration reduces serum RBP4 concentrations.

Methods. Thirty-two male C57BL/6 mice were placed on a control diet (10% kcal as fat; n=16) or high-fat diet (HFD; 60% kcal as fat; n=16) and were maintained on such diets for the duration of the study. After 6 weeks on the control or HFD, vehicle or niacin treatments were begun. Half of the mice from each group received vehicle (water) and the other half received niacin (200 mg/kg/day in drinking water) for 4 weeks. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. Identical studies were conducted concurrently in GPR109A−/− mice.

Results. Niacin decreased serum RBP4 concentrations in obese wild-type mice by 22% (61.8 ± 4.3 vs 48.4 ± 3.3
Niacin also tended to reduce serum RBP4 in the lean wild-type mice, although not significantly, by 16% (52.8 ± 3.7 vs 44.3 ± 3.3 μg/ml; P=0.11). Interestingly, niacin significantly reduced serum RBP4 concentrations by 16% in the lean GPR109A−/− mice (51.1 ± 1.5 vs 43.1 ± 1.6 μg/ml; P<0.01), but had no effect on obese GPR109A−/− mice (46.6 ± 1.6 vs 50.1 ± 3.6 μg/ml; P>0.05). Adipose tissue and liver RBP4 gene and protein expression were unchanged in response to niacin treatment in any group. Niacin (1 μM – 1000 μM) also had no direct effect on the secretion or intracellular levels of RBP4 from isolated rat primary adipocytes, hepatocytes or the human hepatoma (HepG2) cell line.

**Conclusions.** Since niacin was able to reduce serum RBP4 in wild-type and GPR109A null mice, but niacin had no effect on RBP4 gene or protein expression in the liver or fat of any mice, niacin most likely reduces serum RBP4 concentrations in mice in a receptor-independent manner, possibly through increased clearance.

**INTRODUCTION**

With the increasing prevalence of obesity and related derangements in lipid metabolism, pharmacological doses of niacin or related agonists of the niacin receptor figure to play a prominent role in the treatment of dyslipidemia. Indeed, niacin remains the most effective agent to increase serum HDL-C and decrease triglycerides (TAGs) in obese individuals with metabolic syndrome (MetS) (112). Activation of the niacin receptor in adipose leads to a rapid reduction in intracellular cAMP production and subsequent inhibition of lipolysis (36). The
reduction in lipolysis results in a transient decrease in circulating non-esterified fatty acids (NEFAs) which is accompanied by a reduction in the expression of hepatic diacylglycerol acyl transferase-2 (DGAT2), the rate-limiting enzyme in TAG synthesis (79). The reduction in serum NEFAs and decrease in DGAT2 expression are associated with decreased serum TAG concentrations. Our group and others have shown that niacin also increases serum concentrations of the adipocyte-derived protein adiponectin in rodents (171) and humans (170; 226). In addition to its insulin-sensitizing properties, adiponectin increases the oxidation of NEFAs in liver and skeletal muscle and reduces hepatic glucose production (237). Additional studies have shown that niacin decreases circulating levels of total- and phospo-fetuin A, a liver secreted protein which decreases insulin receptor tyrosine kinase activity and increases hepatic lipid accumulation (109). Overall, these findings suggest a complex and coordinated attenuation of hepatic triglyceride formation produced by niacin's effects on adipose- and liver-secreted proteins.

Retinol-binding protein 4 (RBP4) is a 21 kDa protein secreted primarily by liver and adipose tissue which serves as the principal transporter of retinol (vitamin A). A role for RBP4 in obesity and insulin resistance was initially suggested from DNA arrays in adipose-specific GLUT4-/- mice which developed insulin resistance in skeletal muscle and liver (238). Since then, a number of studies demonstrate that circulating RBP4 concentrations are increased in mouse models of obesity and insulin resistance and in obese humans with MetS (51; 123; 173; 238). Mice
with a heterozygous or homozygous deletion of RBP4 have improved insulin sensitivity, while transgenic overexpression of human RBP4 or injection of recombinant RBP4 causes insulin resistance (238). A recently identified functional polymorphism of the human RBP4 promoter causes increased RBP4 expression in adipocytes and is associated with increased plasma RBP4 concentrations and risk for Type 2 diabetes (115; 149). Further studies suggest that elevated circulating RBP4 concentration are involved in the development of dyslipidemia and cardiovascular disease (50; 86; 111).

The peroxisome proliferator-activated receptor α (PPARα) agonist fenofibrate is one of the principal lipid-lowering agents used for the treatment of hypertriglyceridemia (74). Recent studies also show that fenofibrate decreases circulating RBP4 (231) and increases adiponectin through changes in gene expression (181; 231). Niacin increases serum adiponectin through transcriptional and post-translational effects on secretion that appear to be mediated through niacin receptor activation (171). In the current investigation, we examined whether expression levels and circulating RBP4 are regulated by niacin and if so, if the effects are mediated by the niacin receptor. Our results show that niacin administration attenuates high-fat diet-induced increases in circulating RBP4 in the presence of increased body weight and adiposity. The reduction in circulating RBP4 occurs in mice with a genetic deletion of the niacin receptor and in the absence of changes in gene expression, suggesting that
niacin reduces RBP4 by altering the overall metabolic milieu, or by promoting clearance of RBP4.

