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Niacin stimulates adiponectin secretion through the GPR109A receptor

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[1]*Boshell Diabetes and Metabolic Diseases Research Program, Department of Anatomy, Physiology, and Pharmacology, Auburn University, Auburn, Alabama;* [2]*Institute of Pharmacology, University of Heidelberg, Heidelberg, Germany; and* [3]*Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana*

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Plaisance EP, Lukasova M, Offermanns S, Zhang Y, Cao G, Judd RL. Niacin stimulates adiponectin secretion through the GPR109A receptor. *Am J Physiol Endocrinol Metab* 296: E549–E558, 2009. First published January 13, 2009; doi:10.1152/ajpendo.91004.2008.—Niacin (nicotinic acid) has recently been shown to increase serum adiponectin concentrations in men with the metabolic syndrome. However, little is known about the mechanism(s) by which niacin regulates the intracellular trafficking and secretion of adiponectin. Since niacin appears to exert its effects on lipolysis through receptor (GPR109A)-dependent and -independent pathways, the purpose of this investigation was to examine the role of the recently identified GPR109A receptor in adiponectin secretion. Initial in vivo studies in rats demonstrated that niacin (30 mg/kg po) acutely increases serum adiponectin concentrations, whereas it decreases NEFAs. Further in vitro studies demonstrated an increase in adiponectin secretion and a decrease in lipolysis in primary adipocytes following treatment with niacin or β-hydroxybutyrate (an endogenous ligand of the GPR109A receptor), but these effects were blocked when adipocytes were pretreated with pertussis toxin. Niacin had no effect on adiponectin secretion or lipolysis in 3T3-L1 adipocytes, which have limited cell surface expression of the GPR109A receptor. To further substantiate these in vitro findings, wild-type and GPR109A receptor knockout mice were administered a single dose of niacin or placebo, and serum was obtained for the determination of adiponectin and NEFA concentrations. Serum adiponectin concentrations increased and serum NEFAs decreased in the wild-type mice within 10 min following niacin administration. However, niacin administration had no effect on adiponectin and NEFA concentrations in the GPR109A receptor knockout mice. These results demonstrate that the GPR109A receptor plays an important role in the dual regulation of adiponectin secretion and lipolysis.

nicotinic acid; PUMA-G; HM74A; nonesterified fatty acids; lipolysis

NIACIN (NICOTINIC ACID) HAS BEEN USED for more than 50 years as a pharmacological agent for the treatment of dyslipidemia and remains an effective strategy to decrease serum triglycerides and increase HDL cholesterol concentrations (6, 19). In one of the first randomized clinical trials of heart disease prevention, niacin reduced serum triglyceride concentrations by 27% and nonfatal myocardial infarctions and cardiovascular disease (CVD) mortality by 27 and 26%, respectively (6a). More recent studies provide evidence that niacin is associated with a reduction in the number of angiographically documented vascular lesions and carotid artery intima-media thickness in patients with CVD (16, 31).

The cardioprotective benefits of niacin have been attributed primarily to improvements in blood lipid and lipoprotein characteristics and reductions in vascular inflammation and thrombosis (18). However, recent studies have demonstrated that niacin significantly modulates serum adipokine concentrations. Westphal et al. (36) demonstrated that 6 wk of niacin treatment increased serum adiponectin concentrations by 54% in obese men with the metabolic syndrome. Investigations from our laboratory support these findings and further demonstrate that the increase in serum total adiponectin concentrations is due primarily to an increase in the biologically active high-molecular weight (HMW) form of adiponectin (25). These effects of niacin on serum adiponectin concentrations are important because adiponectin is a prominent biomarker of metabolic disease with insulin-sensitizing, anti-inflammatory, and antiatherogenic properties (32). Serum adiponectin concentrations are generally low in obesity and are strongly correlated with CVD (12). Therefore, increases in serum adiponectin concentrations observed following niacin administration may play an important role in the reduction in CVD risk.

Reductions in adipose tissue lipolysis following niacin administration have been attributed to activation of the recently identified G protein-coupled receptor 109A (GPR109A) (27, 33, 37). GPR109A (PUMA-G in mice and HM74A in humans) is a seven-transmembrane G protein-coupled receptor of the G_i family that is expressed mainly in white adipocytes and immune cells such as monocytes and neutrophils (23). A number of exogenous agonists, including niacin, acipimox, and acifran, have been shown to activate the receptor (13). β-Hydroxybutyrate (β-OHB), a ketone body produced by the oxidation of fatty acids, has also been identified as an endogenous ligand of the receptor (30). Although pharmacological inhibition and genetic knockout of GPR109A provides evidence that the receptor is required for niacin-mediated reductions in adipose tissue lipolysis (33, 38), the role of the GPR109A receptor in the regulation of adiponectin secretion is unknown.

Therefore, the purpose of this investigation was to examine the role of the GPR109A receptor in the regulation of adiponectin secretion. Results presented in this paper clearly demonstrate that stimulation of the GPR109A receptor leads to a rapid increase in adiponectin secretion.

MATERIALS AND METHODS

Materials. All cell culture reagents were obtained from Invitrogen (Grand Island, NY). Rabbit monoclonal and polyclonal adiponectin and leptin antibodies were from Affinity Bioreagents (Golden, CO). Resistin polyclonal antibodies were from Biovision Research Products (Mountain View, CA). HM74A antibodies were obtained from

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Santa Cruz Biotechnology (Santa Cruz, CA). Collagenase was from Worthington Biochemical (Lakewood, NJ). Collagen was obtained from INAMED (Fremont, CA). Niacin, β-OHB, and pertussis toxin (PTX) were from Sigma (St. Louis, MO). All other reagents were obtained from commercial sources and were of analytical grade.

In vivo niacin treatments. All experiments were approved by the Auburn University Institutional Animal Care and Use Committee prior to initiation. Male Sprague-Dawley rats (175–225 g) with indwelling femoral vein catheters were purchased from Charles River Laboratories (Wilmington, MA). After a 7-day acclimatization period, rats were fasted for 16 h and then randomly assigned to a niacin-treated ($n = 6$) or placebo control ($n = 6$) group. Niacin was prepared in PBS and orally administered at a concentration of 30 mg/kg. Control animals received PBS as a placebo at an equivalent volume. Blood sampling was conducted immediately before and 10 min and 1, 3, 6, 12, and 24 h following niacin or placebo administration. Catheter patency was maintained by injecting ~200 μl of 0.9% saline into the dead space of the catheter as needed during the course of the study (every 2–3 days) and following each blood sample. Approximately 250 μl of blood was "wasted" prior to sampling to prevent dilution of the blood sample with the 0.9% saline in the catheter.

Blood samples were analyzed for serum total and HMW adiponectin, nonesterified fatty acids (NEFAs), insulin, and glucose concentrations. Adiponectin, leptin, and insulin concentrations were analyzed with ELISA kits from Millipore (Billerica, MA). HMW adiponectin was analyzed with an ELISA kit from Bio-Vender (Candler, NC). Resistin concentrations were analyzed with ELISA kits from B-Bridge International (Mountain View, CA). NEFA assays were conducted using a NEFA C kit by Wako (Richmond, VA). Glucose concentrations were determined using the glucose oxidase method (Raichem, San Marcos, CA). Intra-assay coefficients of variation for adiponectin, leptin, resistin, NEFAs, insulin, and glucose were 3.5, 3.9, 4.2, 5.8, 1.6, and 6.5%, respectively. Interassay coefficients of variation for adiponectin, leptin, resistin, NEFAs, insulin, and glucose were 3.9, 3.5, 3.8, 6.1, 2.1, and 7.2%, respectively.

