Nicotinic acid timed to feeding reverses tissue lipid accumulation and improves glucose control in obese Zucker rats.

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Abstract Nicotinic acid (NiAc) is a potent inhibitor of lipolysis, acutely reducing plasma free fatty acid (FFA) concentrations. However, a major FFA rebound is seen during rapid NiAc washout, and sustained exposure is associated with tolerance development, with FFAs returning to pretreatment levels. Our aim was to find a rational NiAc dosing regimen that preserves FFA lowering, sufficient to reverse nonadipose tissue lipid accumulation and improve metabolic control, in obese Zucker rats. We compared feeding-period versus fasting-period NiAc dosing for 5 days: 12 h subcutaneous infusion (programmable, implantable mini-pumps) terminated by gradual withdrawal. It was found that NiAc timed to feeding decreased triglycerides in liver (−47%; P < 0.01) and heart (−38%; P < 0.05) and reduced plasma fructoseamine versus vehicle. During oral glucose tolerance test, plasma FFA levels were reduced with amelioration of hyperglycemia and hypertriglyceridemia. Furthermore, timing NiAc to feeding resulted in a general downregulation of de novo lipogenesis (DNL) genes in liver. By contrast, NiAc timed to fasting did not reduce tissue lipids, ameliorate glucose intolerance or dyslipidemia, or alter hepatic DNL genes.

In conclusion, NiAc dosing regimen has a major impact on metabolic control in obese Zucker rats. Specifically, a well-defined NiAc exposure, timed to feeding periods, profoundly improves the metabolic phenotype of this animal model.—Kroon, T., T. Baccega, A. Olsén, J. Gabrielsson, and N. D. Oakes. Nicotinic acid timed to feeding reverses tissue lipid accumulation and improves glucose control in obese Zucker rats. J. Lipid Res. 2017. 58: 31–41.

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Lipid accumulation in peripheral nonadipose tissues has been shown to be a major driver of insulin resistance, nonalcoholic steatohepatitis, and dyslipidemia (1–3). Circulating lipids, including plasma free fatty acids (FFAs) and TGs, are important sources of the intracellular lipid pool in muscle and liver (4). Hence, antilipolysis may be an approach for reversing peripheral tissue lipid overload and the downstream negative consequences, including insulin resistance.

Several G protein-coupled receptors (GPRs) are involved in regulating adipocyte lipolysis, including GPR43, GPR81, and GPR109A (5–7). The GPR109A agonist nicotinic acid (NiAc or niacin) potently inhibits lipolysis, resulting in an acute FFA reduction (8). However, prolonged NiAc exposure is associated with tolerance development, with return of FFAs to pretreatment levels (9, 10). Furthermore, during rapid NiAc washout a major FFA rebound is seen, overshooting pretreatment levels (10); this phenomenon is also observed with oral dosing in humans (8, 11). This may be one reason for the apparent worsening of glycemic control with NiAc (12–18). Another important factor may be the currently used extended-release dosing regimen once daily at bedtime to minimize flush (19). FFA increases in the fasting state, and it has recently been suggested that bedtime dosing might limit NiAc efficacy by triggering powerful counter-regulatory mechanisms (20). It is not unreasonable that bedtime dosing might also be involved in the above-mentioned glucose metabolic impairments. Furthermore, an insulin-NiAc synergy on antilipolysis (10) would favor mealtime over bedtime dosing.
We have been interested in determining whether a rational NiAc dosing regimen exists that maximizes FFA lowering and metabolic control. To achieve this, both tolerance and FFA rebound need to be minimized. Recently we tried to reduce tolerance by interspersing abruptly terminated NiAc exposures with drug holidays. This approach was partially successful, retaining acute NiAc-induced FFA lowering and insulin sensitivity but without delivering significant peripheral tissue lipid unloading. This absence of tissue lipid lowering may have reflected a failure to lower average FFA levels over the 24 h period due to a combination of FFA rebound and insufficient containment of tolerance. We speculated that abrupt infusion termination, in the context of NiAc’s short plasma half-life (~2 min in the rat (21)), may exacerbate the FFA rebound. Furthermore, our initial study was performed without timing NiAc exposure to the feeding/fasting periods.