MATERIALS AND METHODS

Animal Studies

Thirty-two 4-week old male C57BL/6J mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were housed in groups of four in filter-top cages and were acclimated to the animal facilities at Auburn University for three days after arrival. Mice were then placed on a control diet (10% kcal as fat; n = 16) or high-fat diet (60% kcal as fat; n = 16) (Research Diets, New Brunswick, NJ) for six weeks. The energy content of the control diet was 16.1 kJ/g, while the high-fat diet was 21.9 kJ/g. Half of the mice from the control and high-fat diet groups received vehicle (water) and the other half received niacin (200 mg/kg/day) dissolved in drinking water for four weeks, while remaining on their respective diets. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. An identical study was conducted in parallel using C57BL/6J mice with a global deletion of the niacin receptor, GPR109A (215). GPR109A−/− mice were a generous gift from Dr. Stefan Offermanns (Bad Nauheim, Germany) and were generated as previously described (215). Thirteen GPR109A−/− mice were placed on the control diet (n = 7 vehicle; n = 6 niacin), and nine GPR109A−/− mice were placed on the high-fat diet (n = 4 vehicle; n = 5 niacin). After five weeks of vehicle or niacin treatment, mice were fasted overnight and euthanized. Energy intake, water intake, and body weight were
measured twice per week. Energy and water intake were calculated per box of four mice. Whole blood was collected into centrifuge tubes and tissues were flash frozen in liquid nitrogen. Isolated serum and tissues were stored at -80°C until analysis. Animal experiments were approved by the Auburn University Institutional Animal Care and Use Committee.

**Cell Culture Studies**

Epididymal fat pads were obtained from male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 175-225 g each. Adipocytes were isolated as previously described (164). DMEM supplemented with 0.2% fatty-acid free bovine serum albumin was added overnight prior to all experiments. HepG2 cells were cultured in Williams E media. Cells were treated with niacin (1 μM – 1000 μM) to establish the concentration-dependent effects of niacin on RBP4 secretion. Hepatocytes were prepared by *in situ* collagenase perfusion of the liver of male Sprague-Dawley rats using a modification of the procedure described by Seglen (192) and were treated as described above. Human recombinant adiponectin was from Axxora Platform (San Diego, CA).

**Serum Analysis**

Serum RBP4 concentrations were measured using an ELISA from AXXORA (San Diego, CA). Serum insulin was measured by ELISA (Millipore, Billerica, MA) and glucose was measured by glucose assay kit (Cayman Chemical, Ann Arbor, MI).
**Real-time PCR**

RNA was isolated from tissues using a Qiagen RNeasy Mini Kit or RNeasy Lipid Tissue Mini Kit (Valencia, CA). RNA (1 μg) was reverse transcribed into cDNA using an iScript cDNA Synthesis Kit from Bio-Rad (Hercules, CA). PCR primers used in the real-time-PCR analysis were as follows:

- RBP4 forward, 5’- GTCCGTCTTTCTGAGCAACTG
- RBP4 reverse, 5’-AGGTGTCGCTAGTCCGTGTC-3’
- 36B4 forward, 5’- CACTGCTGAACATGCTGAAC-3’
- 36B4 reverse, 5’- CCACAGACAATGCCAGGAC-3’.

Analyses were performed on a Bio-Rad iCycler iQ thermocycler. Samples were analyzed in 30 μl reactions using SYBR Green PCR Master Mix (Bio-Rad). All expression levels were normalized to the corresponding 36B4 mRNA levels.

**Immunoblot Analysis**

Epididymal white adipose tissue (EWAT) and liver were homogenized in RIPA buffer (NP40, sodium deoxycholate, SDS, NaCl, Tris pH 6.8, EDTA, protease and phosphatase inhibitors). Primary rat adipocytes, hepatocytes and HepG2 cells were treated with RIPA buffer on ice for 20 minutes prior to cell collection with a rubber cell scraper. Lysates were then centrifuged in a microcentrifuge at 13,000 RPM for 30 minutes at 4°C and infranatant was collected. Dc protein assay (Bio-Rad) was used to measure protein concentrations. Proteins were then separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Membranes were blocked in LI-COR Odyssey (Lincoln, NE).
blocking buffer for 1 h and incubated overnight with primary antibodies. Membranes were then washed three times with PBS-0.1% Tween-20 and incubated with infrared-conjugated secondary antibodies for 1 h. Blots were scanned with a LI-COR Odyssey infrared scanner. Mouse monoclonal RBP4 antibody was from R&D Systems (Minneapolis, MN). Mouse monoclonal actin antibody was from Millipore (Temecula, CA).

Statistical Analysis

Data were analyzed using a one-way ANOVA. When differences were observed, a Bonferroni post hoc test was conducted to determine where differences occurred. Data were analyzed using GraphPad Prism software version 4.0 (GraphPad Software, La Jolla, CA). Significance was accepted at the $P < 0.05$ level.

RESULTS

Effects of diet and niacin on serum RBP4 and metabolic parameters in mice

Previous studies conducted by our laboratory show that niacin reduces lipolysis and increases circulating adiponectin through activation of the GPR109A receptor (171). To determine if the reduction in circulating RBP4 produced by niacin we observed in our human studies (unpublished data) is due to activation of the GPR109A receptor, we placed WT and GPR109A$^{-/-}$ mice on a low fat control or high fat diet for 11 weeks and administered niacin or vehicle in the drinking water during the final 5 weeks. Mice from each genotype, diet group and
treatment were matched for body weight at the start of the study. WT and GPR109A\(^{-/-}\) mice gained a similar amount of body weight and adiposity (\% epididymal fat) following the control diet, but adiposity in the GPR109A\(^{-/-}\) mice was approximately 40\% higher than WT mice on the high fat diet (Table 4). Niacin produced no effect on the accretion of body weight or adiposity in either genotype on the control diet.