Primary rat adipocyte isolation. Epididymal fat pads were obtained from male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 175–225 g each. Adipocytes were isolated as described previously (24). In brief, epididymal fat was minced and digested in HEPES buffer with type I collagenase at 37°C with gentle shaking for 30 min. Adipocytes were resuspended in DMEM supplemented with 1% FBS and incubated for 30 min at 37°C. The isolated adipocytes (150 μl of 2:1 ratio of packed cells to medium) were then transferred to 500 μl of a collagen matrix in six-well culture plates, as described in Ref. 24. After incubating at 37°C for 50 min, DMEM supplemented with 0.2% fatty acid-free bovine serum albumin was added overnight prior to all experiments.

Primary rat adipocyte treatments. Cells were initially treated with niacin (1–100 μM) to establish the concentration-dependent and temporal responses of niacin on adiponectin secretion and lipolysis. Adipocytes were then treated with β-OHB (2–15 mM) to examine the effects of an endogenous GPR109A receptor ligand on adiponectin secretion and lipolysis. Medium was collected at 1, 3, 6, and 24 h for the determination of adiponectin and glycerol concentrations.

To further characterize the role of the GPR109A receptor, primary adipocytes were then pretreated with PTX (an inhibitor of G protein coupling) for 18 h, followed by niacin or β-OHB treatment. Medium was collected at 3 h for the determination of adiponectin and glycerol concentrations. In another series of studies, adipocytes were treated with niacin (10 μM), forskolin (50 μM), or niacin plus forskolin to examine the effects of niacin on basal and stimulated adiponectin and glycerol secretion.

Medium was obtained at designated times and adiponectin in the medium was measured using SDS-PAGE (10%) and immunoblotting. Briefly, proteins were 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 rabbit polyclonal antiadiponectin antibody (1:1,000). Membranes were then washed three times with PBS-0.1% Tween-20 and incubated with infrared-conjugated secondary antibodies (1:20,000) for 1 h. Blots were scanned with a LI-COR Odyssey infrared scanner. Glycerol concentrations were measured using a free glycerol reaction (Sigma), as described by Richman (26).

3T3-L1 fibroblast differentiation. 3T3-L1 mouse fibroblasts were purchased from American Type Culture Collection (Manassas, VA) and were grown in DMEM supplemented with 10% FBS at 37°C in an atmosphere of 5% CO_2, as described previously (2). Differentiation was induced 2 days after confluence by incubating the cells for 2 days in DMEM containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μM dexamethasone, and 10 μg/ml insulin. Adipocytes were used for experimentation 10–12 days postdifferentiation. Adipogenesis was monitored by morphological examination of the cells for the accumulation of lipid droplets with Oil Red O (0.2%) staining.

Previous studies have been unable to detect GPR109A (PUMA-G) expression in 3T3-L1 adipocytes (38). So 3T3-L1 adipocytes were stably transfected with retroviral constructs of the human form of GPR109A (HM74A), as described previously (38). Preadipocytes were then differentiated as described above for native 3T3-L1 adipocytes.

3T3-L1 adipocyte treatments. Native and 3T3-L1 adipocytes expressing the HM74A receptor were serum starved for 3 h with DMEM supplemented with 0.2% BSA prior to all experiments. Cells were treated with vehicle (DMEM + 0.2% BSA) or niacin (1–100 μM). Medium was sampled at 3 h as described previously for primary adipocytes to determine the concentration-dependent and temporal effects of niacin on adiponectin secretion and glycerol release. A final series of experiments was conducted to determine the effects of niacin and β-OHB on adiponectin secretion following forskolin-stimulated lipolysis. Experiments were also conducted to examine the effects of PTX pretreatment on niacin and β-OHB-mediated increases in adiponectin secretion.

Plasma membrane isolation and Western blot analysis of GPR109A. Primary rat adipocytes and 3T3-L1 adipocytes were homogenized using a Dounce homogenizer (10 strokes) and centrifuged in HEPES-EDTA-sucrose buffer to isolate the plasma membrane fraction. Proteins (35 μg) were separated by SDS-PAGE, transferred to nitrocellulose membranes, and incubated with goat polyclonal anti-HM74A (GPR109A) or mouse monoclonal anti-Na^+K^+ATPase α2 (plasma membrane marker) antibodies (1:1,000 dilution for both) overnight. This was followed by incubation with infrared-conjugated secondary antibodies (1:20,000). Blots were scanned with a LI-COR Odyssey infrared scanner.

cAMP measurement. Native 3T3-L1 adipocytes and 3T3-L1 adipocytes expressing the HM74A receptor were treated on 60-mm culture dishes with niacin (10 μM) in the presence or absence of forskolin (50 μM) to determine the effects of niacin on cAMP production. Primary adipocytes were treated similarly in suspension using ~1×10^6 cells/ml, as described previously (14). 3T3-L1 and primary rat adipocytes were also pretreated (3 h) with PTX (50 ng/ml) to examine the effects of G protein uncoupling on cAMP production. Following each of the treatments, adipocytes were lysed in 0.1 M HCl using an EIA kit from Cayman Chemical (Ann Arbor, MI) according to the manufacturer's instructions.

In vivo ($GPR109A^{+/+}$ vs. $GPR109A^{-/-}$) studies. All experiments were approved by the University of Heidelberg Institutional Animal Care and Use Committee prior to initiation. Homozygous PUMA-$G^{-/-}$ mice were generated on a C576BL/6 background, as described previously (33). Eight PUMA-$G^{-/-}$ and six wild-type littermates were randomly assigned to receive a single dose of niacin (30 mg/kg, 150 μl) or an equal volume of vehicle (0.9% saline) by intraperitoneal injection. Following a 7-day washout period, animals were crossed over to the opposing treatment (animals receiving treatment received vehicle, and animals receiving vehicle received treatment). Animals were fasted for 16 h before each experiment. Blood samples were

obtained retroorbitally immediately before and 10 and 60 min following niacin administration. Serum was separated by centrifugation and analyzed for total adiponectin, HMW adiponectin, leptin, resistin, and NEFAs.

Statistical analysis. In vitro studies were analyzed using a one-way ANOVA. In vivo studies were analyzed using multiple repeated-measures ANOVAs. When differences were observed, a Student-Neuman-Keuls post hoc test was conducted to determine where differences occurred. Relationships between baseline physiological characteristics and changes in the dependent variables were determined using Pearson product-moment correlation coefficients. Power analyses suggested that six animals would be required for the in vivo studies as determined using the variance in total adiponectin at an effect size of 0.8 and an α-level of 0.05. Data were analyzed using the Statistical Analysis System (SAS for Windows; SAS Institute, Cary, NC). Significance was accepted at the $P < 0.05$ level.

