A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects

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ABSTRACT

Background: Animal studies suggest a positive role for nicotinamide riboside (NR) on insulin sensitivity and hepatic steatosis in models of obesity and type 2 diabetes. NR, an NAD^+ precursor, is a member of the vitamin B-3 family now available as an over-the-counter supplement. Although data from preclinical trials appear consistent, potential effects and safety need to be evaluated in human clinical trials.

Objective: The aim of this study was to test the safety of dietary NR supplementation over a 12-wk period and potential to improve insulin sensitivity and other metabolic parameters in obese, insulin-resistant men.

Design: In an investigator-initiated randomized, placebo-controlled, double-blinded, and parallel-group designed clinical trial, forty healthy, sedentary men with a body mass index (BMI) > 30 kg/m^2, age-range 40–70 y were randomly assigned to 12 wk of NR (1000 mg twice daily) or placebo. We determined the effects of NR supplementation on insulin sensitivity by a hyperinsulinemic euglycemic clamp and substrate metabolism by indirect calorimetry and labeled substrates of tritiated glucose and palmitate. Body composition and fat mass distribution were determined by whole-body dual-energy X-ray absorptiometry (DXA) and MRI scans, and measurements of intrahepatic lipid content were obtained by MR spectroscopy.

Results: Insulin sensitivity, endogenous glucose production, and glucose disposal and oxidation were not improved by NR supplementation. Similarly, NR supplementation had no effect on resting energy expenditure, lipolysis, oxidation of lipids, or body composition. No serious adverse events due to NR supplementation were observed and safety blood tests were normal.

Conclusion: 12 wk of NR supplementation in doses of 2000 mg/d appears safe, but does not improve insulin sensitivity and whole-body glucose metabolism in obese, insulin-resistant men. This trial was registered at clinicaltrials.gov as NCT02303483. *Am J Clin Nutr* 2018;108:1–11.

Keywords: insulin sensitivity, hyperinsulinemic euglycemic clamp, human, obesity, hepatic steatosis, nicotinamide riboside

INTRODUCTION

A continuous effort in the search for new potential therapeutic approaches is required to curb the health hazards of obesity and related conditions. Insulin resistance is central to the pathogenesis of obesity-related conditions such as type 2 diabetes. When overweight individuals are successfully coached to lose >7% of their body weight in 24-wk lifestyle interventions or given metformin, improvements in insulin sensitivity can be achieved (1, 2). Nicotinamide riboside (NR), a member of the vitamin B-3 family, has antiobesogenic properties and has been shown to improve insulin sensitivity in rapidly induced rodent models of metabolic diseases (3).

Similar to classical forms of vitamin B-3—nicotinic acid (NA) and nicotinamide (NAM)—NR is a nicotinamide adenine dinucleotide (NAD^+) precursor (4), and a naturally occurring

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Supplemental Figure 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ALT, aminotransferase; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; EE, energy expenditure; GIR, glucose infusion rate; HFD, high-fat diet; HLC, hepatic lipid content; LWR, lipid to water ratio; MRM, multiple reaction monitoring; NA, nicotinic acid; NAD, nicotine adenine dinucleotide; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; NEFA, nonesterified fatty acid; NR, nicotinamide riboside; Ra, rate of appearance; Rd, rate of disappearance; SAT, subcutaneous adipose tissue; TG, triglyceride; VAT, visceral adipose tissue.

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TABLE 1
Baseline characteristics of the placebo and NR groups[1]

	Placebo ($n = 20$)	NR ($n = 20$)	P[2]
Age, y	60 ± 2.0	58 ± 1.6	0.42
Weight, kg	104.6 ± 2.0	104.8 ± 2.2	0.94
BMI, kg/m^2	33.3 ± 0.6	32.4 ± 0.5	0.28
Waist:hip ratio	1.01 ± 0.01	1.01 ± 0.01	0.77
Lean mass, kg	67.2 ± 1.1	68.8 ± 1.6	0.41
Fat mass, kg	32.2 ± 1.4	31.1 ± 1.2	0.55
Fat, %	31.3 ± 1.0	30.2 ± 0.9	0.41
Visceral adipose tissue, cm^2	320.4 ± 17.0	295.5 ± 16.3	0.30
Subcutaneous adipose tissue, cm^2	225.1 ± 13.6	229.6 ± 12.9	0.81
Hepatic lipid content,[4] %	14.1 ± 1.9	11.3 ± 1.8	0.30
HOMA-IR[3,4]	2.8 ± 0.3	2.5 ± 0.2	0.42
Fasting glucose,[4] mmol/L	5.7 ± 0.1	5.5 ± 0.1	0.25
Fasting insulin,[3] pmol/L	75.3 ± 5.6	71.4 ± 6.6	0.57
HbA1c, mmol/mol	39.8 ± 0.8	37.7 ± 1.0	0.10
HbA1c, %	5.8 ± 0.1	5.6 ± 0.1	0.10
Total cholesterol, mmol/L	5.3 ± 0.2	5.3 ± 0.2	0.88
LDL cholesterol, mmol/L	3.3 ± 0.2	3.4 ± 0.2	0.74
HDL cholesterol, mmol/L	1.3 ± 0.1	1.2 ± 0.1	0.34
Triglycerides, mmol/L	1.6 ± 0.1	1.5 ± 0.1	0.45

[1]Data presented as mean ± SEM. HbA1c, glycated hemoglobin.
[2]Baseline comparisons were made with unpaired 2-sample t test.
[3]Statistical test performed on log-transformed data.
[4]Placebo group ($n = 19$).

substance in the human diet (5). NR utilizes a unique 2-step biosynthetic pathway through the NR kinases (NRK) 1 and 2 leading to NAD^+ formation (6). Oral administration of NR increases NAD^+ concentrations in vivo in rodents (7–15) and has recently been shown to increase NAD^+ concentrations in human blood (16, 17). NR has undergone formal genotoxicity and toxicology studies in animals (18), and is safe in humans in single doses ≤1000 mg (16). However, no data are available regarding safety and potential side effects of long-term NR supplementation in healthy or overweight humans.

NAD^+ is recognized as an essential coenzyme in redox reactions in beta-oxidation, glycolysis, and in the citric acid cycle (19). Furthermore NAD^+ serves as a rate-limiting substrate for a range of regulatory proteins, including the sirtuin enzyme family, which are intricately involved in cellular metabolic regulation (20). NAD^+ availability is compromised in circumstances associated with increased risk of metabolic disorders including aging (21–24) and high-fat diet (HFD)-induced obesity (25). Nicotinamide phosphoribosyltransferase (NAMPT), a key NAD^+ biosynthetic enzyme, is influenced by lifestyle factors that affect cellular NAD^+ content. NAMPT abundance is increased by exercise (26–28), caloric restriction (29), and fasting (30), whereas obesity (31, 32) and aging (25) are associated with reduced NAMPT abundance. Decreased NAD^+ availability thus seems to play a significant role in disorders involving metabolic stress including heart disease (33), type 2 diabetes (9), and brain injury (34), suggesting the possibility that repletion with a NAD^+ precursor could correct imbalances of NAD^+ availability and provide health benefits.