WT mice receiving niacin on the high fat diet weighed less than the vehicle-treated group by the end of the study, but these differences resulted from random differences in body weight gain between high fat diet groups and not due to the niacin treatment itself. No differences in body weight or adiposity were observed in GPR109A\(^{-/-}\) mice following niacin on the high fat diet. Average energy intake was approximately 49\% higher in WT mice on the high fat diet compared to the control diet as expected. In contrast, average energy intake was lower in the GPR109A\(^{-/-}\) mice on the high fat diet compared to the control diet despite significantly higher body weight and adiposity suggesting that energy expenditure may be attenuated in GPR109A\(^{-/-}\) mice placed on a high fat diet.

High fat diet increased serum glucose and insulin concentrations in both genotypes. Niacin tended to increase serum glucose concentrations in both genotypes on the control diet but produced no changes in glucose on the HFD. Serum insulin concentrations were not significantly increased following niacin for either diet or genotype. Serum RBP4 concentrations were similar between
genotypes on the control diet treated with vehicle. High fat diet increased serum RBP4 in WT mice but not in GPR109A−/− mice.

Niacin reduced circulating RBP4 by 21.6% in WT mice on the high fat diet ($P < 0.05$), and by 16.2% in WT mice on the control diet; however, this did not reach statistical significance ($P = 0.11$). While niacin reduced RBP4 in GPR109A−/− mice to a similar extent as WT mice on the control diet, vehicle-treated mice on the HFD did not experience an increase in RBP4 and niacin failed to reduce circulating RBP4 (Table 4). These results suggest that niacin reduces RBP4 through GPR109A-independent mechanisms and that the absence of the receptor prevents HFD-induced increases in circulating RBP4.

*Niacin does not affect RBP4 gene or protein expression in the adipose tissue or liver*

To determine if the reduction in circulating RBP4 was due to reductions in adipose tissue and/or liver expression, we examined mRNA and protein levels of RBP4 in WT and GPR109A−/− mice. HFD decreased RBP4 gene (Table 4) and protein (Figure 15A & B) expression in EWAT of both genotypes. Niacin had no significant effect on RBP4 gene or protein expression in EWAT of WT or GPR109A−/− mice on the control diet or HFD (Table 4 & Figure 15A & B). Hepatic RBP4 gene expression was similar between genotypes and was not altered by HFD or niacin, while hepatic RBP4 protein was increased by HFD, yet remained unaltered by niacin (Table 4 & Figure 16A & B).
<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>GPR109A^{+/−}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD Vehicle</td>
<td>CD Niacin</td>
</tr>
<tr>
<td><strong>Body Weight, g</strong></td>
<td>32.1 ± 1.1</td>
<td>33.3 ± 1.1</td>
</tr>
<tr>
<td><strong>Epididymal Fat Pad Wt, g</strong></td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td><strong>Epididymal Fat Pad Wt, %</strong></td>
<td>3.4 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td><strong>Energy Intake</strong></td>
<td>190 ± 3</td>
<td>195 ± 23</td>
</tr>
<tr>
<td><strong>Water Intake</strong></td>
<td>12.8 ± 1.0</td>
<td>9.4 ± 0.6&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td>150 ± 10</td>
<td>193 ± 17</td>
</tr>
<tr>
<td><strong>Insulin (ng/mL)</strong></td>
<td>0.78 ± 0.3</td>
<td>0.81 ± 0.1</td>
</tr>
<tr>
<td><strong>RBP4 (µg/mL)</strong></td>
<td>52.8 ± 4.8</td>
<td>44.3 ± 3.3</td>
</tr>
<tr>
<td><strong>EWAT RBP4 mRNA (AU)</strong></td>
<td>2.0 ± 0.2</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td><strong>Liver RBP4 mRNA (AU)</strong></td>
<td>6.0 ± 0.4</td>
<td>6.0 ± 0.3</td>
</tr>
</tbody>
</table>

Table 4. Effects of HFD and niacin on metabolic parameters in mice. <sup>†</sup>P<0.05 Compared to mice of same genetic makeup and on same diet receiving vehicle. <sup>a</sup>P<0.05; <sup>b</sup>P<0.01; <sup>c</sup>P<0.001 compared to mice of the same genetic makeup on control diet receiving vehicle. All data are represented as mean ± SEM. Comparisons were made between mice of the same genetic makeup receiving vehicle on CD vs HFD (a, b, c) and between mice of same genetic make-up and diet receiving vehicle vs niacin (*). CD Control diet; HFD High fat diet.
Figure 15. Effects of HFD and niacin on adipose tissue RBP4 protein expression. Effect of niacin on RBP4 protein expression in EWAT of wild-type (A) or GPR109A<sup>−/−</sup> mice (B).
Figure 16. Effect of HFD and niacin on hepatic RBP4 protein expression. Effect of niacin on RBP4 protein expression in liver of wild-type (A) or GPR109A<sup>−/−</sup> mice (B); *P<0.05; **P<0.01 compared to mice on the control diet.
Niacin has no effect on RBP4 secretion from adipocytes or hepatocytes

Niacin concentrations ranging from 1 μM to 1000 μM, which had previously been shown to produce a concentration-dependent increase in adiponectin secretion (171), were used to determine the direct effects of niacin on RBP4 secretion in rat primary adipocytes and rat primary hepatocytes and in the human HepG2 cell line. Niacin concentrations within this range had no effect on the secretion (Figure 17A-C) or intracellular levels (data not shown) of RBP4 in primary adipocytes, hepatocytes or HepG2 cells. Primary adipocytes isolated using collagenase digestion express the niacin receptor and respond normally to niacin as determined by reductions in isoproterenol stimulated intracellular cAMP production and glycerol release (171). In order to determine if the reductions in circulating RBP4 occurred in response to the increased serum adiponectin seen after niacin treatment, hepatocytes were treated with recombinant adiponectin. Treatment of rat primary hepatocytes with human recombinant adiponectin had no effect on RBP4 secretion (Figure 17D).