RESULTS

Niacin acutely increases total adiponectin concentrations and decreases lipolysis in rats. Short-term administration of extended-release niacin increases adiponectin secretion in obese humans (25, 36). Since a single dose of niacin acutely reduces NEFA concentrations to a similar extent as short-term administration (4, 26, 34), we hypothesized that a single dose of niacin might also increase serum adiponectin concentrations. In the current investigation, total serum adiponectin concentrations increased by 37% within 10 min, peaked within 1 h, and remained elevated above baseline for ≤24 h (Fig. 1*A*). In a similar fashion, the HMW adiponectin multimer increased from 4.7 ± 0.8 μg/ml to 6.9 ± 0.7% (47%; $P < 0.05$) within 10 min following niacin administration and remained elevated above baseline throughout the blood-sampling period. The increase in total serum adiponectin concentrations was accounted for primarily by a 56–64% increase in the HMW multimer of adiponectin. Serum NEFA concentrations decreased by 62% within 10 min and "rebounded" back to baseline within 3 h (Fig. 1*B*). Interestingly, adiponectin followed a reciprocal pattern that correlated with the reduction in serum NEFAs. The increase in total and HMW adiponectin concentrations was strongly correlated with the reduction in serum NEFAs observed at 10 min ($r = -0.89$, $P < 0.05$; $r = -0.87$, $P < 0.05$, respectively). Serum insulin and glucose concentrations were not significantly different from control following niacin administration (data not shown).

Serum leptin and resistin concentrations were measured to characterize the effects of niacin on other adipokines. Leptin concentrations were similar between control and treated animals at baseline (6.4 ± 0.8 vs. 6.7 ± 0.6, $P > 0.05$) and were not altered in either group following niacin administration throughout the blood-sampling period. Resistin concentrations were also similar between control and treated animals at baseline (4.9 ± 0.8 vs. 4.3 ± 1.0, $P > 0.05$) and were unaltered by the niacin treatment. These results suggest that a single dose of niacin may specifically target intracellular pathways that increase serum adiponectin concentrations.

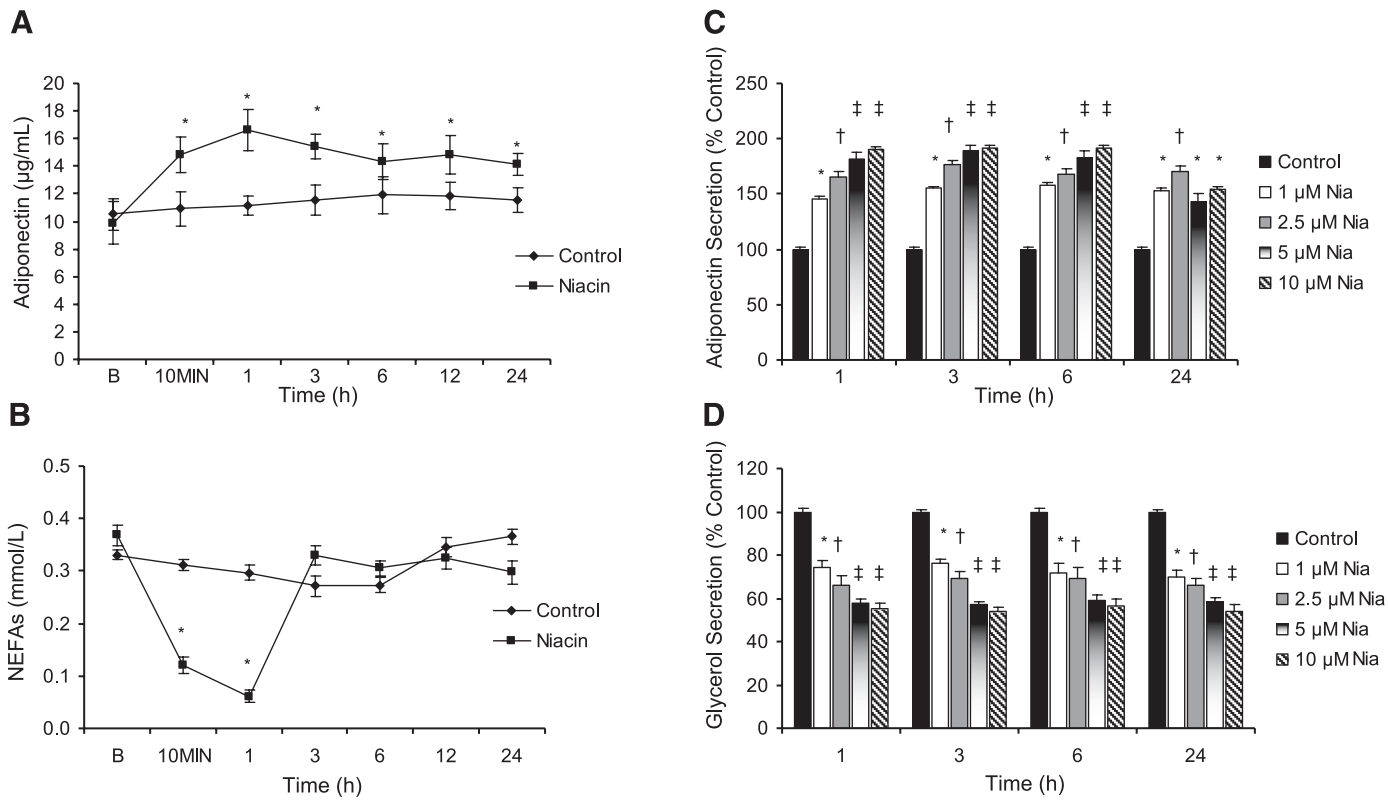

Fig. 1. Serum adiponectin (*A*) and nonesterified fatty acid (NEFA) concentrations (*B*) following a single dose of niacin (Nia) at 30 mg/kg vs. placebo control over the course of 24 h. Data are expressed as means ± SE. *Significant difference from control ($P < 0.05$ for all). *C*: Nia increases adiponectin secretion in primary rat adipocytes in a concentration-dependent manner. Adipocytes were treated with control medium or 1–10 μM Nia. Medium was sampled at 1, 3, 6, and 24 h. Adiponectin secretion into the medium was measured using SDS-PAGE and immunoblotting. *D*: Nia decreases lipolysis in a concentration-dependent manner. Lipolysis was measured by the release of glycerol into the culture medium. Data are expressed as means ± SE from 3 different experiments. Values with different symbols are significantly different ($P < 0.05$).

Niacin increases adiponectin secretion in primary rat adipocytes. To address the role of the GPR109A receptor in adiponectin secretion, primary rat adipocytes were initially treated with 1–300 μM niacin to determine a concentration range for effects on adiponectin secretion and lipolysis. Niacin concentration dependently increased adiponectin secretion and decreased lipolysis in primary rat adipocytes over the course of 24 h (Fig. 1, *C* and *D*). Maximal stimulation of both adiponectin secretion and glycerol release (marker of lipolysis) occurred between 5 and 10 μM. Niacin concentrations ranging from 50 to 300 μM produced no additional effects on adiponectin or glycerol release (data not shown).

β-OHB increases adiponectin secretion in primary rat adipocytes. Based on the recent discovery that β-OHB is an endogenous ligand of the GPR109A receptor, we determined whether physiological concentrations of β-OHB (2–15 mM) achieved during starvation might increase adiponectin secretion. β-OHB concentration dependently increased adiponectin secretion and decreased glycerol release ≤15 mM (15 mM represented; Fig. 2, *A* and *B*). Similarly to our in vivo observations, β-OHB and niacin produced no significant changes in leptin or resistin secretion, suggesting that short-term treatment with agonists of the GPR109A receptor have little influence on leptin or resistin secretion (data not shown).