In the present study, we used a preclinical animal model of peripheral tissue lipid overload-induced insulin resistance, the obese Zucker rat (22). Our aim was to find a NiAc dosing regimen that would preserve robust FFA lowering sufficient to reverse nonadipose tissue lipid accumulation and improve metabolic control. In study I, we address the issue of FFA rebound during NiAc withdrawal. Acute metabolic responses to either rapid or gradual NiAc withdrawal in the basal fasting or glucose-infused situation were identified. The aim was to identify the protocol with the lowest FFA rebound for deployment in study II. In study II, we investigated the metabolic consequences of dosing NiAc to feeding versus fasting using a 12 h time-shifted but otherwise identical administration protocol. The results demonstrate a remarkable influence of dosing regimen on metabolic control.

MATERIALS AND METHODS

Animals

Experimental procedures were approved by the local Ethics Committee for Animal Experimentation (Gothenburg region, Sweden). Male obese fa/fa Zucker rats (Charles River) were housed in groups of five in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility with environmental control (20–22°C, relative humidity 40–60% and 12 h light-dark cycle) with free access to water and rodent chow (R70; Laktamin AB, Stockholm, Sweden). At 12–13 weeks of age, animals were weighed, and a tail vein blood sample was taken for determination of glycated hemoglobin (A1cNOW+; PTS Diagnostics, Indianapolis, IN). Animals with glycated hemoglobin <6.2% were housed individually until study completion, with a 3 day recovery period before treatment start. General health status, body weight, and food intake were monitored and recorded daily.

NiAc formulation

Fresh NiAc (pyridine-3-carboxylic acid; Sigma-Aldrich, St. Louis, MO) infusion solution was prepared to a concentration of ~350 mM using procedures described in (10). Vehicle, for control animals, consisted of sodium chloride solutions at equimolar concentrations.

Study overview

Study I was performed to address the issue of FFA rebound during NiAc withdrawal. Specifically, the influence of two potentially important factors were examined: 1) the rate of plasma NiAc decay upon acute drug withdrawal, either rapidly (NiAc-Off) or gradually (NiAc-Stp-Dwn), and 2) the metabolic status at the time of withdrawal (i.e., either during fasting state or glucose infusion), a situation that partially mimics the fed state. Metabolic responses were assessed in overnight-fasted, anesthetized obese Zucker rats. The results of study I were used to select the NiAc termination protocol (NiAc-Off vs. NiAc-Stp-Dwn) and the metabolic state (fed vs. fasted) in which NiAc was to be terminated in study II.

Study II was performed to test the metabolic consequences of NiAc dosing timed to either fasting (daytime) or feeding (nighttime). Chronically jugular catheterized obese Zucker rats, with food freely available during nighttime only (to entrain defined periods of feeding and fasting in these hyperphagic animals), were treated for 5 days with NiAc (NiAc Day vs. NiAc Night). Acute experiments were then performed in the conscious state. Metabolic control was assessed using an oral glucose tolerance test (OGTT).

Study I

The study I protocol is diagrammed in Fig. 1 (top panel). The day before the acute study, food was removed at 17:00, and at 01:00 the preprogrammed implanted pump began infusing NiAc at a constant rate (170 nmol/min/kg, corresponding to 29.1 µl/hr/kg) for 12 h. At 09:30 animals were anesthetized and surgically prepared with jugular and carotid catheters as previously described (10).

Design of the step-down NiAc termination protocol. We hypothesized that a gradual withdrawal of NiAc during washout would attenuate FFA rebound. To test this, we compared a NiAc washout exposure that delayed the plasma NiAc decay toward in vivo IC50 for FFA lowering by several hours versus abrupt termination. A specific step-down infusion protocol needed to produce this profile was identified with the aid of a previously developed model describing acute NiAc pharmacokinetics and FFA response (21). The step-down NiAc infusion rates were 88.9, 58.3, 43.7, 34.0, 24.3, 17.0, and 9.7 nmol/min/kg.