DISCUSSION

The results of this investigation indicate that five weeks of niacin decreases serum concentrations of RBP4. In an effort to better understand the molecular action of niacin on serum levels and tissue production of RBP4, we placed WT and GPR109A null mice on a low fat control or high fat diet for 11 weeks with niacin or vehicle administered over the final 5 weeks of the diets. Niacin decreased serum RBP4 concentrations on both diets and genotypes. However,
Figure 17. Effect of niacin or adiponectin treatment on RBP4 secretion from adipocytes or hepatocytes. A-C Effect of varying concentrations of niacin (μM) on RBP4 secretion from primary rat adipocytes (A), primary rat hepatocytes (B), and HepG2 cells (C). D Effect of varying concentrations of recombinant human adiponectin on primary rat hepatocytes. Media was sampled 6 h following niacin or adiponectin treatment. A1, 3.5 μg recombinant human adiponectin; A2, 7 μg recombinant human adiponectin; A3, 14 μg recombinant human adiponectin.
mRNA and protein levels of RBP4 were not decreased in either EWAT or liver of either genotype providing evidence that the reduction in circulating RBP4 is not due to reductions in tissue expression. In support of these findings, niacin produced no effect on the intracellular content or secretion of RBP4 in isolated primary rat adipocytes, hepatocytes or human HepG2 cells. Interestingly, we demonstrated that HFD resulted in reduced RBP4 gene and protein expression in EWAT of both wild-type and GPR109A<sup>−/−</sup> mice. The reduction in RBP4 expression did not correlate with circulating RBP4 protein concentrations but is in agreement with previous reports in humans (29; 105). Hepatic RBP4 gene expression was unaltered by diet, however hepatic RBP4 protein expression was significantly increased by HFD. Overall, these results suggest that the reductions in serum RBP4 concentrations following niacin are not due to direct actions of niacin on adipose tissue or liver but instead may be caused by indirect effects that occur in parallel with overall improvements in the metabolic milieu.

Niacin has been used for over 50 years in the treatment of dyslipidemia, producing reductions in serum triglycerides and increases in HDL-C that exceed those of more frequently used lipid-lowering agents (112). Niacin’s effects on blood lipids have been primarily linked to transient reductions in serum NEFAs, but little is known regarding the molecular action of niacin. Recent studies demonstrate that niacin increases serum total and HMW adiponectin concentrations in obese humans and decreases serum concentrations of IL-6 and fetuin-A (109; 170; 226). Adiponectin increases the oxidation of fatty acids in
liver and skeletal muscle and has been shown to repress hepatic gluconeogenesis (237). Niacin increases adiponectin secretion from adipocytes through activation of the niacin (GPR109A) receptor in a cAMP/PKA-dependent manner (36). Additional studies suggest that GPR109A activation is relatively specific to adiponectin with little effect on the secretion of other adipokines such as leptin or resistin (171).

In the current investigation, niacin decreased serum RBP4 concentrations. Since RBP4 is secreted by both targets of niacin (adipose and liver) and is positively associated with circulating triglycerides and insulin concentrations, our hypothesis was that niacin reduces circulating RBP4 in a coordinated effort with the regulation of other adipokines such as adiponectin to lower hepatic and circulating triglycerides. Results from our in vivo animal studies demonstrate that niacin decreases circulating RBP4 through GPR109A receptor-independent mechanisms which are not associated with changes gene expression levels in liver or adipose tissue. Furthermore, cell culture studies reveal that niacin has no direct effect on the secretion or intracellular levels of RBP4 in primary hepatocytes and adipocytes or human HepG2 cells suggesting that niacin’s ability to reduce serum RBP4 is due to indirect improvements in other aspects of the metabolic milieu or due to changes in the renal clearance of RBP4.

RBP4 is a compact 21 kDa protein that can be freely filtered through the renal glomerular membrane (215). However, RBP4 normally binds to the larger
(56 kDa) protein transthyretin (TTR) which reduces the renal clearance of the complex. Targeted deletion of the TTR gene or compounds that interfere with TTR-RBP4 binding profoundly reduce serum RBP4 levels (146). Our lab has observed that in humans, serum TTR is not affected by niacin (unpublished observations) suggesting that the reduction in serum RBP4 is not due to a reduction in serum TTR concentrations. However, it remains possible that niacin increases the renal clearance of RBP4 by interfering with TTR-RBP4 binding affinity.

Since niacin has direct effects on the regulation of adiponectin and because adiponectin is inversely associated with RBP4 levels, we then wanted to determine if adiponectin itself has a direct effect on RBP4 secretion. Recombinant human adiponectin had no effect on the secretion or intracellular levels of RBP4 in isolated rat hepatocytes suggesting that niacin-mediated increases in serum adiponectin are not responsible for the reduction in serum RBP4.

Several limitations are inherent to these studies. First, in our rodent studies, the shorter duration of the study, an end-point analysis without baseline values, and the use of an immediate-release form of niacin may have reduced the magnitude of the effect. Additionally, by chance, the wild-type mice on the HFD receiving vehicle weighed more at the completion of the study than those receiving niacin. This was due mainly in part to one mouse in the HFD group receiving niacin
which weighed 29.1 g at completion of the study compared to the average of the rest of the group of 40.9 g. He was not excluded as an outlier because all of his other metabolic and phenotypic parameters were similar to other mice in his group.