PTX blocks niacin- and β-OHB-mediated increases in adiponectin secretion. PTX is a nonselective G protein-uncoupling agent frequently used to examine the function of G protein-coupled receptors. Pretreatment with PTX blocked the niacin-mediated elevation in adiponectin secretion and reduction in glycerol release in primary rat adipocytes (Fig. 3, *A* and *B*). Similar effects were observed when primary adipocytes were pretreated with PTX followed by β-OHB administration (Fig. 3, *C* and *D*). These results provide further evidence that niacin and β-OHB increase adiponectin secretion and decrease adipose tissue lipolysis similarly via the GPR109A receptor.

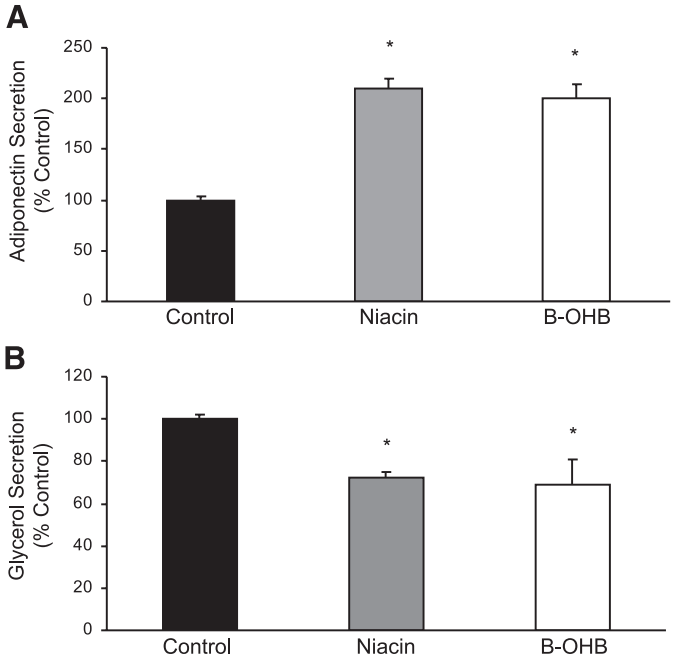

Fig. 2. *A*: β-hydroxybutyrate (β-OHB) increases adiponectin secretion and decreases glycerol secretion similarly to Nia. Rat adipocytes were treated with vehicle, 10 μM Nia, or 15 mM β-OHB. Medium was sampled at 3 h. Adiponectin secretion was measured using SDS-PAGE and immunoblotting. *B*: β-OHB decreases lipolysis similarly to Nia. Lipolysis was measured by the release of glycerol into the culture medium. Data are expressed as means ± SE from 3 different experiments. $*P < 0.05$.

Niacin does not increase adiponectin secretion in 3T3-L1 adipocytes. Zhang et al. (38) have reported previously that differentiated 3T3-L1 adipocytes do not express the endogenous mouse form of the GPR109A receptor (PUMA-G) and found that niacin had virtually no effect on lipolysis in these cells. However, stable 3T3-L1 clones expressing HM74A (human form of GPR109A) receptors demonstrate a concentration-dependent inhibition of lipolysis (38). In line with these previous findings, we found that native 3T3-L1 adipocytes possess a lower cell surface expression of the GPR109A receptor compared with primary adipocytes or 3T3-L1 adipocytes expressing the human form of GPR109A (HM74A; Fig. 4). Niacin had no effect on basal adiponectin secretion or glycerol release in native 3T3-L1 adipocytes (Fig. 5, *A* and *B*), whereas in primary rat adipocytes, niacin increased adiponectin secretion and decreased glycerol release at 10 μM (5, *C* and *D*, respectively). Niacin also increased adiponectin secretion and decreased glycerol release in 3T3-L1 adipocytes expressing the HM74A receptor (Fig. 5, *E* and *F*). Niacin concentrations in the range of 50–300 μM had no effect on adiponectin or glycerol release in 3T3-L1 adipocytes (data not shown). Forskolin, a direct activator of adenylate cyclase activity, significantly decreased adiponectin secretion and increased glycerol release compared with control in all cell types (Fig. 5, *A–F*). Niacin increased forskolin-stimulated reductions in adiponectin secretion and elevations in lipolysis in primary adipocytes (Fig. 5, *C* and *D*) and 3T3-L1 adipocytes expressing the HM74A receptor (Fig. 5, *E* and *F*) but not native 3T3-L1 adipocytes (Fig. 5, *A* and *B*).

cAMP concentrations are decreased in primary adipocytes and 3T3-L1 adipocytes expressing the HM74A receptor following niacin administration. In preliminary studies, basal levels of cAMP were measurable (300–350 pmol/ml), and niacin reduced basal levels below detectable levels of the high-sensitivity cAMP EIA (data not shown). Therefore, in subsequent studies we employed forskolin to increase cAMP levels prior to niacin exposure. Intracellular cAMP concentrations were dramatically increased within minutes following the administration of forskolin (50 μM) in primary adipocytes (Fig. 6*A*) and 3T3-L1 adipocytes with HM74A expression (Fig. 6*C*). Niacin (10 μM) decreased forskolin-stimulated cAMP production in both cell types. Conversely, niacin did not reduce intracellular cAMP production in native 3T3-L1 adipocytes following forskolin stimulation (Fig. 6*E*). Pretreatment with PTX attenuated niacin-mediated reductions in forskolin-stimulated cAMP production in primary adipocytes (Fig. 6*B*) and 3T3-L1 adipocytes expressing the HM74A receptor (Fig. 6*D*). Niacin did not alter forskolin-stimulated cAMP production in native 3T3-L1 adipocytes in the presence or absence of PTX treatment (Fig. 6, *E* and *F*). The observation that niacin alters the release of adiponectin and lipolysis in primary adipocytes and in 3T3-L1 adipocytes expressing the HM74A receptor, but not native 3T3-L1 adipocytes, provides further evidence that niacin modulates basal adiponectin secretion and lipolysis through the GPR109A receptor.