After a postsurgery stabilization period of at least 1.5 h, blood sampling was initiated at 12:00. At 12:30, half the animals (Glu+ groups) began to receive an intravenous glucose infusion based on lean body mass (lbm) (20.6 mg/min/kg, corresponding to 41.2 µl/min/kg) delivered using an external syringe pump (Pump 11 Elite Series; Harvard Apparatus, Cambridge, MA). The remaining animals did not receive glucose (Glu− groups). At 13:00, NiAc infusion was either programmed to switch off (NiAc-Off) or to decrease in a stepwise manner (see above), with final switch-off at 16:30 (NiAc-Stp-Dwn). All NiAc protocols were...
matched with saline-infused controls. Blood samples were drawn at 12:00, 12:30, 12:45, 13:00, 13:15, 13:30, 13:45, 14:00, 14:30, 15:00, 15:30, 16:00, 16:30, 16:45, 17:00, 17:15, 17:30, and 18:00. Samples (30–150 µL, with total loss <5% of blood volume) were collected in potassium-EDTA tubes and centrifuged. Plasma was stored at −80°C pending analysis for NiAc, FFAs, glucose, and insulin.

Study II

The study II protocol is diagrammed in Fig. 1 (bottom panel). During 5 days of treatment, food was restricted to the 12 h dark period, and a daily NiAc delivery profile, with gradual stepwise termination, was timed either to fasting (NiAc Day group) or feeding (NiAc Night group), including a 12 h drug holiday period. On the day 5, the acute study was performed with repeated blood sampling initiated at 13:00 and an oral glucose load at 19:00. NiAc protocols were matched with saline-infused controls.

Fig. 1. Study protocols in obese Zucker rats. Study I: Implantation of programmable mini-pumps containing nicotinic acid (NiAc) at −3 days. On the acute study day, the pump began delivering a constant subcutaneous NiAc infusion for 12 h. At 13:00, NiAc was either switched off (NiAc-Off) or decreased in a stepwise manner with final switch-off at 16:30 (NiAc-Stp-Dwn). At 12:30, glucose-infused groups (Glu+) received a constant-rate intravenous glucose infusion. Study II: A pump and jugular catheter were implanted 3 days before treatment start. During 5 days of treatment, food was restricted to the 12 h dark period, and a daily NiAc delivery profile, with gradual stepwise termination, was timed either to fasting (NiAc Day group) or feeding (NiAc Night group), including a 12 h drug holiday period. On the day 5, the acute study was performed with repeated blood sampling initiated at 13:00 and an oral glucose load at 19:00. NiAc protocols were matched with saline-infused controls.

Analytical methods

Plasma concentrations of NiAc, clinical chemistry biomarkers, and tissue TG content were measured according to previously described methods (10) with the exception of plasma glucose and fructoseamine (Horiba ABX, Montpellier, France). Area under the concentration-time curves (AUCs) for plasma NiAc, FFAs, insulin, glucose, and TG were calculated by trapezoidal approximation using GraphPad Prism 6.01 (GraphPad Software Inc., La Jolla, CA). RNA was extracted, and cDNA was prepared as previously described (10). Gene expression was determined by quantitative RT-PCR using a QuantStudio7 Flex system (Applied Biosystems, Foster City, CA). Premade primer/probe TaqMan assays (Applied Biosystems) were used for the following genes (alias/gene: Applied Biosystems assay ID): carbohydrate-responsive element-binding protein (ChREBP/Mkixp: Rn00591943_m1), sterol regulatory element binding protein-1c (SREBP-1c/Srebfl: Rn01495769_m1), fatty acid synthase (FAS/Fasn: Rn00569117_m1), fatty acid elongase 6 (Elov6: Rn01522302_g1), stearoyl-CoA desaturase-1 (SCD1: Rn00594894_g1), PPARγ2 (Pparg: Rn0040945_m1), cluster of differentiation 36/fatty acid translocase (CD36/Fat: Rn0142640_g1), fatty acid binding protein-4 (Fabp4/Albp: Rn00670361_m1), and perilipin-1 (PLIN1: Rn00586762_m1). The housekeeping gene ribosomal protein large P0 (RPLP0; also known as 36B4) was used for normalization of gene expression data; forward (5′-CGC TGT CTA AGC AGT CAA CTC-3′), reverse (5′-GTC TGT CGT CTA TGT CAT GC-3′).
compared the effects of NiAc termination on plasma NiAc, glucose, and insulin AUCs. For study II, the primary objective was to determine whether NiAc timed to feeding could induce a substantial reversal of liver TG accumulation compared with control animals with hepatic steatosis. Using historical data from obese Zucker rats (CV for hepatic lipid content, 32%) (25), a minimum sample size of eight per group was calculated to be required to detect a 50% reduction in liver TG between two of the three means with 80% power at the 0.05 level of significance. Secondary endpoints of key interest from the efficacy standpoint were metabolic responses to a glucose tolerance test, especially plasma glucose and TG. Based on historical meal challenge data in obese Zucker rats (CVs for plasma glucose and TG AUCs, 4% and 9%, respectively) (25), it was estimated that group sizes of n = 8 would be adequate, having at least an 80% chance of detecting reductions at the 0.05 level of 17% and 35% or more for glucose and TG AUCs, respectively. Several other secondary measurements were also measured in study II, with estimated variability based on similar experiments in the range of the above-mentioned measurements: food intake, body weight, fructosamine, heart TG content, tissue mRNA levels (see Materials and Methods), and AUCs for NiAc and insulin. P < 0.05 was considered statistically significant. Throughout, results are reported as mean ± standard error of the mean (SEM).