In conclusion, these studies provide evidence that niacin decreases serum RBP4 concentrations. The reduction in RBP4 appears to be mediated by an improvement in the overall metabolic milieu or interference with TTR-RBP4 binding affinity and not due to direct effects of niacin on GPR109A activation or gene expression in adipose or liver.
ABSTRACT

Introduction. GPR109A (PUMA-G, NIACR1, HCA₂) and GPR81 (HCA₁) are G protein-coupled receptors located predominantly on adipocytes that mediate anti-lipolytic effects. These cell surface receptors give the adipocyte the ability to “sense” its surrounding environment and respond through lipolytic regulation and release of products including free fatty acids and pro- or anti-inflammatory adipokines. The endogenous ligands for GPR109A and GPR81 are β-hydroxybutyrate and lactate, respectively, both of which are hydroxycarboxylic acids and intermediates of energy metabolism. Circulating β-hydroxybutyrate levels are increased during a 2-3 day fast and prolonged starvation, while lactate levels are elevated during times of intense exercise. Therefore, regulation of expression of these receptors is crucial for the metabolic sensing ability of the adipocyte and ultimately whole body energy homeostasis. Objective. Therefore, we investigated the effects of high fat diet-induced obesity and niacin treatment on adipose tissue expression of GPR109A and GPR81. Methods. Thirty-two male C57BL/6 mice were placed on a control (10% kcal fat; n=16) or a high fat (60% kcal fat; n=16) diet for 6 weeks. After 6 weeks on the diets, half of the mice
from each group received vehicle (water) and the other half received niacin (200 mg/kg/day in drinking water) for 4 weeks. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. **Results.** We demonstrated that high fat diet resulted in significantly reduced GPR109A and GPR81 gene and protein expression in epididymal fat. While niacin had no significant effect on gene expression of GPR109A and GPR81, niacin significantly increased GPR109A and GPR81 protein expression in adipose tissue of lean and obese mice. **Conclusions.** In conclusion, niacin treatment was able to partially reverse or further prevent obesity-induced reductions in GPR109A and GPR81 expression in the adipose tissue.

**INTRODUCTION**

Adipocytes express a number of cell surface receptors that contribute to their ability to sense the surrounding environment, including GPR109A and GPR81. In 2003, GPR109A was identified as the receptor for the beneficial lipid-altering drug, niacin (200; 215; 228). However, under physiological conditions, plasma concentrations of niacin do not reach levels high enough to activate the receptor, making it unlikely to be the endogenous ligand. In 2005, Taggart *et al.* demonstrated that β-hydroxybutyrate, a ketone body produced by the liver, is an endogenous ligand for GPR109A with an EC$_{50}$ of 767 ± 57 µM (206). β-hydroxybutyrate activates GPR109A and inhibits adipocyte lipolysis at concentrations seen during a 2-3 day fast (206). β-hydroxybutyrate may represent a homeostatic mechanism for surviving starvation in which it acts in a
negative feedback manner to inhibit lipolysis. In this manner, \(\beta\)-hydroxybutyrate can regulate its own production by decreasing the serum level of fatty acid precursors available for hepatic ketogenesis (206) and possibly preserving lipid stores during a prolonged fast (193). GPR109A is predominantly expressed in adipocytes of white and brown adipose tissue, and is expressed to a lesser extent in keratinocytes and immune cells, including dermal dendritic cells, monocytes, macrophages and neutrophils (90; 136; 200; 209; 215; 228).

GPR81 shares a 52% amino acid sequence identity in humans to GPR109A (200; 215; 228). In addition, GPR81 is localized more specifically to the adipose tissue (228). In 2008, lactate was discovered to be the endogenous ligand for GPR81 (38; 125). Plasma lactate levels reach concentrations capable of activating the receptor during bouts of intense exercise. Infusion of lactate reduces lipolysis \textit{in vivo} (28; 82; 97) as does treatment of adipocytes \textit{in vitro} (22; 58). The effect of lacate to reduce lipolysis is mediated through activation of GPR81 (38; 125), although the receptor has recently been shown to not be involved in the regulation of lipolysis during intensive exercise when lactate concentrations are elevated (5).

Both GPR109A and GPR81 are located on chromosome 12q24 (228) and mediate anti-lipolytic effects through coupling to \(G_i\)-type G proteins (80). It is important to note that with the recent deorphanization of these receptors, there has been a recommendation to the International Union of Basic and
Clinical Pharmacology (IUPHAR) to rename this group of receptors, GPR81, GPR109A, and GPR109B (a human-specific, low-affinity niacin receptor) to the hydroxy-carboxylic acid (HCA) receptor family, whereby GPR81 is HCA₁, GPR109A is HCA₂, and GPR109B is HCA₃ (154). Studies have revealed that all three receptors bind hydroxy-carboxylic acids as their endogenous ligands, leading to the proposed nomenclature for this receptor family.