Experimental work in animals shows beneficial effects of NAD^+ boosting strategies on metabolic endpoints. NR supplementation enhances oxidative metabolism, attenuates insulin resistance, and protects against weight gain in mice on a HFD (7). NR improves glycemic control and provides resistance to weight gain, hepatic steatosis, and hypercholesterolemia in mouse models of prediabetes and diabetes (9, 35). Moreover, NR effectively reduces ectopic lipid deposition in the liver in mouse models of nonalcoholic fatty liver disease (NAFLD) (8, 10).

Here we tested whether healthy obese men would tolerate 2000 mg of NR per day over a 12-wk period and whether this nutritional intervention would be sufficient to improve insulin sensitivity or other metabolic parameters.

METHODS

Study design

The study was designed as an investigator-initiated randomized, double-blinded, placebo-controlled, and parallel-group trial in which participants received oral supplementation with NR (NIAGEN^{tm}, ChromaDex) 1000 mg twice daily or placebo (capsules identical to NR in number and external appearance) for 12 wk.

Study population

Forty healthy male Caucasian volunteers were recruited. Inclusion criteria were: male, 40–70 y of age, obese (BMI > 30 kg/m^2), sedentary (<30 min exercise per day), nonsmokers, and no prescribed medicine. Participants underwent a physical examination by a trained physician including routine clinical biochemistry and electrocardiography to evaluate eligibility for the study.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki after approval by the local Research Ethics Committee (H-3-2014-130) and the Danish Data Protection Agency (1-16-02-714-14). The study was registered at clinicaltrials.gov (NCT02303483) before recruitment was commenced. Participants received oral and written information before written consent was obtained.

Study procedure

Participants were examined on 3 separate days at baseline and at the end of the trial. The same physician and laboratory personnel undertook all examinations with the use of the same equipment. Examinations included dual-energy X-ray absorptiometry (DXA) scan, MR imaging and spectroscopy, and a clamp study day consisting of a hyperinsulinemic euglycemic clamp with infusion of radioactive isotope-labeled tracers of glucose and palmitate, indirect calorimetry, repetitive blood sampling, and muscle and adipose tissue biopsies.

After completion of baseline investigations, participants were randomized to 12 wk supplementation with NR or placebo with 2 daily administrations (morning and evening). The last administration took place on the morning of the final clamp study day. Surplus trial medication was returned, and compliance rate

TABLE 2
Markers of substrate metabolism—indirect calorimetry, circulating hormones, and metabolites[1]

	Placebo		NR	
	Pretreatment	Post-treatment	Pretreatment	Post-treatment
Glucose oxidation, kcal/24 h				
Basal	434.9 ± 47.4	501.1 ± 46.2	414.6 ± 46.2	527.0 ± 46.2
Clamp	681.5 ± 50.1*	801.0 ± 49.3*	605.5 ± 51.5*	650.4 ± 51.5*
Delta	239.8 ± 51.5	299.9 ± 49.0	207.6 ± 51.6	138.3 ± 51.6
Lipid oxidation, kcal/24 h				
Basal	987.6 ± 61.9	971.0 ± 60.4	1014.7 ± 60.4	856.8 ± 60.4
Clamp	784.1 ± 54.2*	688.2 ± 53.0*	846.7 ± 55.7*	790.1 ± 55.7
Delta	−181.7 ± 53.2	−282.8 ± 50.6	−199.0 ± 53.3	−66.7 ± 53.3
Protein oxidation, kcal/24 h				
Basal	428.8 ± 36.2	413.4 ± 35.5	450.0 ± 35.5	471.9 ± 35.5
Clamp	417.5 ± 27.3	401.6 ± 27.3	458.2 ± 28.0	424.4 ± 28.5*
Delta	−11.3 ± 36.9	−11.9 ± 36.1	5.8 ± 37.0	−57.4 ± 37.9
Resting energy expenditure, kcal/24 h				
Basal	1850 ± 44	1886 ± 44	1879 ± 44	1856 ± 44
Clamp	1886 ± 44	1891 ± 44	1906 ± 45	1872 ± 45
Delta	36 ± 17	5 ± 17	18 ± 18	7 ± 18
Respiratory exchange ratio				
Basal	0.80 ± 0.01	0.81 ± 0.01	0.80 ± 0.01	0.82 ± 0.01
Clamp	0.84 ± 0.01*	0.86 ± 0.01*	0.83 ± 0.01*	0.84 ± 0.01*
Delta	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Insulin, pmol/L				
Basal	59.4 ± 5.6	68.8 ± 5.6	56.3 ± 5.6	65.9 ± 5.6
Clamp	262.6 ± 10.0*	272.5 ± 10.0*	244.9 ± 10.2*	275.6 ± 10.4*
Delta	203.2 ± 7.9	203.8 ± 7.9	188.8 ± 8.1	212.0 ± 8.2
NEFA, mmol/L				
Basal	0.50 ± 0.03	0.45 ± 0.03	0.48 ± 0.03	0.42 ± 0.03
Clamp	0.08 ± 0.01*	0.07 ± 0.01*	0.07 ± 0.01*	0.06 ± 0.01*
Delta	−0.42 ± 0.02	−0.38 ± 0.02	−0.40 ± 0.03	−0.35 ± 0.03
Palmitate flux, μmol/min				
Basal	286.0 ± 20.0	257.5 ± 20.0	296.4 ± 21.2	219.4 ± 21.2
Clamp	60.9 ± 4.9*	56.4 ± 4.9*	58.9 ± 5.5*	51.0 ± 5.5*
Delta	−224.5 ± 19.0	−199.7 ± 19.0	−234.7 ± 20.7	−168.7 ± 20.7

[1]Data estimated mean ± SEM. Pretreatment and post-treatment values with placebo ($n = 18$–20) or NR ($n = 16$–20) supplementation. Each parameter was measured in the basal and clamp periods during a hyperinsulinemic euglycemic clamp, referring to periods with and without insulin stimulation. Delta = clamp – basal. Circulating parameters were measured in triplicates at the end of the basal and clamp periods. Oxidation rates of glucose, lipid, and protein, resting energy expenditure, and respiratory exchange ratio as measured by indirect calorimetry. Palmitate flux, reflecting lipolysis, as determined by [9,10-^{3}H]-palmitate tracer infusion. Effects of NR treatment were assessed between groups by repeated-measurement mixed model analysis. Comparisons were performed on basal, clamp, and delta values, respectively. There were no significant treatment effects of NR. Effects of insulin stimulation (basal compared with clamp) during the hyperinsulinemic euglycemic clamp were tested by paired 2-sample *t* tests within each group and visit. As expected, well-known insulin effects were observed including changes in substrate metabolism, reflected by increased glucose oxidation and decreased lipid oxidation, as well as suppressed lipolysis and NEFA concentrations during insulin stimulation (*$P \leq 0.01$). NEFA, nonesterified fatty acid; NR, nicotinamide riboside.

was calculated as the proportion of capsules ingested relative to the intended number.