RESULTS

Study I

**Plasma NiAc exposure.** The target plateau plasma NiAc concentration of ~1 µM was successfully achieved in all NiAc-infused groups (Fig. 2A, B). After preprogrammed initiation of the step-down protocol at 13:00, plasma NiAc concentrations declined gradually in the NiAc-Step-Dwn groups, taking ~3.8 h to reach acute in vivo IC₅₀ for FFA lowering (~0.07 µM) versus ~1.2 h for abrupt termination in the NiAc-Off groups. During this period (13:00–18:00), NiAc-Step-Dwn groups had significantly increased NiAc exposures (~3-fold vs. the corresponding NiAc-Off group) (Fig. 2C). In control animals (saline infused), endogenous NiAc levels were below the detection limit (6 nM).

**Plasma FFAs.** Upon abrupt NiAc withdrawal (NiAc-Off groups), a FFA rebound was observed in both no-glucose (Glu−) and glucose-infused (Glu+) groups (Fig. 2D, E), with FFA AUC greater than corresponding saline-infused control groups (P < 0.01 and P < 0.001, respectively) (Fig. 2F). Upon gradual NiAc withdrawal in the Glu− groups, the FFA AUC was unexpectedly higher in the NiAc-Step-Dwn versus the corresponding NiAc-Off group (P < 0.001) (Fig. 2F), despite a ~3-fold higher NiAc exposure (P < 0.001) (Fig. 2C). By contrast, in the Glu+ groups, NiAc step-down successfully attenuated the FFA AUC versus abrupt withdrawal (P < 0.05) (Fig. 2F).

**Plasma insulin.** Abrupt NiAc withdrawal induced a pronounced insulin rebound in the no-glucose group (Fig. 2G), where insulin AUC was profoundly raised versus NiAc-Step-Dwn group (P < 0.01) (Fig. 2F). The marked hyperinsulinemia provides a likely explanation for the lower FFA exposure in the NiAc-Off versus NiAc-Step-Dwn groups (Fig. 2F). In the Glu+ groups, over the whole study period (13:00–18:00), the glucose-induced hyperinsulinemia was unaffected and was of similar magnitude in both Off and Step-Dwn groups (Fig. 2I).

**Plasma glucose.** Abrupt termination of NiAc had remarkably little influence on plasma glucose levels in the no-glucose situation (Fig. 2J) despite the impressive changes in FFA and insulin levels. There was no difference in the glucose AUC between NiAc-Off and NiAc-Step-Dwn groups. In the glucose-infused state, gradual and abrupt NiAc withdrawal worsened glucose control similarly (Fig. 2K), with glucose AUC significantly elevated versus saline control for both NiAc groups (P < 0.001) (Fig. 2L).

**Selection of NiAc withdrawal protocol for study II.** Gradual NiAc withdrawal during glucose loading displayed the lowest FFA AUC versus all other NiAc groups. Thus, to minimize FFA rebound, gradual NiAc withdrawal should occur during the fed state in study II.