G protein-coupled receptor synthesis is regulated by gene transcription, translation, and posttranslational processing, which may be regulated by the ligand itself and by other hormones and factors (145). GPR109A gene expression in adipocytes is reduced with age in mice (126). While the regulation of GPR109A gene expression in the adipose tissue in various disease states (including diabetes or obesity) has not been demonstrated, a down-regulation of GPR81 mRNA expression in mouse adipose tissue has been demonstrated in response to acute treatment with inflammatory stimuli (70). In addition, ob/ob mice, a mouse model of obesity and type 2 diabetes, demonstrate reduced adipose tissue expression of GPR81 (70). We and others have demonstrated that niacin possess anti-inflammatory properties, independent of its lipid-modifying capabilities (64; 65; 133; 171). Therefore, we investigated the effects of high fat diet-induced obesity and niacin treatment on adipose tissue expression of GPR109A and GPR81.
MATERIALS AND METHODS

Materials

Niacin (nicotinic acid) was purchased from Sigma-Aldrich (St. Louis, MO). Rabbit polyclonal GPR109A and goat polyclonal GPR81 antibodies were from Santa Cruz Biotechnology, inc. (Santa Cruz, CA). Mouse monoclonal actin antibody was from Millipore (Temecula, CA).

Animal Studies

Thirty-two male C57BL/6 mice were purchased at three weeks of age from Charles River Laboratories (Wilmington, MA). After three days of acclimatization, mice were either placed on a high fat diet (HFD; 60% kcal as fat; n=16) or a control diet (10% kcal as fat; n=16) obtained from Research Diets (New Brunswick, NJ) and were maintained on such diets for the duration of the study. After six weeks on the control or high fat diets, vehicle or niacin treatments were begun. Half of the mice from the control and high fat diet groups received vehicle (water) and the other half received niacin (200 mg/kg/day) dissolved in drinking water for four weeks. Niacin concentrations were increased to 360 mg/kg/day for the fifth week of treatment. After five weeks of vehicle or niacin treatments, mice were fasted overnight and sacrificed. Epididymal fat pads were excised, flash frozen in liquid nitrogen, and stored at -80°C until analysis.
**Real-time PCR**

RNA was isolated from epididymal white adipose tissue (EWAT) using Qiagen RNeasy Lipid Tissue Mini Kit (Valencia, CA). RNA (1 μg) was reverse transcribed into cDNA using iScript cDNA Synthesis Kit from Bio-Rad. PCR primers used in the real-time-PCR analysis are listed in Table 5.

Analyses were performed on a Bio-Rad iCycler iQ thermocycler. Samples were analyzed in 30 μl reactions using SYBR Green PCR Master Mix (Bio-Rad). Relative changes in gene expression were calculated by the comparative $C_T$ method using the $2^{\Delta\Delta C_T}$ equation with results normalized to the corresponding 36B4 mRNA levels.

**Immunoblot Analysis**

Frozen EWAT pads (~100 mg) were homogenized with a handheld homogenizer in RIPA buffer (NP40, sodium deoxycholate, SDS, NaCl, Tris pH 6.8, EDTA, protease and phosphatase inhibitors) and the protein fractions of the epididymal fat were isolated. Protein content was determined by the Bio-Rad DC protein assay method. Proteins were then separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Membranes were blocked in LI-COR Odyssey (Lincoln, NE) blocking buffer for 1 h and incubated overnight with primary antibodies (1:1,000). Membranes were then washed three times with PBS-0.1% Tween-20 and incubated with infrared-conjugated secondary antibodies (1:10,000) for 1 h. Following three more washes, blots
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer 5’-3’</th>
<th>Reverse Primer 5’-3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR109A</td>
<td>GCTGCCTGCCGTTCTTGAC</td>
<td>GTTCAAGAAGTGGTGGATGG</td>
</tr>
<tr>
<td>GPR81</td>
<td>ATCCTGGTCCTCGTTGCTTTG</td>
<td>GTCTGCGTGATGGTAGTC</td>
</tr>
<tr>
<td>36B4</td>
<td>CACTGCTGAACATGCTGAAC</td>
<td>CCACAGACAATGCCAGGAC</td>
</tr>
</tbody>
</table>

**Table 5.** Primers used for real-time RT-PCR.
were scanned with a LI-COR Odyssey infrared scanner. Band density was determined using the LI-COR Odyssey software with actin as the protein loading control.

Statistical Analysis
Data were analyzed using a t-test or one-way ANOVA. When differences were observed, a Bonferroni post hoc test was conducted to determine where differences occurred. Data were analyzed using GraphPad Prism software version 4.0 (GraphPad Software, La Jolla, CA). Significance was accepted at the $P < 0.05$ level.

RESULTS

Effects of HFD and niacin on serum and metabolic parameters in mice
HFD caused significant increases in body weight, epididymal fat pad weight, serum glucose and insulin concentrations in mice (Table 6). Niacin had no effect on epididymal fat pad weight, serum glucose or insulin concentrations (Table 6). Interestingly, niacin tended to increase serum glucose concentrations in lean mice, although this did not reach statistical significance. Niacin is known to suppress the release of free fatty acids from the adipocyte, resulting in a transient reduction in circulating NEFAs (41; 43). In the current investigation, niacin reduced serum NEFA concentrations in the lean, but not obese mice (Table 6).
**Table 6.** Effects of HFD and niacin on metabolic parameters in mice.  
†P<0.01, ‡P<0.001 vs mice on the control diet receiving vehicle; §P<0.05 vs mice on the high fat diet receiving vehicle. Comparisons were made between mice receiving vehicle on control or high fat diets and between vehicle and niacin treatments in mice on same diet.
**HFD reduces GPR109A and GPR81 gene and protein expression**

We examined the effect of HFD-induced obesity on gene expression of GPR109A and GPR81 in the epididymal fat (Figure 18). HFD reduced adipose tissue gene expression of both GPR109A and GPR81. Similar to the gene expression, HFD resulted in reduced adipose tissue protein expression of GPR109A and GPR81 (Figure 18).