The Pharmacy at Aarhus University Hospital undertook randomization, blinding, packaging, and labeling of the trial dietary supplement. Block randomization was used with a block size of 4; within each block 2 participants were allocated to NR supplementation, and 2 participants to placebo in a random pattern. Participants and data collectors were blinded to treatment. Once all participants had completed the study, the randomization code was released.

Safety

Participants had direct access to a physician throughout the study, and were seen at a midway safety checkup after 6 wk, when a blood sample was drawn to monitor for adverse events. The blood sample included creatinine, sodium, potassium, urea nitrogen, albumin, alanine aminotransferase (ALT), bilirubin, alkaline phosphatase, hemoglobin, white blood cell count, and platelets. Oral information about adverse events was recorded after 6 wk and at the end of the trial.

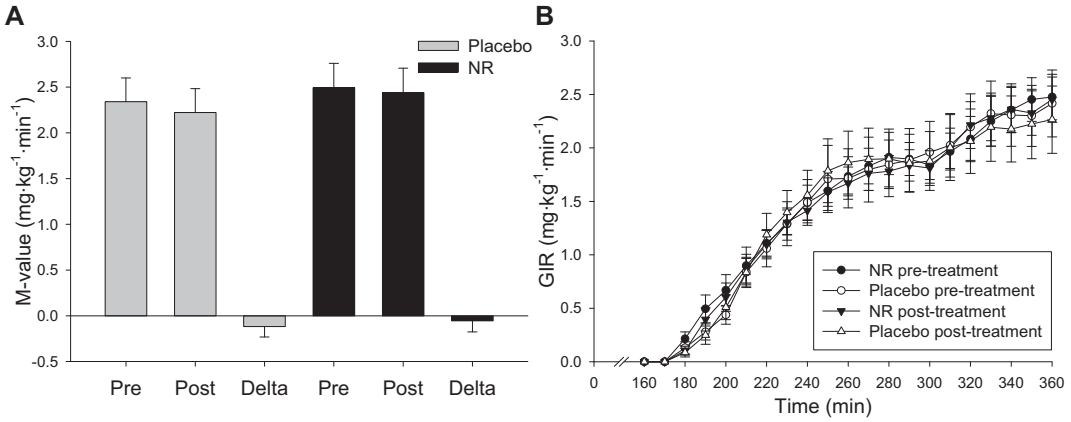

FIGURE 1 Insulin sensitivity parameters, pretreatment (Pre), post-treatment (Post), and delta (Post – Pre) values with placebo ($n = 20$) or NR ($n = 18$–19) supplementation. (A) The M-value (estimated mean ± SEM), reflecting whole-body insulin sensitivity, calculated as the mean GIR during the last 30 min of the clamp. Treatment comparison was made by repeated-measurement mixed model analysis. No significant treatment effect was observed. (B) The GIRs (mean ± SEM) necessary to maintain euglycemia at 5 mmol/L during the 3-h clamp period, pretreatment and post-treatment with placebo ($n = 20$) or NR ($n = 18$–19). Gray bars, white dots = placebo; black bars/dots = NR. GIR, glucose infusion rate; NR, nicotinamide riboside.

Study preparations

Two wk preceding and during the trial, participants were asked to refrain from taking vitamins or other dietary supplements. Because the study aimed to test the sufficiency of NR supplementation to improve metabolic parameters, the significance of maintaining their normal way of living in terms of diet and physical exercise was emphasized.

To avoid variations in composition of diet and intake of naturally occurring forms of vitamin B-3, participants kept a diet journal for 3 d preceding the clamp study day at baseline and repeated the dietary pattern at the end of the trial. The diet journal included detailed information about time of intake, type of food/drink, amount, and type of preparation. Participants—already preselected as adhering to a sedentary lifestyle—were instructed to abstain from alcohol and physical exercise for 72 h preceding the clamp study day.

Hyperinsulinemic euglycemic clamp

After a 10-h overnight fast, participants arrived at the Medical Research Laboratory, Aarhus University Hospital, Denmark at approximately 0700 and were studied in supine position for 6.5 h. Intravenous catheters were inserted: 1 in an antecubital vein for infusions and 1 in a heated dorsal hand vein for sampling of arterialized blood. Blood samples were drawn at 0, 160, 170, 180, 300, 340, 350, and 360 min.

The clamp study day was divided into a 3-h basal period (0–180 min) and a 3-h clamp period (180–360 min) with continuous insulin infusion at 0.5 $mU \cdot kg^{-1} \cdot min^{-1}$ (Humulin® Regular, Eli Lilly). Plasma glucose concentrations were measured every 10 min during insulin infusion and the glucose infusion rate (GIR) adjusted with a 20% glucose infusion to maintain euglycemia at 5 mmol/L. The primary endpoint measure, the M-value, reflecting whole-body insulin sensitivity, was calculated as mean GIR at 340–360 min.

Tracers

A 0.74 MBq bolus followed by continuous infusion at 0.44 MBq/h of [3-^{3}H]-glucose (GE Healthcare) was given from 0–360 min. In addition, [3-^{3}H]-glucose was added to the infused glucose used during the hyperinsulinemic euglycemic clamp in order to avoid rapid dilution (3.7 MBq [3-^{3}H]-glucose in 500 ml 20% glucose). Specific activities of [3-^{3}H]-glucose were measured in triplicates at the end of the basal (160–180 min) and clamp (340–360 min) periods. Glucose rate of appearance (Ra) and rate of disappearance (Rd) were calculated with the use of Steele's non-steady-state equation (36). Endogenous glucose production (EGP) equals Ra under the basal period and is calculated by subtracting GIR from Ra during the clamp period. Nonoxidative glucose disposal (NOGD) was calculated by subtracting oxidative glucose disposal (derived from indirect calorimetry) from total glucose disposal (Rd). Systemic palmitate turnover was determined as previously described (37). A continuous infusion of [9,10-^{3}H]-palmitate (GE Healthcare) was infused at 0.0111 MBq/min in the basal (120–180 min) and clamp (300–360 min) periods. Blood samples for measurement of palmitate concentration and specific activity were drawn before the infusions (0 and 300 min) and in triplicates at the end of the infusions (160–180 min and 340–360 min).

Indirect calorimetry

Indirect calorimetry (Oxycon Pro, Intramedic) was applied in order to measure the nonthermal component of resting energy expenditure (EE) and respiratory exchange ratio (RER) during the basal and clamp periods (120 min and 270 min). Mean values of a 15-min period collection of respiratory gases were used for calculations. Substrate oxidation rates were calculated after correction for protein oxidation, determined on the basis of urinary excretion of urea nitrogen (38).