Study II

**Food intake and body weight.** Pump/catheter implantation did not result in reduced food intake or body weight loss during the 3 day recovery period (day –2 to 0) (Fig. 3). Coincident with treatment start (day 0) until termination, food was restricted to nighttime only. Initial dips in food intake and body weight were fully compensated by the end of the 5 day treatment period. Importantly, food intake and body weight trajectories were practically identical in all groups (Fig. 3).

**Plasma NiAc exposure.** Target plateau plasma NiAc concentrations were similar at ~1 µM in both NiAc-infused groups (Fig. 4A). After preprogrammed initiation of the step-down protocol at 14:30, plasma NiAc levels declined gradually in the Day group. Overall, the NiAc exposure results confirm reliable pump performance according to preprogramming. In control animals, endogenous NiAc levels were below the detection limit (6 nM).

**Plasma FFAs.** In the NiAc Day group, FFA AUC(13:00–19:00) was higher versus Night and Saline groups (P < 0.05) (Fig. 4D) despite having the highest NiAc AUC(13:00–19:00). In the Night group, during the last half of the drug holiday, FFA AUC(13:00–19:00) was similar to the Saline group. As expected, in the Saline group the oral glucose load at 19:00 reduced FFA levels (Fig. 4C). Strikingly, Day versus Night dosing had opposite effects on FFA levels during the OGTT. In the Day group, FFA AUC(19:00–21:00) was increased versus Saline (P < 0.05), whereas in the Night group it was reduced (P < 0.01) (Fig. 4D). Dosing NiAc to feeding resulted in 60% lower FFA AUC(19:00–21:00) versus fasting period dosing (P < 0.001).

**Plasma glucose.** NiAc Night improved glycemia in the 15:00–19:00 fasting period, with lower glucose AUC(15:00–19:00) versus Day and Saline groups (P < 0.01) (Fig. 4F). After OGTT, the Night group displayed a remarkable improvement in glucose control (19:00–21:00) (Fig. 4E),
Timing nicotinic acid to feeding improves metabolic control

Fig. 2. Acute plasma concentration responses to abrupt versus gradual cessation of a constant rate, 12 h nicotinic acid (NiAc) infusion, in the presence or absence of glucose loading in obese Zucker rats. At 13:00, NiAc infusions were either switched off (NiAc-Off) or decreased in a stepwise manner with final switch-off at 16:30 (NiAc-Stp-Dwn). At 12:30, glucose-infused groups (Glu+, middle column) received a constant-rate intravenous glucose infusion, whereas the other groups did not (Glu−, left column). AUC for each plasma variable during the withdrawal period (13:00–18:00) is shown in the right-hand column. **P < 0.01, ***P < 0.001 versus Saline Glu−; "P < 0.01, ""P < 0.001 versus Saline Glu+; †††P < 0.001 versus NiAc-Off Glu−; †P < 0.05, ††P < 0.001 versus NiAc-Off Glu+; ‡‡‡P < 0.001 versus NiAc-Stp-Dwn Glu−. Data are presented as mean ± SEM (n = 5–7 per group).

Plasma insulin. During both the fasting and OGTT periods, NiAc Night exhibited lower insulin AUCs versus NiAc Day (P < 0.05) (Fig. 4H). These data indicate that feeding-period NiAc dosing 1) decreases insulin secretory burden versus fasting-period dosing and 2) enhances insulin sensitivity, given the reduced fasting and OGTT levels of glycemia.

Plasma TG. During the 13:00–19:00 fasting period, there was a nonsignificant tendency for the NiAc groups to exhibit lower plasma TG levels versus Saline. During the OGTT period, NiAc dosed to feeding decreased TG AUC(19:00–21:00) versus Saline (P < 0.05) (Fig. 4J). This was not observed with dosing to fasting.

Fructosamine. Consistent with a long-term improvement in glucose control, feeding-period NiAc dosing resulted in lower fructosamine levels versus Saline and Day groups (P < 0.05 and P < 0.001, respectively) (Fig. 5), whereas fasting-period NiAc dosing had no effect.