**Niacin increases GPR109A and GPR81 protein expression**

Niacin had no significant effect on GPR109A or GPR81 gene expression (data not shown). However, short-term niacin treatment resulted in significantly increased protein expression of both GPR109A and GPR81 (Figures 19 and 20) in lean and obese mice. GPR81 expression in adipose tissue is reduced by inflammatory stimuli (70), and niacin has been shown to possess anti-inflammatory effects, independent of its lipid-modifying capabilities (64; 65). Short-term niacin treatment either partially reversed obesity-associated down-regulation of adipose tissue protein expression of GPR109A and GPR81, or prevented further HFD-induced reductions in receptor expression. Niacin treatment of these mice also resulted in increased adipose tissue expression of the anti-inflammatory cytokine adiponectin, and reduced expression of the pro-inflammatory cytokine and chemokine interleukin-1β (IL-1β) and monocyte chemoattractant protein-1 (MCP-1). Niacin treatment also reduced expression of the pro-inflammatory macrophage marker CD11c in obese mice, indicating anti-
Figure 18. Effects of HFD on GPR109A and GPR81 gene and protein expression. High fat diet reduces EWAT GPR109A (A) and GPR81 (B) gene expression. High fat diet reduces EWAT GPR109A (C) and GPR81 (D) protein expression; *P<0.05, **P<0.01.
Figure 19. Effects of niacin on GPR109A protein expression. Niacin increases EWAT GPR109A protein expression in mice on control diet (A) and high fat diet (B); *P<0.05, **P<0.01.
Figure 20. Effects of niacin on GPR81 protein expression. Niacin increases EWAT GPR81 protein expression in mice on control diet (A) and high fat diet (B); *P<0.05.
inflammatory effects of niacin that may explain the increase in receptor expression (Chapter II).

**DISCUSSION**

Here, we demonstrate, for the first time, that HFD results in down-regulation of adipose tissue GPR109A and GPR81 gene and protein expression. Down-regulation of GPR109A and GPR81 during states of obesity and adipose tissue inflammation may contribute to the dysregulation of adipocyte lipolytic function often seen in obesity.

GPR109A and GPR81 give the adipocyte the ability to “sense” its surrounding environment and respond through lipolytic regulation and release of products including free fatty acids and pro- or anti-inflammatory adipokines. We previously demonstrated that activation of GPR109A results in release of the anti-inflammatory cytokine, adiponectin, from the adipocyte (171). Adiponectin is involved in the regulation of energy homeostasis through increasing glucose uptake in adipocytes and skeletal muscle and reducing hepatic glucose production (45; 232). Regulation of expression of these receptors is crucial for the metabolic sensing ability of the adipocyte and ultimately whole body energy homeostasis.

Obesity is associated with a chronic low-grade inflammation of the adipose tissue, characterized by increased production of pro-inflammatory cytokines and
chemokines like MCP-1, TNF-α, IL-1β, and IL-6. GPR81 down-regulation in ob/ob mice (a mouse model of obesity) has previously been demonstrated (70). GPR81 expression is also down-regulated in response to the inflammatory stimuli LPS, zymosan, and turpentine (70). While GPR81 expression is reduced by the inflammatory stimuli LPS, zymosan and turpentine, its expression does not appear to be regulated by the usual cytokines that induce metabolic changes during obesity-associated inflammation. Specifically, treatment of adipocytes with the pro-inflammatory cytokines typically elevated in the adipose tissue during obesity, including TNF-α, IL-1β, IL-6, or interferon γ fails to reduce the expression of GPR81 (70). Here, we demonstrate for the first time that high fat diet-induced obesity also reduces GPR81 gene and protein expression. The exact mechanism for the down-regulation of GPR81 in obesity has yet to be fully elucidated, but it does not appear to be mediated through the increased production of pro-inflammatory cytokines produced abundantly in adipose tissue of obese subjects.

To date, no studies have demonstrated any effect of obesity or inflammation on GPR109A expression in adipose tissue. Our findings demonstrate that HFD-induced obesity decreases adipose tissue GPR109A gene and protein expression. Contradictory to our findings, Digby et al. report that exposure of 3T3-L1 adipocytes to TNF-α (1.0 ng/ml) for four hours up-regulates GPR109A mRNA expression (65). We demonstrated that HFD-induced obesity results in a marked increase in TNF-α mRNA (Chapter II), concomitant with reduced
GPR109A gene and protein expression. Others have shown an increase in GPR109A expression in retinal pigment epithelial cells from \textit{db/db} mice and streptozotocin-induced diabetic mice compared to normal controls (77). Additionally, GPR109A expression is increased in macrophages treated with interferon $\gamma$ (188). The adipose tissue of the mice on the HFD has been infiltrated by macrophages (Chapter II). Hence, in the obese mice there are two cell types expressing GPR109A in the adipose tissue: adipocytes and macrophages. Since we examined whole epididymal white adipose tissue, we cannot differentiate the effects of HFD and niacin on GPR109A expression in adipocytes and macrophages. We demonstrated a dramatic reduction in GPR109A gene and protein expression in the adipose tissue in response to HFD. Others would say that GPR109A expression in macrophages would increase during HFD because of all the inflammatory stimuli present. It is possible that HFD has opposing effects in the two cell types.