Blood analysis

Analyses of insulin (Insulin ELISA, Mercodia) and nonesterified fatty acids (NEFAs) (Wako Chemicals) were performed with the use of commercially available kits. Plasma glucose was measured immediately after collection on a YSI 2300 Stat Plus

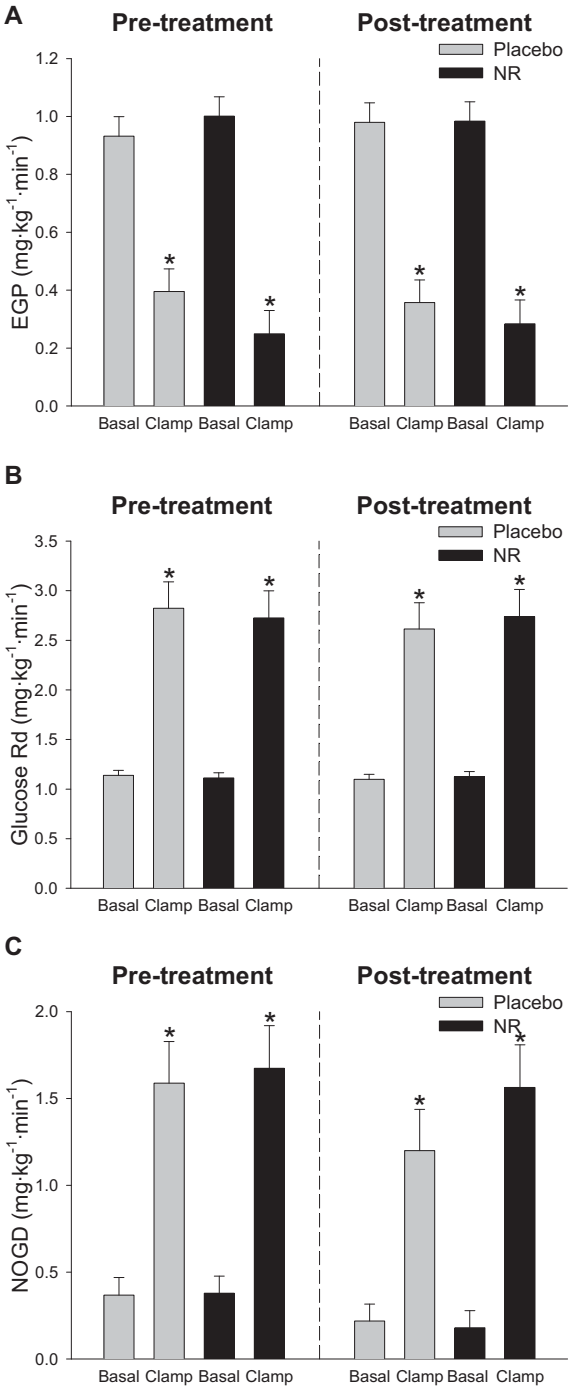

FIGURE 2 Glucose turnover parameters (estimated mean ± SEM) determined by [3-^{3}H]-glucose tracer infusion in the basal and clamp periods. Pretreatment and post-treatment values with placebo (n = 18–20) or NR (n = 17–20) supplementation. (A) EGP. (B) Glucose rate of disappearance (Rd), corresponding to whole-body glucose uptake. (C) NOGD. Effects of NR treatment were assessed between groups by repeated-measurement mixed model analysis. Comparisons were performed on basal, clamp, and delta (clamp – basal) values, respectively. There were no significant treatment effects of NR. Effects of insulin stimulation (basal compared with clamp) during the hyperinsulinemic euglycemic clamp were tested by paired 2-sample t tests within each group and visit. As expected, insulin stimulation suppressed EGP and increased whole-body glucose uptake and NOGD (*$P \leq 0.001$). Gray bars = placebo; black bars = NR. EGP, endogenous glucose production; NOGD, nonoxidative glucose disposal; NR, nicotinamide riboside.

Glucose Analyzer (YSI Inc.). Glycated hemoglobin (HbA1c), ALT, total cholesterol, HDL, LDL, and triglycerides (TGs) were analyzed at the Department of Clinical Biochemistry, Aarhus University Hospital, with the use of standard methods. Insulin resistance determined by HOMA-IR was calculated through the use of the equation: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5 (39).

Urine sampling

Urine was collected at the end of the basal and clamp periods for quantification of urine urea nitrogen with absorption photometry (Cobas 6000, Roche). First morning urine was collected at the end of the trial approximately 12 h after ingestion of trial capsules for quantitative assessment of NR- and NAD-derived metabolites by liquid chromatography–mass spectrometry (LC-MS) analysis. An internal standard solution containing 200 pmol of d3 N-methyl-4-pyridone-3-carboxamide (Me-4-PY), 400 pmol of ^{18}O NR and ^{18}O, d3 N-methyl nicotinamide (MeNAM), and 800 pmol of ^{18}O-NAM and d4-NA in 0.5% formic acid was added to 100 µL of urine. Standards and controls were prepared in water with the above amounts of internal standard, 0.4–800 pmol of nicotinic acid riboside (NAR), and 4–8000 pmol of all other analytes. Over range samples were reanalyzed by diluting 10 µL of urine to 100 µL with water. All samples were evaporated to dryness under vacuum through the use of a CentriVap (Labconco). LC-MS was carried out as described (40) on a Waters TQD using the modified multiple reaction monitoring (MRM) transitions of NR(255→123); NAM(123→80); NA(124→80); MeNAM(137→94); Me-4-PY(153→136); Me-2-PY(153→110); NAM oxide(139→106); NAR(256→124); ^{18}O-NAM(125→80); ^{18}O-NR(257→125); d4-NA(128→84); ^{18}O, d3-MeNAM(142→97); and d3-Me-4-PY(156→139). Urine metabolites were normalized to creatinine concentrations measured with absorption photometry (Cobas 8000, Roche).

MR imaging and spectroscopy

Hepatic lipid content (HLC), abdominal subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) were quantified with the use of MR techniques on a Signa HDxt 1.5 Tesla twin speed scanner (GE Medical Systems).

HLC was determined with ^{1}H-MR liver spectroscopy as previously described (41). Full width at half maximum of the water peak was 7.7 ± 2.8 Hz (mean ± SD). LCModel software (version 6.2-1, Stephen Provencher) was applied to quantify the spectra with the use of a dedicated liver spectroscopy fitting model, providing an estimate of the lipid to water ratio (LWR) in the tissue within the voxel (42). HLC, which is the fraction of signal from fat over the total signal, was calculated as: HLC = LWR/(LWR + 1).