Liver and heart TG. Five days of programmed NiAc delivery, applied during nighttime feeding periods but not significantly reducing the glucose AUC(19:00–21:00) versus Day and Saline groups (−27%, P < 0.001 and −22%, P < 0.01, respectively) (Fig. 4F).
of the intermittent NiAc exposure profile have a major impact on metabolic control. Specifically, timing a well-defined NiAc exposure to feeding periods, terminated by an engineered gradual washout profile, results in marked improvements in the metabolic phenotype of the obese Zucker rat. These include plasma FFA and TG lowering as well as improved glycemic control. Perhaps most significant, however, is the substantial peripheral tissue lipid unloading, implying a long-term, fundamental improvement of metabolic control. These effects are achieved without any measurable change in food intake or body weight. By contrast, improvements are not observed with NiAc timed to fasting periods, with a 12 h time-shifted but otherwise identical administration protocol. The current study builds on our previous work, where intermittent dosing was applied without timing administration to feeding periods or gradual withdrawal to terminate the infusion periods (10). Although acute NiAc-induced insulin sensitization in conjunction with FFA lowering was preserved, there was no evidence of longer-term, more fundamental improvements in metabolic control. In particular, tissue TG levels were not lowered, suggesting a failure to lower 24 h FFA exposure.

We suggest that postprandial FFA lowering is the primary mechanism driving the metabolic improvements resulting from NiAc timed to feeding. Reduced FFA supply to the tissues lowers substrate competition with glucose (26) and improves insulin sensitivity (10) just when it is needed the most (i.e., during the influx of dietary carbohydrate in the postprandial phase), resulting in reduced postprandial hyperglycemia and hyperinsulinemia (Fig. 4E, G). Timing NiAc to feeding successfully reduces peripheral lipid accumulation (Fig. 6). In the heart, TG stores parallel the FFA exposure during the period leading up to tissue collection (Fig. 4D), with reductions when NiAc is dosed to feeding and strikingly the opposite response is seen when timed to fasting. This likely reflects the quantitative dependence of the cardiac lipid pool on plasma FFA and its high turnover rate. Unloading of the much larger liver TG pool (Fig. 6) likely results from both direct and indirect effects of the postprandial FFA lowering. The latter from the longer-term effects of repeated reductions in postprandial hyperglycemia and hyperinsulinemia leading to reduced hepatic DNL. This pathway is heavily regulated via transcriptional control by glucose and insulin (27). Strong evidence for reduced hepatic DNL with feeding-period NiAc dosing is provided by the observed downregulation of the master regulatory genes ChREBP and SREBP-1c and by four of their regulated genes directly involved in DNL (ACC1, FAS, Elovl6, and SCD1) (Fig. 7). The absence of a significant change in adipose DNL genes suggests that excess dietary glucose is partitioned away from liver and into adipose tissue. Hepatic TG lowering might be an important driver of the observed reduction in postprandial hypertriglyceridemia (Fig. 4J), which would also tend to reduce lipid accumulation in nonadipose tissue and its negative consequences (1).

Why does NiAc timed to feeding benefit metabolic control? One possibility is that FFA lowering would most likely be enhanced due to the relative hyperinsulinemia associated with this state. Thus, the antilipolytic effects of NiAc...
and insulin are mediated via adipocyte cAMP lowering, but their combined effects are theoretically synergistic with NiAc, decreasing cAMP formation (28), and insulin, increasing cAMP breakdown (29). An insulin-NiAc synergy is not just a theoretical possibility but was in fact seen in our previous hyperinsulinemic clamp studies (10). Although
the potential for FFA lowering might in theory be greater in the fasting state, it has been suggested that antilipolysis in this situation may invoke a more powerful counter-regulatory response to defend the supply of the predominant oxidative fuel (20, 30). Indeed, plasma epinephrine levels significantly increase (about 2.5-fold) after oral ingestion of NiAc in fasting humans (31). In a rat study, FFA was acutely lowered in the fasting state either by NiAc, an $\alpha_1$ adenosine receptor agonist, or by insulin in combination with glucose infusion to maintain euglycemia (32). In support of the above idea, increases in plasma adrenocorticotropic hormone and the lipolytic hormone corticosterone were attenuated with insulin/glucose (a situation somewhat akin to the fed state) compared with the other interventions.