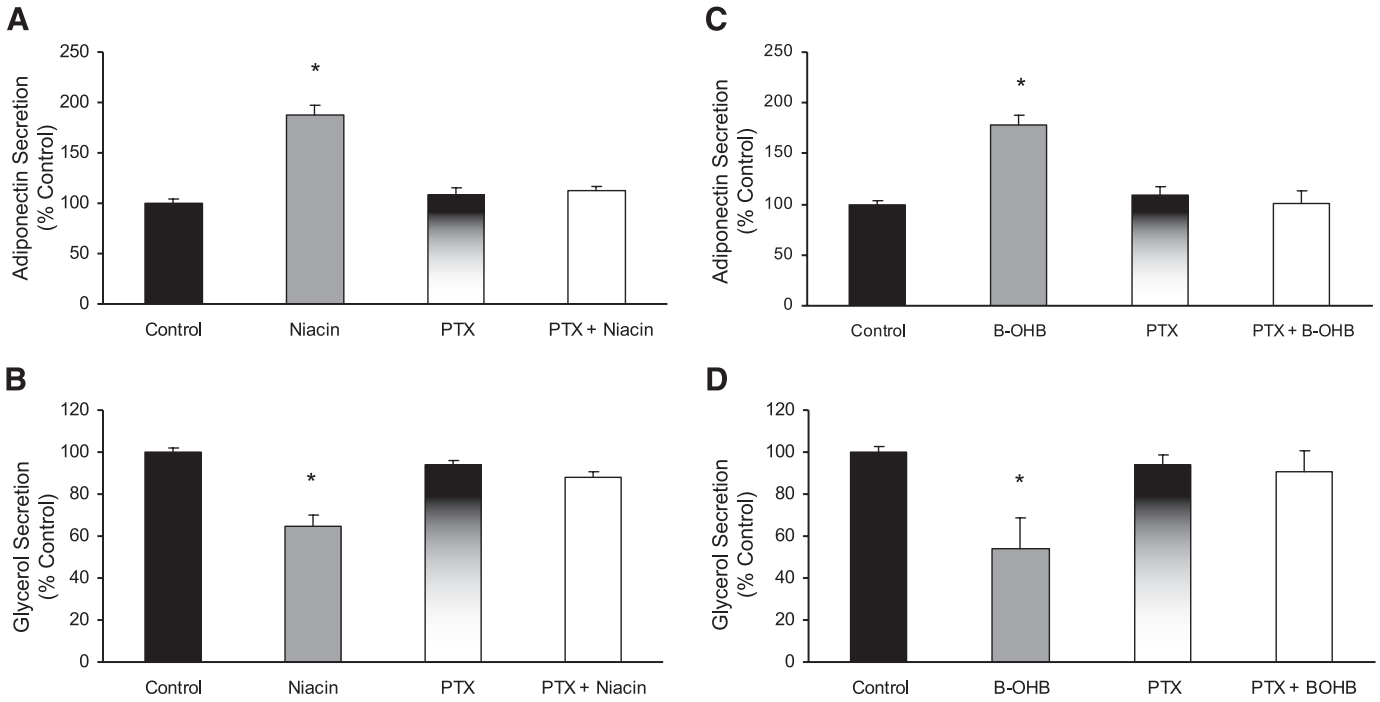

Fig. 3. *A*: pertussis toxin (PTX) blocks Nia-mediated increases in adiponectin secretion. Rat adipocytes were pretreated with vehicle or PTX for 18 h. Medium from each condition was removed and replaced with vehicle, 10 μM Nia, 50 ng/ml PTX, or Nia + PTX. Medium was sampled at 3 h. Adiponectin secretion was measured from the medium using SDS-PAGE and immunoblotting. *B*: PTX inhibits Nia-mediated lipolysis. Lipolysis was measured by the release of glycerol into the culture medium. *C*: PTX blocks β-OHB-mediated increases in adiponectin secretion. *D*: PTX blocks Nia-mediated decreases in glycerol secretion. Data are expressed as means ± SE from 3 different experiments. $*P < 0.05$.

Niacin does not increase total adiponectin concentrations in PUMA-G$^{-/-}$ (GPR109A$^{-/-}$) mice. Since our findings indicate that a single dose of niacin acutely increases serum adiponectin concentrations in rats expressing the GPR109A receptor and that pharmacological inhibition of the receptor attenuates the effects of niacin on adiponectin secretion and lipolysis in primary rat adipocytes, our final objective was to examine the effects of a single dose of niacin in PUMA-G$^{-/-}$-deficient mice. Niacin increased serum total adiponectin concentrations by 44% within 10 min in the PUMA-G$^{+/+}$ mice. The increase in adiponectin concentrations was accounted for primarily by a 57% increase in the HMW adiponectin multimer (Fig. 7*B*). Niacin decreased serum NEFA concentrations by 65% within 10 min following administration compared with control in the wild-type (PUMA-G$^{+/+}$) mice (Fig. 7*C*). The increase in serum adiponectin concentrations was correlated with the decrease in serum NEFAs ($r = -0.77$, $P < 0.05$). However, niacin had no effect on serum total adiponectin, HMW adiponectin, or NEFA concentrations in the PUMA-G$^{-/-}$ mice (Fig. 7, *A*, *B*, and *C*, respectively). Furthermore, niacin had no effect on serum leptin or resistin concentrations (Fig. 7, *D* and *E*). These studies clearly demonstrate the importance of the GPR109A receptor in the regulation of adiponectin secretion and lipolysis by niacin.

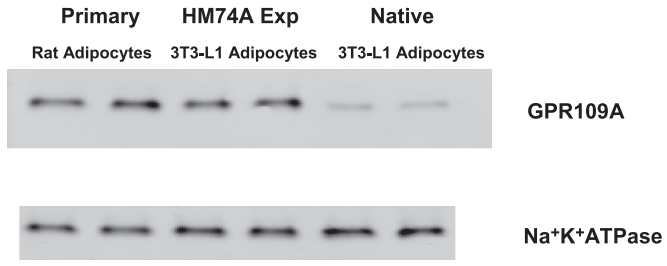

Fig. 4. G protein-coupled receptor 109A (GPR109A) cell surface expression was greater in primary adipocytes and 3T3-L1 adipocytes expressing the GPR109A receptor compared with native 3T3-L1 adipocytes. Na^+K^+ATPase (α2 form) was used as a protein loading control for the plasma membrane preparation.

DISCUSSION

The present investigation indicates for the first time that niacin increases serum total and HMW adiponectin concentrations by activating the recently identified GPR109A (PUMA-G in mice, HM74A in humans) receptor. We also demonstrate that β-OHB, a ketone body produced by the β-oxidation of fatty acids and the only known endogenous ligand of the GPR109A receptor, increases adiponectin secretion and reduces adipose tissue lipolysis at concentrations observed during prolonged fasting or starvation. The effects of niacin and β-OHB on basal adiponectin secretion and lipolysis were blocked when primary and 3T3-L1 adipocytes expressing the HM74A receptor were pretreated with PTX. These in vitro studies were corroborated with studies in GPR109A knockout mice, in which niacin administration had no effect on adiponectin secretion or lipolysis. Therefore, activation of the GPR109A receptor by endogenous and exogenous ligands results in an increase in adiponectin secretion and a decrease in lipolysis.