Abdominal SAT and VAT were quantified based on a single axial image at the L2-L3 vertebral interspace by MRI with the use of a 16-element abdominal coil and a fast spin-echo T1-weighted IDEAL/DIXON sequence: echo time: 13 ms; repetition time: 700 ms; slice thickness: 5 mm; field of view: 40–48 cm; pixel resolution: 1.4 × 2.3 mm. The VAT area based on a single slice correlates highly with the total VAT volume as assessed

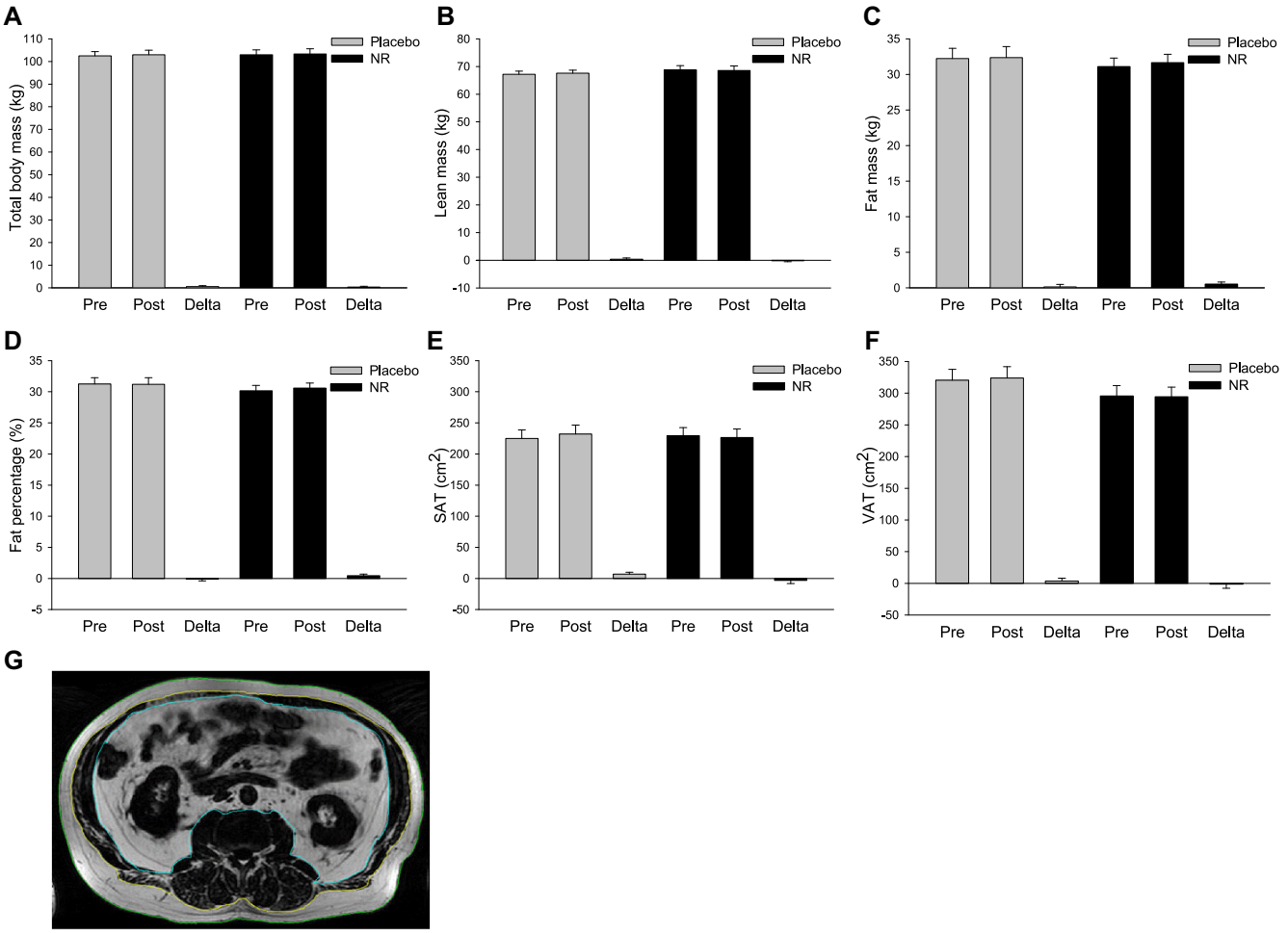

FIGURE 3 Body composition measures (estimated mean ± SEM), pretreatment (Pre), post-treatment, (Post), and delta (Post – Pre) values with placebo ($n = 20$) or NR ($n = 20$) supplementation. (A) Total body mass, (B) lean mass, (C) fat mass, and (D) fat percentage as determined by whole-body dual-energy X-ray absorptiometry scans. (E) Abdominal SAT and (F) VAT determined on the basis of a single axial image at the L2-L3 vertebral interspace by MR imaging. (G) Defining the external and internal contours for quantification of SAT and VAT with the use of Hippo Fat software. Treatment comparison was made by repeated-measurement mixed model analysis. No significant treatment effects were observed. Gray bars = placebo; black bars = NR. NR, nicotinamide riboside; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

by a multislice protocol (43). Data were processed with the use of Hippo Fat software (version 1.3, Vincenzo Positano) (44), allowing for semiautomatic quantification. Each slice was visually inspected and the segmentation manually adjusted when necessary. Analysis was performed blinded to treatment.

DXA

Body composition and bone mineral density (BMD) were assessed by a whole-body DXA scan (Hologic Discovery, Hologic).

Power calculation

The Power calculation was based on the primary endpoint, which was insulin sensitivity as defined by the M-value obtained in the hyperinsulinemic euglycemic clamp. Sample size was calculated on the basis of a *t* test for comparing 2 population means. The significance level was set at 0.05 with a power of 0.8. The SD of the examination was estimated at 1.6 based on previous in-house experience. To determine whether NR could provide improvements in insulin sensitivity comparable with those of insulin-sensitizing drugs or exercise (45, 46), 19 participants would be required in each group, to detect a statistically significant treatment difference of 1.5 $mg \cdot kg^{-1} \cdot min^{-1}$.