Several design aspects of the dosing regimen were addressed to maximize FFA lowering.

A therapeutically relevant NiAc exposure

We aimed at, and achieved, plateau plasma NiAc concentrations of ~1 µM, based on the concentration-response relationship for FFA lowering, delivering a close-to-maximal FFA suppression in substance naive rats (21, 33). It is important to have a sufficient but not excessive level of NiAc for two reasons. First, loss of FFA lowering might theoretically be exacerbated by sustained supra-maximally effective levels of target engagement (e.g., by ligand-induced GPR109A desensitization and internalization) (34). Second, excessive exposures can invoke additional complicating mechanisms beyond FFA lowering. Examples include hepatic DGAT2 inhibition with an $IC_{50}$ of 100 µM (13, 35), suppression of glucose-mediated insulin secretion via direct activation of GPR109A in the islet observed at 100 µM (36, 37), downregulation of phosphodiesterase-3B in adipose tissue reported at an ~10-fold higher dose of NiAc than used in the current study (9) but not occurring at the present dose level (10), and Toll-like receptor-4 signaling inhibition (38) observed at 100 µM.

![Fig. 5.](image1.png) Plasma fructosamine after 5 days of programmed NiAc delivery given either during fasting (NiAc Day) or feeding (NiAc Night) in obese Zucker rats. *$P < 0.05$ vs. Saline; †††$P < 0.001$ versus NiAc Day. Data are presented as mean ± SEM ($n = 8$ per group).

![Fig. 6.](image2.png) TG content in liver (left) and heart (right) after 5 days of programmed NiAc delivery given either during fasting (NiAc Day) or feeding (NiAc Night) in obese Zucker rats. *$P < 0.05$ vs. Saline; †$P < 0.05$ and †††$P < 0.001$ versus NiAc Day. Data are presented as mean ± SEM ($n = 8$ per group).

![Fig. 7.](image3.png) mRNA expression analysis of genes involved in DNL in liver (top) and epididymal adipose tissue (bottom). Tissues were collected after 5 days of programmed NiAc delivery given either during fasting (NiAc Day) or feeding (NiAc Night) in obese Zucker rats. *$P < 0.05$, **$P < 0.01$ versus Saline; †$P < 0.05$; ††$P < 0.01$ versus NiAc Day. Data are normalized to housekeeping gene (RPLP0) and are presented as mean ± SEM ($n = 8$ per group).

![Fig. 8.](image4.png) PPARγ-regulated genes in white adipose tissue. Tissues were collected after 5 days of programmed NiAc delivery given either during fasting (NiAc Day) or feeding (NiAc Night) in obese Zucker rats. Data are normalized to housekeeping gene (RPLP0) and presented as mean ± SEM ($n = 8$ per group).
Limiting tolerance

The standard pharmacological approach of engaging the target mechanism (GPR109A activation) for 24 h/day fails in the case of NiAc because prolonged exposure induces tolerance development, with FFAs returning to pretreatment levels (9). Therefore, we applied intermittent dosing with 12 h drug holidays, resulting in containment but not elimination of tolerance (10). Thus, tolerance still developed during the second half of the infusion period when NiAc was applied in the fasting state (Fig. 4C). Most likely this did not occur to the same extent when NiAc was given with feeding (discussed above) given the magnitude of tissue lipid unloading. Drug holidays might be important for another reason. Because the products of lipolysis are thought to provide ligands for PPARγ activation (39), sustained and robust antilipolytic therapy might reduce the activation of PPARγ. Depending on its magnitude, this could undermine the beneficial effects by impairing adipose tissue function and perturbing systemic metabolic control, as seen in humans with insufficiencies of functional PPARγ or HSL (40, 41). However, we found that expression of PPARγ2 as well as several regulated genes [perilipin 1 (42), CD36 (43), and FABP4 (44)] in adipose tissue was unaffected by NiAc treatment. Thus, our data indicate that the intermittent NiAc administration protocol used in the current study did not reduce the activation of adipose tissue PPARγ. This fits well with data showing that haploinsufficiency of HSL in mice did not deactivate adipose tissue PPARγ (45).