Niacin has been used for more than 50 years in the treatment of atherogenic dyslipidemia (5). Improvements in blood lipid and lipoprotein characteristics have primarily been credited for the well-described reduction in CVD morbidity and mortality

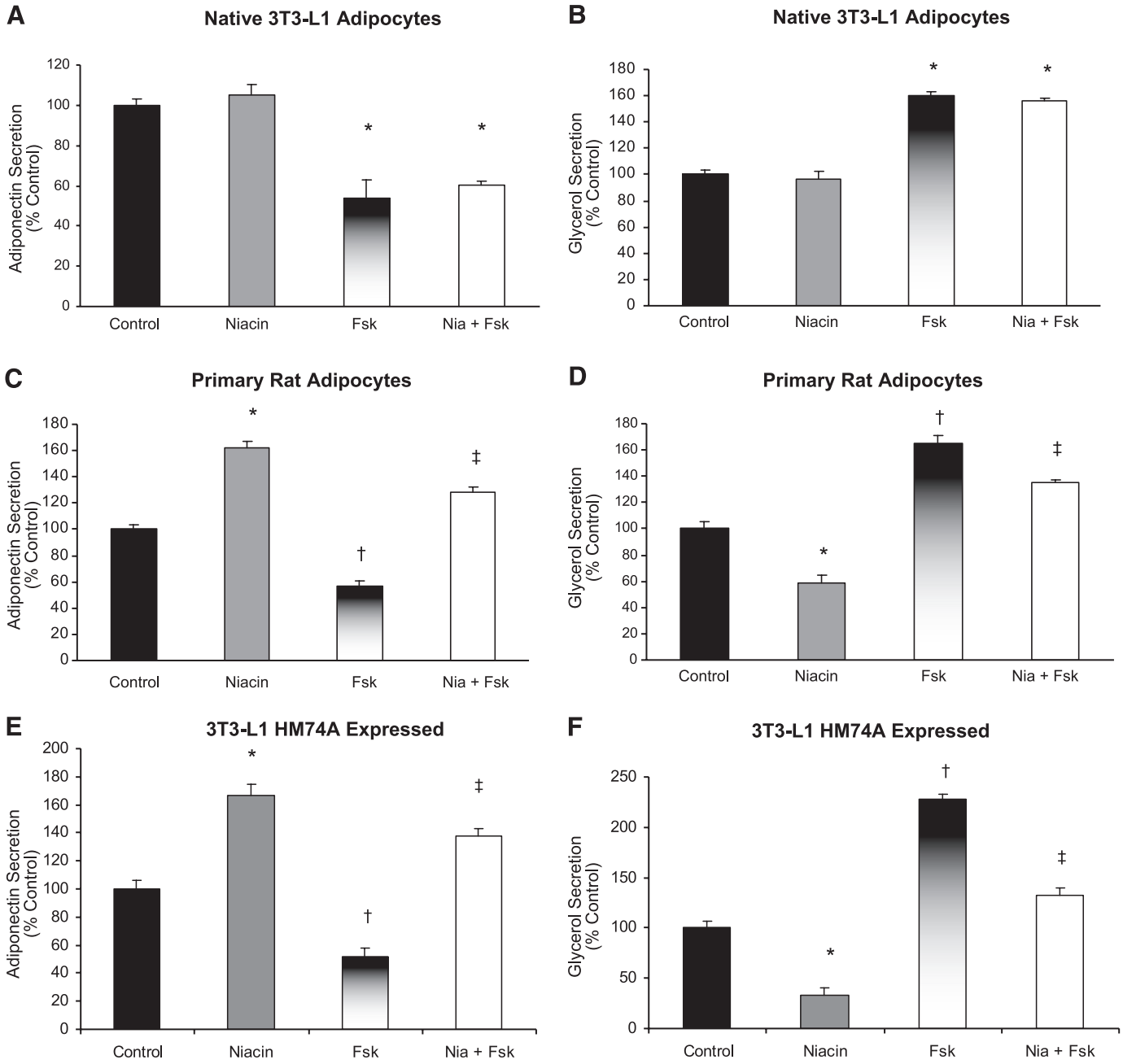

Fig. 5. Nia increases adiponectin secretion and decreases lipolysis in primary adipocytes but not 3T3-L1 adipocytes. Primary rat adipocytes and 3T3-L1 adipocytes were treated with 10 μM Nia, 50 μM forskolin (Fsk), or Nia + Fsk. Medium was sampled at 3 h. *A*: Nia had no effect on basal adiponectin secretion or Fsk-stimulated reductions in adiponectin secretion in 3T3-L1 adipocytes. *B*: Nia had no effect on basal or Fsk-stimulated elevations in lipolysis in 3T3-L1 adipocytes. *C*: Nia increased basal adiponectin secretion and Fsk-stimulated reductions in adiponectin secretion in primary adipocytes. *D*: Nia decreased basal and Fsk-stimulated lipolysis in primary rat adipocytes. *E*: Nia increased basal adiponectin secretion and Fsk-stimulated reductions in adiponectin secretion in 3T3-L1 adipocytes expressing the HM74A receptor. *F*: Nia decreases basal and Fsk-stimulated lipolysis in 3T3-L1 adipocytes expressing the HM74A receptor. Data are expressed as means ± SE from 3 different experiments. Values with different symbols are significantly different ($P < 0.05$).

associated with niacin administration. However, emerging evidence suggests that niacin may also modulate the trafficking and secretion of a number of adipokines, including leptin and adiponectin (25, 35, 36). Since serum adiponectin concentrations are inversely associated with the metabolic syndrome and CVD (32), understanding the mechanisms by which niacin increases serum adiponectin concentrations could provide a better understanding of the regulation of adiponectin trafficking and secretion and assist in the development of additional pharmacological or molecular biology strategies to increase adiponectin secretion in individuals at risk for CVD.

In the present investigation, we demonstrate that niacin acutely regulates adiponectin secretion. Previous studies in humans and rodents have consistently shown that a single dose of immediate-release niacin decreases lipolysis within 5–15 min by 70–85% for ≤4 h, depending on the dosage administered (4, 26). This is followed by a "rebound" in serum NEFAs as niacin levels diminish in the blood. Niacin (30 mg/kg)