Statistics

Data are presented as estimated mean ± SEM unless stated otherwise. Treatment effect was assessed between groups by repeated-measurement mixed model analysis that included systematic factors: intervention (NR/placebo), visit (pretreatment/post-treatment), and the interaction between intervention and visit, and the subjects' unique ID as the random effect. The Wald test was applied to test interaction. The likelihood ratio test was applied to check for distinct residual variances

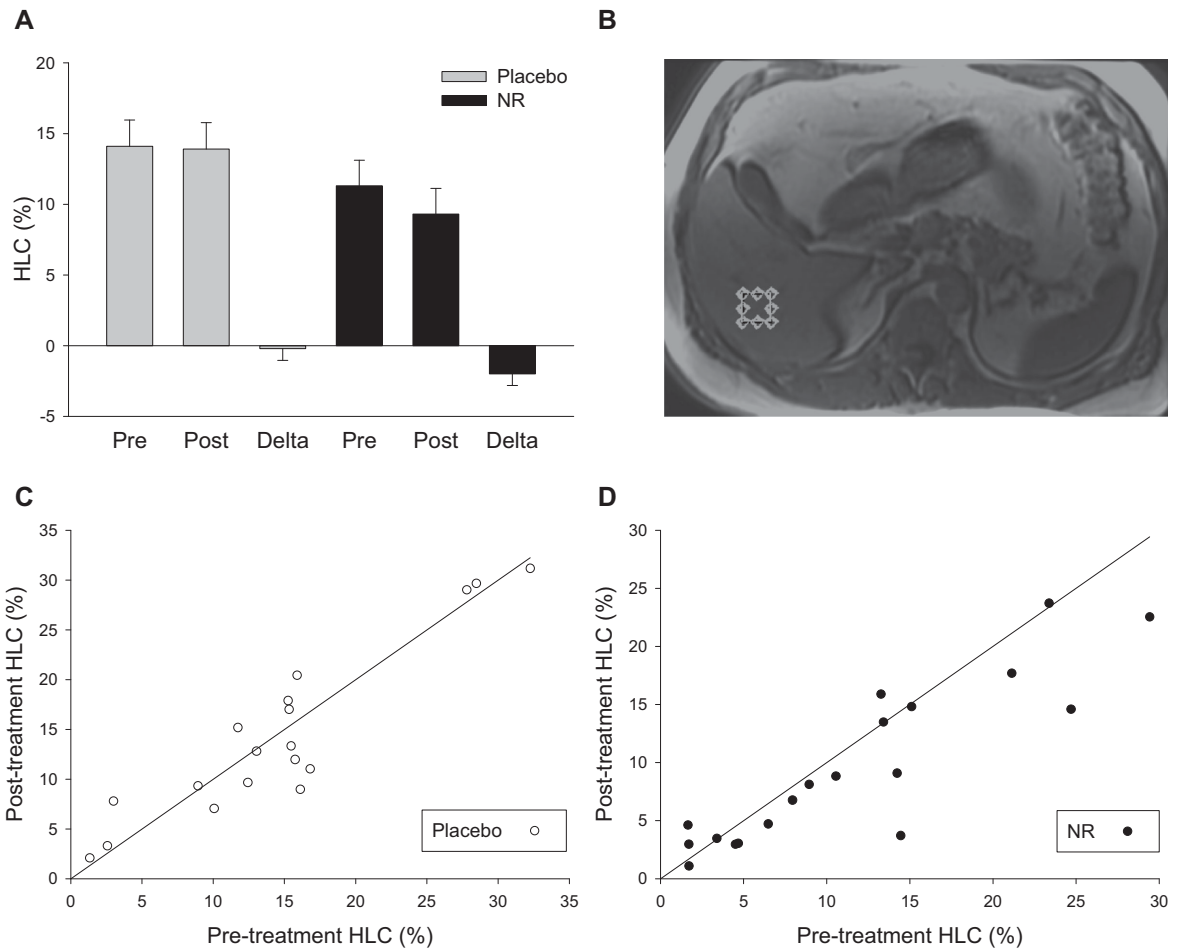

FIGURE 4 HLC determined by ^{1}H-MR-spectroscopy (estimated mean ± SEM). (A) Pretreatment (Pre), post-treatment (Post), and delta (Post – Pre) values with placebo (n = 18–19) or NR (n = 19–20) supplementation. (B) 2 × 2 × 3 cm^3 voxel positioned in the lower posterior part of the liver for quantification of HLC by ^{1}H-MR-spectroscopy. (C–D) Changes in HLC for each subject in the placebo group (C) and NR group (D) displayed with pretreatment values on the x axis and post-treatment values on the y axis. Subjects below the diagonal have reduced HLC, whereas subjects above the diagonal have increased HLC. Treatment comparison was made by repeated-measurement mixed model analysis. No significant treatment effects were observed. Gray bars, white dots = placebo; black bars/dots = NR. HLC, hepatic lipid content; NR, nicotinamide riboside.

in the intervention and placebo groups, and model assumptions were evaluated by inspection of diagnostic plots of residuals and fitted values. In case of a significant interaction, linear pairwise comparisons were performed to compare differences within and between interventions and visits. Baseline characteristics and urinary NR metabolites were compared through the use of the unpaired 2-sample t test. Furthermore, to validate the hyperinsulinemic euglycemic clamp model, insulin effects (basal compared with clamp) were tested independently with paired 2-sample t tests within interventions and visits. When appropriate, data were logarithmically transformed before statistical testing. All available data were included in each analysis. A 2-tailed P value of <0.05 was considered statistically significant. The dataset has also been tested with 2-factor repeated-measures ANOVA, including only participants contributing a full dataset for a given repeated measurement. The result of this statistical analysis did not differ from the mixed model analysis. Statistical analyses were performed with Stata (version 14.1, StataCorp) and graphical presentations with SigmaPlot (version 11.0, Systat Software Inc.).

RESULTS

Inclusion, completion, and compliance

Recruitment and data collection took place from January 2016 to April 2017. One hundred and ninety-seven requests for participation were received in response to advertisements. Forty-five volunteers were screened, of which forty were found eligible and included in the study. All enrolled participants completed the study (**Supplemental Figure 1**). Two participants did not complete the hyperinsulinemic euglycemic clamp at the end of the trial due to technical errors. The compliance rate (mean ± SD) was 95.5% ± 5.0% for the NR group and 98.3% ± 3.0% for the placebo group.

TABLE 3
Blood biochemistry[1]

	Placebo		NR		
	Pretreatment	Post-treatment	Pretreatment	Post-treatment	P[2]
Fasting glucose, mmol/L	5.7 ± 0.1	5.8 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	0.92
HbA1c, mmol/mol	39.8 ± 0.9	40.3 ± 0.9	37.7 ± 0.9	38.2 ± 0.9	0.92
HbA1c, %	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	0.92
Total cholesterol, mmol/L	5.3 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	0.96
HDL, mmol/L	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.64
LDL, mmol/L	3.3 ± 0.2	3.3 ± 0.2	3.4 ± 0.2	3.3 ± 0.2	0.13
Triglycerides, mmol/L	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.2	0.01[3]
ALT, U/L	31.7 ± 3.4	32.0 ± 3.4	33.1 ± 3.6	29.6 ± 3.6	0.21

[1]Data presented as estimated mean ± SEM. Pretreatment and post-treatment values with placebo (n = 19–20) or NR (n = 20) supplementation. ALT, alanine aminotransferase; HbA1c, glycated hemoglobin; NR, nicotinamide riboside.
[2]Treatment comparison was made by repeated-measurement mixed model analysis. P value denotes interaction.
[3]Post hoc tests showed significant difference between pretreatment and post-treatment values for the NR group (P = 0.001); all other comparisons were nonsignificant.