Damage control by minimizing FFA rebound

We hypothesized that the FFA rebound is potentiated by a rapid decay of NiAc drug level toward the FFA-lowering potency. Indeed, FFA rebound is minimized by gradually versus abruptly withdrawing NiAc levels in combination with glucose loading (Fig. 2F). In contrast, when NiAc was gradually withdrawn under fasting conditions, despite higher NiAc exposures, FFA exposure was increased (compared with abrupt withdrawal), probably as a result of the much lower insulin levels. One possible explanation for the virtual absence of an insulin rebound in this situation is that NiAc inhibits insulin secretion. Indeed, NiAc-induced blunting of glucose-stimulated insulin secretion has been demonstrated in vitro (36, 37), albeit at considerably higher NiAc concentrations than those achieved in the present study. Thus, timing gradual NiAc withdrawal to the fed state should minimize FFA rebound. A caveat is that withdrawal under conditions of glucose loading worsens glycemic control (Fig. 2E). To minimize the impact of this effect, withdrawal was timed to occur late in the feeding period.

Our data raise the question of whether alternative dosing schedules in humans might improve the current clinical use of niacin (NiAc). This is important because, due to the negative outcomes of two recent clinical trials (46–48), prescriptions have been declining (49), and there is now considerable uncertainty about the value of niacin therapy (50–52). Bedtime dosing has been standard since the late 1990s after the introduction of prescription extended-release niacin (20). Even if taken together with a low-fat snack, in accordance with prescriber’s information, this will presumably have the result that a substantial fraction of the niacin exposure occurs during the fasting state. However, we observed hepatic and plasma TG lowering when treatment was timed to feeding and not when it was timed to fasting. Indeed, mealtime dosing has previously been suggested as an approach to augment niacin’s clinical efficacy (20, 53). Extended-release niacin given immediately before a fat meal suppressed postprandial FFA and plasma TG excursions (53), whereas bedtime dosing failed (54). In addition, patient studies continue to report impairments in insulin sensitivity and/or glycemic control (12–17) and even increased risk of new-onset diabetes (18). Based on the current results, it seems reasonable to speculate that mealtime dosing might reverse these negative effects on glucose control. The specific exposure profile of NiAc used in our preclinical study markedly differs from exposures achieved after oral niacin dosing in patients (55). Clinical niacin dosing has not been optimized for FFA lowering, and therefore simply giving currently available formulations at mealtimes may not reproduce the beneficial effects seen in the present study. Another reason caution should be applied in extrapolating the present results to the clinical setting is that the quantitative role of DNL is likely to be less in a human compared with a rat on a high-carbohydrate diet. Therefore, the potential efficacy for improving TG and glucose control may not be as great in the broader patient population. On the other hand, there may be subpopulations characterized by impaired postprandial suppression of lipolysis and elevated rates of DNL (2, 56), resembling the responses seen in the obese Zucker rat. Aside from timing, the current data also indicate the importance of a prolonged drug washout to minimize the FFA rebound in the fed state. This might at least partly explain improvements in insulin sensitivity or glucose control seen with acipimox (57, 58) given the longer plasma half-life of acipimox compared with niacin (59, 60).

In conclusion, we have shown that dosing regimen (in the context of a fixed daily dose) has a critical influence on whether or not nicotinic acid improves metabolic control in the obese Zucker rat. Specifically, the results demonstrate that timing a well-defined intermittent nicotinic acid infusion protocol to feeding periods can reverse peripheral tissue lipid accumulation and improve lipid and glucose control. These data support the concept that antilipolysis, applied in conjunction with feeding, can be an effective means of reversing lipid overload-induced insulin resistance and dyslipidemia. Finally, our data have important general implications for pharmacological approaches to treatment of metabolic disease. We recently showed that the approach of around-the-clock exposure fails in the case of NiAc to deliver durable FFA lowering due to complete tolerance development. The current study revealed that the details of the intermittent exposure profile (timing and shape) play a critical role in metabolic outcomes. The results also suggest that pharmacological principles for the treatment of metabolic disease may require careful fine tuning of drug exposure and dosing regimen.
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