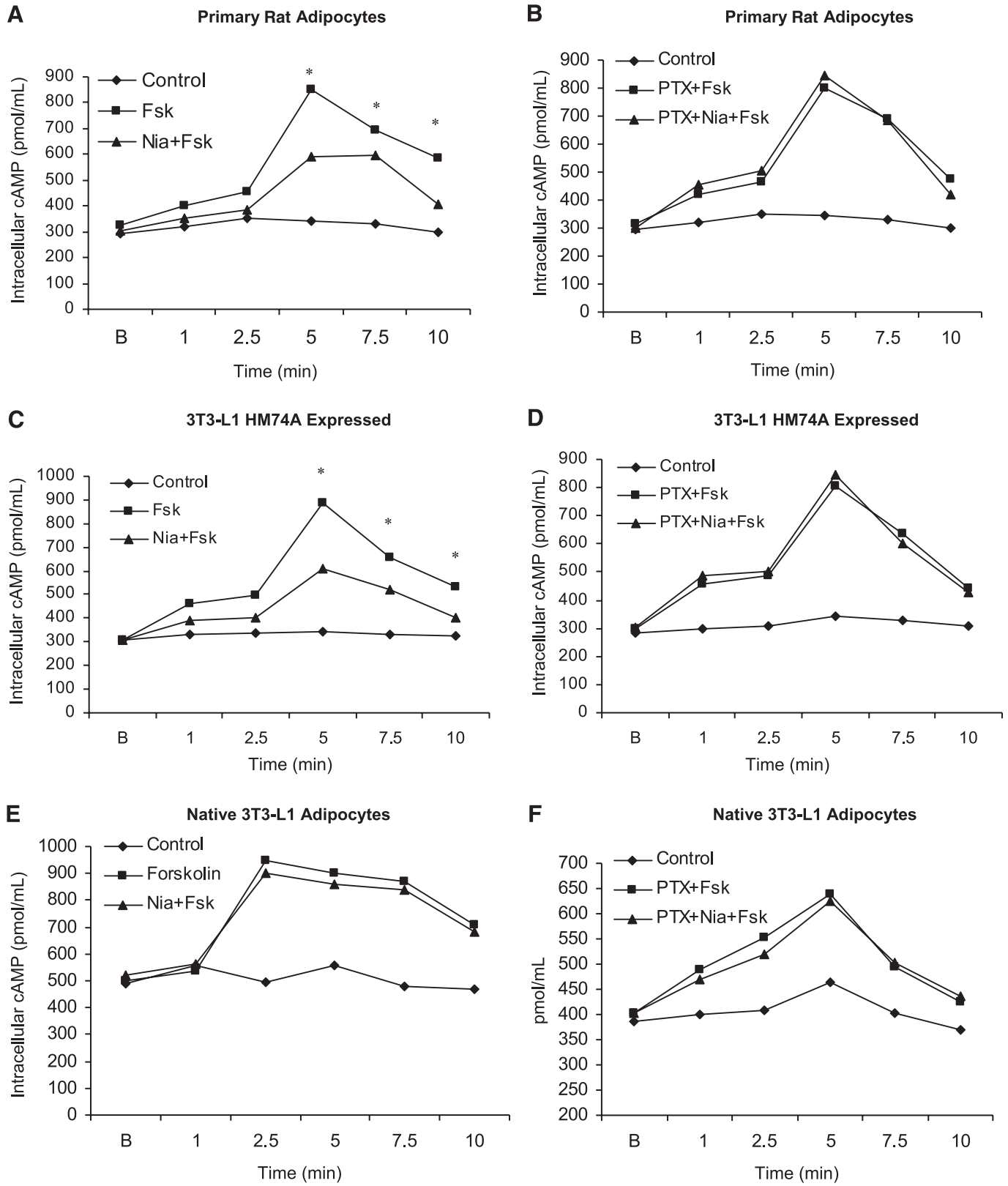

Fig. 6. Nia decreases Fsk-stimulated increases in intracellular cAMP in primary (*A*) and 3T3-L1 adipocytes (*C*) expressing the HM74A receptor. Pretreatment with PTX ameliorated the suppressive effects of Nia on intracellular cAMP production in primary (*B*) and 3T3-L1 adipocytes (*D*) expressing the HM74A receptor. Nia does not decrease Fsk-stimulated increases in intracellular adiponectin in 3T3-L1 adipocytes in the absence (*E*) or presence (*F*) of PTX. Values are means ± SE from at least 2 experiments. SE bars are not visible due to minimal variability. *Significant difference between Fsk and Nia + Fsk conditions.

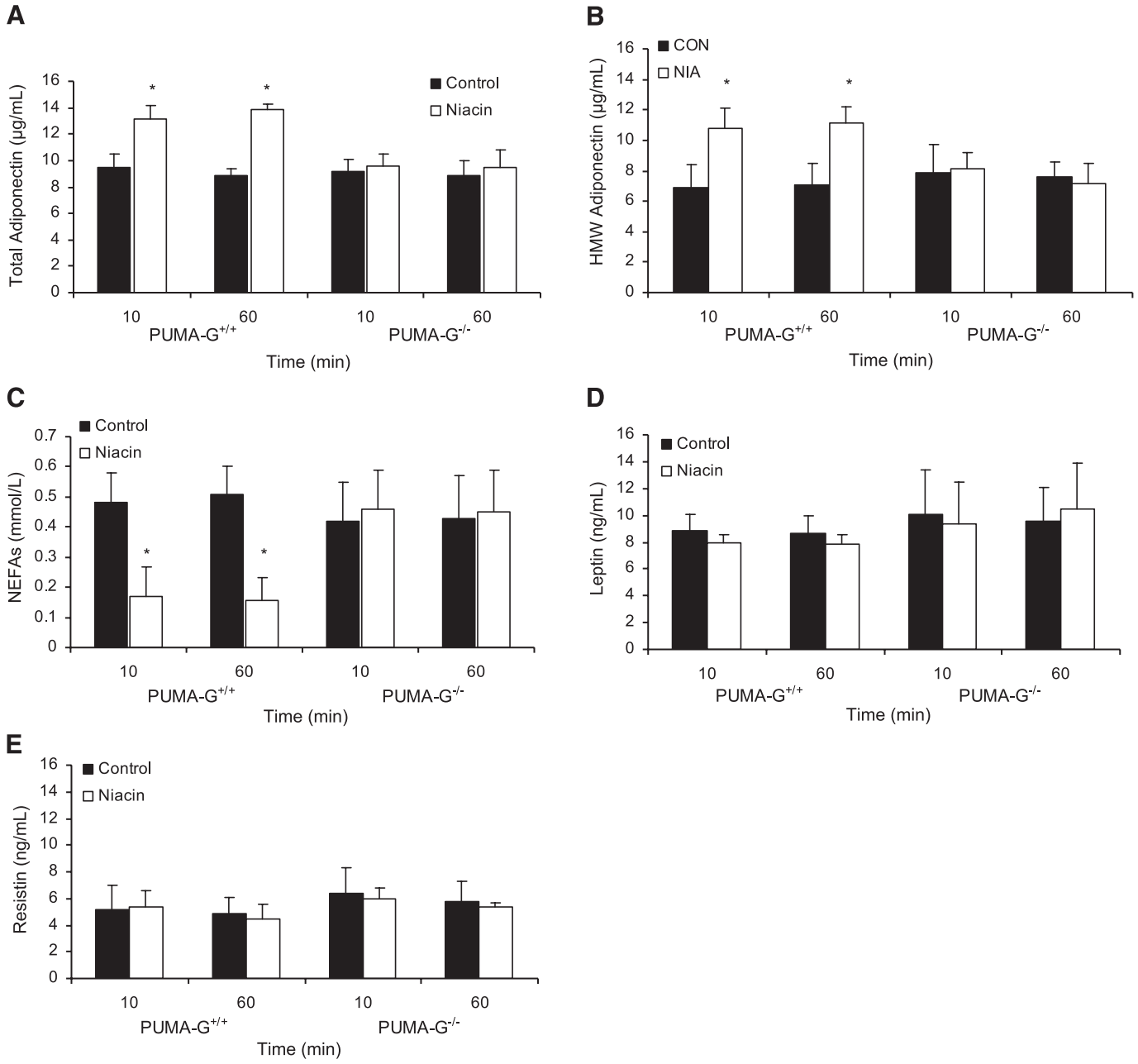

Fig. 7. Nia (30 mg/kg) acutely increases serum adiponectin concentrations and decreases serum NEFA concentrations in PUMA-G wild-type but not knockout mice. Total (*A*) and high-molecular weight (HMW; *B*) adiponectin concentrations increased within 10 min following Nia administration and remained elevated at 60 min in the PUMA-G$^{+/+}$ but not the PUMA-G$^{-/-}$ mice compared with control. *C*: NEFA concentrations decreased within 10 min following Nia administration and remained lower than control at 60 min in the PUMA-G$^{+/+}$ but not the PUMA-G$^{-/-}$ mice. Serum leptin (*D*) and resistin (*E*) concentrations were similar between control and Nia-treated groups at each time point. Values are means ± SE. *Significant difference from control ($P < 0.05$ for all).

increased serum adiponectin concentrations by 37% and decreased serum NEFAs by 62% within 10 min following administration in the current investigation. Adiponectin concentrations remained above baseline for ≤24 h, whereas NEFA concentrations returned to baseline between 1 and 3 h following niacin administration. Additional studies from our laboratory demonstrate that higher dosages of niacin (300 mg/kg) decrease serum NEFAs for ≤3 h but consequently result in serum NEFA concentrations that are ~80% above baseline within 5 h following administration (data not shown). The increase in serum NEFAs is associated with a more rapid decline in serum adiponectin concentrations. Indeed, NEFAs may act directly on the adipocyte and inhibit adiponectin secretion (22). Thus, the rebound increases in serum NEFAs with niacin may return the initial increases in adiponectin concentrations back toward normal.