Safety

NR at 2000 mg/d was well tolerated. There were reports of minor adverse reactions from 4 participants in the NR group and from 2 participants in the placebo group. Adverse reactions in the NR group included pruritus, excessive sweating, bloating, and transient changes in stool, and in the placebo group acid reflux and periodic loose stools. The severity was mild in every case. Blood samples drawn to check for adverse events after 6 wk revealed no abnormalities in the NR group. Three participants from the placebo group displayed mild thrombocytosis (platelet count: $<700 \times 10^9$/L).

Baseline

Baseline characteristics of the participants are displayed in **Table 1**. The groups were generally well matched at baseline.

Insulin sensitivity

Insulin infusion during the hyperinsulinemic euglycemic clamp increased insulin concentrations from the basal state to comparable levels in both groups during the clamp period (**Table 2**). Insulin sensitivity, determined as the M-value, was not affected by NR supplementation (interaction: $P = 0.71$), and no significant changes in the M-value occurred during the study (**Figure 1**).

As expected, there was a significant effect of insulin stimulation with reduced EGP and increased glucose uptake (glucose Rd) and NOGD during the clamp (**Figure 2**). However, no effects of NR supplementation were observed. Likewise, palmitate flux (the rate of lipolysis) was reduced and NEFA concentrations dropped in both groups during insulin stimulation with no observed effects of NR (Table 2).

Substrate metabolism

Resting EE and the RER were not affected by NR supplementation. No treatment effects on oxidation rates of glucose, lipid, or protein were observed. As expected, insulin stimulation significantly increased glucose oxidation whereas lipid oxidation decreased during the clamp (Table 2).

Body composition

Twelve wk of NR supplementation did not affect body composition. No differences in total body mass, lean mass, total fat mass, or fat percentage were observed (**Figure 3**). There were no changes in the amount or distribution of abdominal VAT and SAT. No changes in BMD or T-score were observed (data not shown).

HLC

A 2% reduction in HLC in the NR supplemented group was observed compared with a 0.2% reduction in the placebo group; however, no significant difference between groups was found (interaction: $P = 0.13$) (**Figure 4**). In a mouse model, in which obesity was rapidly induced by a diet in which 60% of the calories derived from lard, NR had a profound effect in reducing hepatic steatosis (9). We therefore considered whether our data might suggest that there are responders and nonresponders of NR to decrease hepatic steatosis. As shown in Figure 4C, there were 15 individuals in the placebo group with pretreatment HLC > 5%. Six of these individuals showed apparent decreases in HLC after placebo. In contrast, 13 individuals in the NR group (Figure 4D) had pretreatment HLC > 5% of whom 9 showed an apparent reduction in HLC after 12 wk on NR.

Biochemistry

NR elevated plasma TGs from a pretreatment mean of 1.5 mmol/L to a post-treatment mean of 1.8 mmol/L (**Table 3**). All participants remained within the normal reference range according to the national Danish reference intervals for clinical biochemistry (TG < 2 mmol/L). Total cholesterol and LDL in plasma were abnormal at baseline in both groups (total cholesterol > 5.0 mmol/L, LDL > 3.0 mmol/L) whereas HDL was within the normal reference range (HDL > 1.0 mmol/L).