The question arises: “Why would HFD reduce expression of GPR109A and GPR81 in the adipose tissue?” Obese humans have elevated circulating concentrations of $\beta$-hydroxybutyrate and lactate, the endogenous ligands for GPR109A and GPR81, respectively (46; 88). It is quite possible that elevated levels of $\beta$-hydroxybutyrate and lactate activate their receptors and induce their internalization.
In this study, we also demonstrated niacin’s novel ability to increase GPR109A and GPR81 protein expression in the adipose tissue. Niacin possesses anti-inflammatory properties, independent of its lipid-modifying capabilities (65; 133). Activation of the nuclear receptor PPARγ enhances GPR109A and GPR81 expression in adipocytes (106). It has been demonstrated that niacin treatment increases PPARγ expression and transcriptional activity (113), identifying a possible mechanism for niacin’s effects on receptor expression. Since niacin increased expression of both receptors and it is not a ligand for GPR81, the increase in receptor expression is probably not due to niacin’s binding to the receptors directly, but rather indirect effects of niacin on receptor expression, possibly through improvements in the metabolic milieu of the adipose tissue microenvironment.

It may appear counterintuitive to conventional pharmacology that niacin treatment did not reduce GPR109A expression, as it is the ligand for this receptor. However, there are examples of receptor up-regulation which occurs in response to chronic agonist stimulation including the β3-adrenergic receptor (211), gonadotropin-releasing hormone receptor (132), angiotensin II receptor (68), dopamine receptor (56), and endogenous somatostatin receptors (99; 172). Niacin treatment results in rapid GPR109A internalization in a dose- and time-dependent manner via the clathrin-coated pit pathway. Internalized niacin receptors are then recycled upon agonist removal (121). Whether a desensitized and internalized G protein-coupled receptor recycles or is downregulated can
depend on the length of agonist treatment (110). Prolonged exposure of cells to receptor agonists results in down- or up-regulation of receptor expression due to alterations in the rate of receptor degradation and synthesis (85). It is possible that the niacin-mediated increases in GPR109A are due to ligand binding of the receptor, but improvements in the inflammatory state of the adipose tissue are more likely.

GPR109A and GPR81 are not the only receptors that give the adipocyte the ability to sense its environment. Other receptors expressed highly in the adipose tissue that give the adipocyte metabolic sensing abilities are GPR43 and GPR120, receptors for endogenous free fatty acids (31; 91). Cornall et al. measured their expression in skeletal muscle, liver and cardiac tissue in response to HFD. Interestingly they demonstrated HFD-induced increases in GPR43 gene expression in the skeletal muscle and liver, while GPR120 expression was increased in the skeletal muscle and cardiac tissue (55). They did not examine receptor expression in the adipose tissue, however others have reported increased adipose tissue expression of GPR43 and GPR120 in response to high fat diet (84; 92).

The mechanism by which HFD reduces and niacin treatment increases GPR109A and GPR81 expression remains to be elucidated. However, the current and previous investigations indicate that inflammation may play a key role in regulation of receptor expression.
Obesity, the accumulation of excess adipose tissue, has reached epidemic proportions in the U.S. Adipose tissue is an endocrine organ that actively participates in whole body energy metabolism through production and secretion of biologically active molecules termed adipokines. Obesity results in adipose tissue dysfunction and a dysregulation of adipokine secretion, contributing to low-grade inflammation and insulin resistance. Niacin is used in the treatment of obesity-related comorbidities, such as the metabolic syndrome, for its ability to lower plasma levels of total and LDL-cholesterol, triglycerides, lipoprotein(a), and to increase HDL-cholesterol. In addition to beneficial effects on blood lipids, anti-inflammatory effects of niacin have been demonstrated in the lung, kidney, vascular endothelial cells, monocytes, retinal pigment epithelial cells, and 3T3-L1 adipocytes. However, no studies have been conducted to look into anti-inflammatory properties of niacin in the adipose tissue. Additionally, recent evidence demonstrates that niacin can alter the production and secretion of certain adipokines, independently of its effects on plasma lipids. Further, the obesity-related adipose tissue dysregulation may result from altered metabolic sensing abilities of the adipocyte due to changes in expression of receptors.
involved in metabolism. Therefore, we examined the effects of high fat diet and niacin on adipose tissue inflammation and the adipokines adiponectin and RBP4. In addition, we assessed the impact of high fat diet and niacin on the expression of GPR109A and GPR81, receptors involved in the metabolic sensing ability of the adipocyte.

Initially, we demonstrated niacin receptor-dependent anti-inflammatory effects of niacin in the adipose tissue as evidenced by reduced CD11c, MCP-1, and IL-1β and increased adiponectin. We then discovered that serum concentrations of RBP4, a cytokine linked to insulin resistance, were reduced in response to niacin treatment, and this effect occurred independently of activation of the niacin receptor. Niacin had no effect on RBP4 gene or protein expression in mice, nor did it have an effect on RBP4 secretion from isolated adipocytes or hepatocytes. Lastly, we showed a high fat diet-induced reduction in adipose tissue gene and protein expression of GPR109A and GPR81. Interestingly, niacin increased adipose tissue protein expression of GPR109A and GPR81, possibly due to improvements in the inflammatory state of the adipose tissue.

Future studies should include knocking out or neutralizing adiponectin in mice to determine the role of adiponectin in the anti-inflammatory effects of niacin. Additionally, to more completely elucidate the effects of niacin in the adipose tissue, it will be necessary to separate the stromal vascular fraction from the adipocyte fraction of the adipose tissue to delineate the effects of niacin on
adipocytes and macrophages independently, as well as in the whole adipose tissue. Furthermore, using flow cytometry in future studies will allow for quantitation of macrophage number in the adipose tissue, as well as separation of macrophages by polarity. Elucidating the mechanism of niacin’s anti-inflammatory effects in the adipose tissue could provide insight into treatment for insulin resistance resulting from obesity-associated adipose tissue dysfunction.
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