Previous investigations in humans report no change or reductions in serum adiponectin concentrations following the administration of a single dose of acipimox despite similar reductions in adipose tissue lipolysis (3, 28). We propose that niacin and β-OHB may selectively activate distinct signal effectors that can lead to similar reductions in adipose tissue

lipolysis but different capacities to regulate adiponectin secretion (26). The concept that a G protein-coupled receptor may selectively activate only a subset of signal effectors or have multiple conformations upon activation that activate parallel effector pathways is not uncommon and is referred to as "agonist-directed trafficking of receptor signals" (15, 26). Although our results suggest that the reduction in lipolysis and increase in adiponectin serum concentrations are inversely correlated, it is possible that the differential activation of downstream activators may be required to reduce lipolysis and increase adiponectin secretion.

Despite the dramatic effects of niacin on serum adiponectin concentrations in humans and rodents, there is no information regarding the mechanisms by which this occurs. Niacin has recently been shown to modulate adipose tissue lipolysis through the GPR109A receptor (33). In contrast, niacin also reduces diacylglycerol acyltransferase 2 activity in hepatocytes and vascular inflammation in endothelial cells that do not exhibit GPR109A receptor expression (7, 8), suggesting that niacin may possess receptor-dependent and -independent properties. On the basis of the fact that adiponectin is secreted predominantly by white adipose tissue and is a primary target for niacin, we hypothesized that activation of the GPR109A receptor modulates not only adipose tissue lipolysis but also the regulation of adiponectin secretion. In agreement with our hypothesis, niacin and β-OHB increased adiponectin secretion in cultured rat primary adipocytes. The increase in adiponectin secretion was blocked when niacin and β-OHB were administered following pretreatment with PTX. Niacin administration increased adiponectin secretion by >40% and reduced lipolysis by 65% within 10 min in GPR109A wild-type animals but had no influence on adiponectin concentrations or lipolysis in GPR109A-deficient animals.

Our hypothesis is further supported by comparative studies with 3T3-L1 adipocytes and primary rat adipocytes. Niacin had no effect on adiponectin secretion or lipolysis in native 3T3-L1 adipocytes that have limited cell surface expression of the receptor. Conversely, niacin dramatically increased adiponectin secretion and decreased lipolysis in 3T3-L1 adipocytes expressing the human form of the GPR109A receptor (HM74A) and in primary rat adipocytes. The GPR109A receptor is expressed abundantly on the surface of primary adipocytes (37). However, the expression and cell membrane density in native 3T3-L1 adipocytes is controversial. Zhang et al. (38) were unable to detect gene expression of the GPR109A (PUMA-G) receptor in 3T3-L1 adipocytes. Conversely, Ge et al. (9) recently reported that 3T3-L1 adipocytes express the GPR109A receptor gene. Our findings demonstrate the presence of the GPR109A receptor on the cell membrane of 3T3-L1 adipocytes using immunoblotting and immunofluorescence microscopy (data not shown). Comparative quantification with primary adipocytes showed a much higher receptor concentration in primary adipocytes than in native 3T3-L1 adipocytes. Functional studies in the current investigation support the lack of or decreased concentration of the GPR109A receptor in native 3T3-L1 adipocytes, because niacin and β-OHB had no effect on lipolysis or adiponectin secretion in these differentiated cells. These results provide further support that the GPR109A receptor is required for niacin-mediated regulation of adiponectin secretion.

The effects of β-OHB on adipose tissue lipolysis and adiponectin secretion may provide insight into differential activation by downstream effectors. β-OHB is a ketone body produced by the β-oxidation of fatty acids following prolonged fasting or starvation (30). It has been hypothesized that β-OHB signaling via the GPR109A receptor serves as a negative feedback mechanism to reduce serum fatty acids and hepatic ketogenesis (21). Feedback would potentially conserve fat stores during extended starvation and attenuate excessive formation of ketones from unrestrained lipolysis and ketogenesis (30). Fasting periods >20 h have been associated with increased β-OHB and adiponectin concentrations (11). Adiponectin concentrations have been observed in cerebrospinal fluid and lead to an increase in the activation of AMP-activated protein kinase in the arcuate nucleus of the brain to stimulate feeding (20). Thus, increased β-OHB associated with fasting may stimulate the GPR109A receptor to release adiponectin from adipose tissue and decrease lipolysis. We hypothesize that niacin mimics the effects of β-OHB, leading to an increase in adiponectin secretion and a decrease in lipolysis. Additional studies will be required to further test this hypothesis and to evaluate the downstream mediators that are required for the secretion of adiponectin.

In the current investigation, we found that niacin had no effect on leptin or resistin serum concentrations (in vivo studies) or in vitro. Previous investigations using short-term (6–8 wk) niacin administration in humans demonstrated an increase (36) and no change (35) in serum leptin concentrations. Furthermore, serum resistin concentrations were unchanged (36) or slightly reduced (35) following niacin administration. Additional studies will be required to determine whether other adipokines are regulated via the GPR109A pathway.

The results of the present investigation are limited in that we only investigated the effects of GPR109A activation in epididymal fat pads from lean animals. Since adiponectin secretion from visceral but not subcutaneous adipocytes is reduced in obesity (1, 29), it is possible that niacin may preferentially increase adiponectin secretion in visceral adipose tissue. This is supported by the observation that rosiglitazone-stimulated adiponectin secretion is higher in visceral adipocytes than subcutaneous adipocytes (21). Future studies will also be required to examine the influence of adipose tissue distribution and adipocyte size. Studies will be required to examine the regional adipose tissue distribution of the GPR109A receptor in humans and the influence of diet, weight loss, and weight gain on receptor expression. Additional studies will be required to examine the influence of sustained-release formulations of niacin on adiponectin secretion. Further studies are also necessary to investigate the role of other G protein-coupled receptors (GPR81, GPR41, GPR43) on adipokine expression and secretion. Although the efficacy of immediate-release and sustained-release formulations of niacin for improving blood lipid and lipoprotein characteristics is similar despite differences in their rate of absorption (17), studies will be required to examine the dose-dependent and temporal differences between niacin formulations.

Overall, our results provide evidence that niacin increases serum total and HMW adiponectin concentrations and decreases lipolysis within minutes following GPR109A receptor activation. Because adiponectin concentrations are generally low and adipose tissue lipolytic activity high in obese individ-

uals with the metabolic syndrome, niacin may be an ideal pharmacological approach to reduce CVD in this population. Determining the downstream GPR109A signaling events that regulate lipolysis and adiponectin secretion may provide important clues to modulate adiponectin and other adipokines by other pharmacological and molecular biology strategies.

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