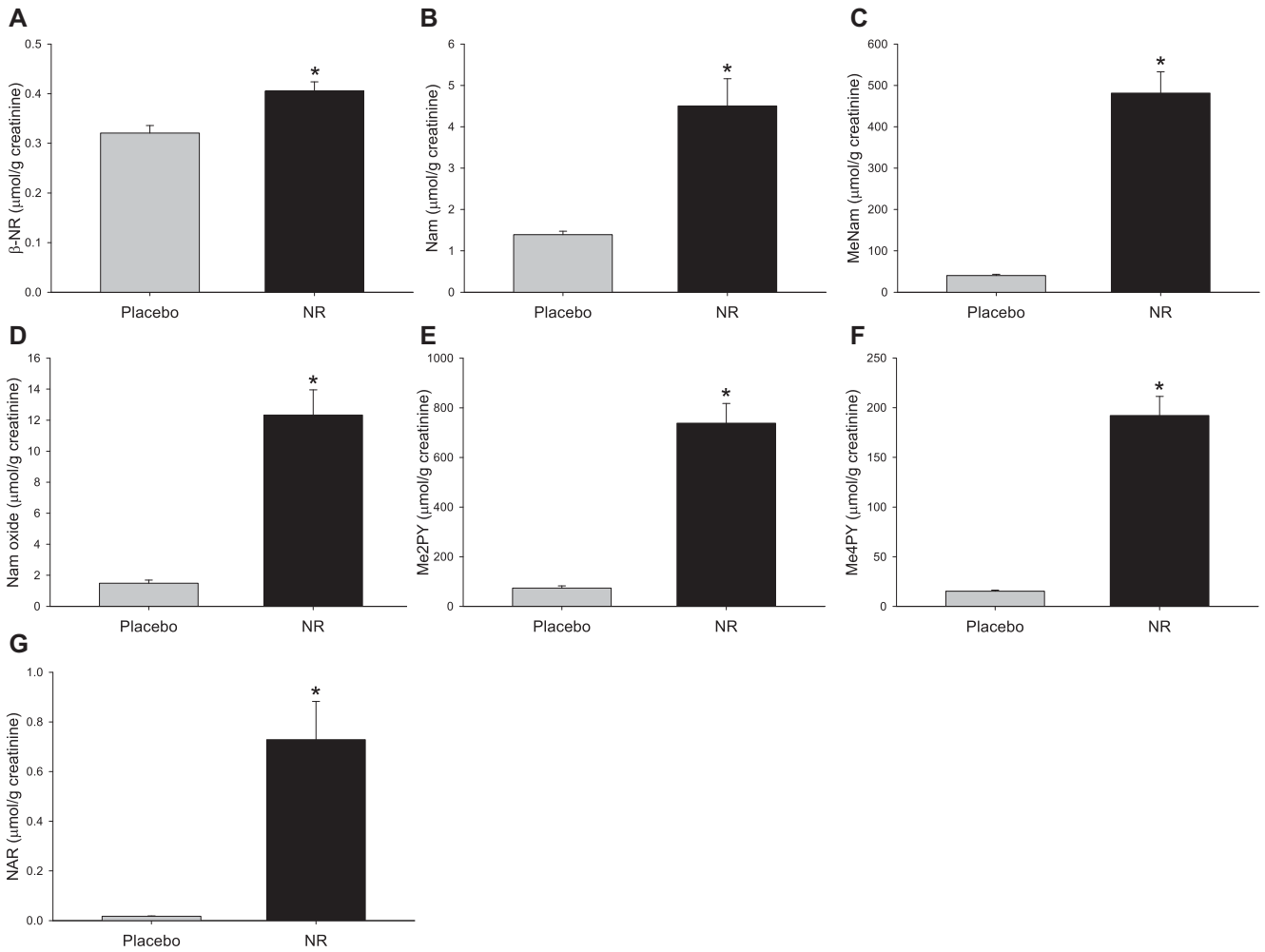

FIGURE 5 Elevation of the urinary NAD^+ metabolome (mean ± SEM) after 12 wk with placebo ($n = 20$) or NR 1000 mg × 2 ($n = 20$) supplementation. NR significantly increased all NAD^+- and NR-derived metabolites (*$P < 0.01$). Urine was collected in the morning approximately 12 h after oral ingestion of NR 1000 mg or placebo. Concentrations are normalized to creatinine concentrations. (A) β-NR, (B) nicotinamide (Nam), (C) N-methylnicotinamide (MeNam), (D) nicotinamide oxide (Nam oxide), (E) N-methyl-2-pyridone-5-carboxamide (Me2PY), (F) N-methyl-4-pyridone-5-carboxamide (Me4PY), (G) nicotinic acid riboside (NAR). Treatment effect assessed via unpaired 2-sample *t* test. Gray bars = placebo; black bars = NR. NAD, nicotine adenine dinucleotide; NR, nicotinamide riboside.

These, however, were unaffected by 12 wk of NR supplementation. No treatment-associated changes were observed in HbA1c, fasting glucose, fasting insulin, or fasting NEFA concentrations. Similarly, no significant change in the liver marker ALT was recorded.

Urinary NR metabolites

The concentrations of NR- and NAD-derived metabolites in urine were determined after the 12-wk study period. All measured metabolites were significantly increased owing to NR supplementation as compared with the placebo group ($P < 0.01$) (**Figure 5**).

DISCUSSION

Changes in body weight, insulin sensitivity, and hepatic steatosis are correlated in rodents and in people. In people, obesity typically develops over decades of imbalance between energy intake and energy expenditure. During this time period, insulin sensitivity decreases, hepatic lipids accumulate, and many other changes occur that simultaneously produce the metabolic syndrome (47). In rodents, NR has been investigated as essentially a monotherapy for rapidly induced diabesity and its complications (9). Thus, a key difference between common human metabolic diseases and animal models is the time frame. While NR has remarkable activity in these models, especially in reducing hepatic steatosis, mice on NR show substantial resistance to HFD-induced weight gain (7, 9), such that it is difficult to know whether the insulin-sensitizing or lipid-mobilizing effects of NR are primary effects or are secondary to weight management. Because people rarely lose weight without an intervention, we aimed to see whether NR might be as powerful as metformin or intense lifestyle changes as a monotherapy.

Safety data on chronic NR supplementation were lacking. Safety data on use of NR by obese individuals were also

lacking and the single dose safety data only went up to 1000 mg/d (16). The safety of ingestion of NR at 2000 mg/d for a 12-wk period was supported in this study by the lack of clinically relevant findings in the blood biochemistry and hematology parameters as well as a lack of difference in incidence, nature, and severity of test article adverse events as well as no reported serious adverse events. Cutaneous flushing, a well-known side effect of NA, present already at a single dose of 500 mg (48), was not reported in relation to NR supplementation in this study.

Insulin sensitivity for glucose utilization was chosen as the primary endpoint of this clinical trial. Peripheral insulin resistance is of primary importance in the events leading to type 2 diabetes and the condition precedes the disease by decades (49, 50). The participants in this study were insulin resistant based on HOMA-IR $\geq$ 2.5 (39). Insulin stimulation during clamp conditions increased glucose oxidation independently of treatment. The capacity to shift between lipids and carbohydrates as substrates for oxidation has been termed metabolic flexibility, and measures of this capacity may give more detailed information on metabolic health (51). Our indirect calorimetry data on substrate oxidation did not reveal effects of NR supplementation, and this observation therefore supports our primary endpoint obtained by the hyperinsulinemic euglycemic clamp. Insulin resistance is defined as a less than biological response to normal concentrations of insulin. Insulin resistance is thus not confined to glucose utilization. To test for tissue-specific effects of insulin, we therefore used substrate tracers that allowed for determination of insulin sensitivity in liver, skeletal muscle, and adipose tissue. The combined results of these investigations all showed no effect of NR supplementation, and we thus did not find evidence of organ-specific effects of NR. However, our findings do not preclude that NR supplementation affects cellular mechanisms in insulin-sensitive tissues other than those measured with the use of substrate tracers. Furthermore, we only included men in this study, and cannot exclude that NR supplementation may have effects on insulin sensitivity or other metabolic parameters in women.

We observed a slight increase in the TG concentration in the NR group mirrored by a slight reduction in the placebo group. Pre- and post-treatment values were within the normal reference range in both groups. Assessed by MR spectroscopy, we observed an absolute reduction in the HLC of 2% (relative reduction 18%) in the NR supplemented group, although this did not reach statistical significance as compared with the minor reduction of 0.2% (relative reduction 1.4%) observed in the placebo group ($P = 0.13$). This effect size is potentially of significance given that the NR group began the trial with 2.8% lower HLC than the placebo group. Inspection of the individual data (Figure 4D) suggests that most participants in the NR group experienced a drop in HLC after 12 wk. In rodents NR displays potent capabilities in reducing hepatic steatosis in HFD-induced NAFLD (8–10). The power calculation for the study was not based on this endpoint, and the possibility of a type II error cannot be excluded. Thus, future sufficiently powered studies should address this endpoint with long-term NR supplementation. In addition, the fate of the hepatic lipid and the question of whether it is transiently mobilized to the circulation can be addressed by collection of urine, feces, and other methods.

In conclusion, 12 wk of dietary NR supplementation is safe at 2000 mg/d but does not improve insulin sensitivity and other metabolic parameters in insulin-resistant, obese men. Longer-term studies are warranted with endpoints that relate to mobilization and disposition of hepatic lipids.

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The authors' contributions were as follows—JTT, NJ, and BC: conceived the study; OLD, BC, MS, NM, JTT, and NJ: designed the study; OLD: conducted the experiments; SR and HS-J: provided technical support regarding the MR techniques and aided in MR data analysis; MSS and CB: performed metabolomics on urine samples; KS: analyzed the data; OLD, JTT, CB, and NJ: interpreted the data; OLD, JTT, CB, and NJ: wrote the manuscript; and all authors: reviewed and accepted the manuscript. NJ is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. CB serves as chief scientific adviser of ChromaDex, Inc., holds stock in ChromaDex, Inc., and has received research grants from ChromaDex, Inc. ChromaDex, Inc. had no role in the study design, data collection, analysis, or preparation of the manuscript. The other authors have no conflicts of interest to declare in relation to this work.

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