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Effects of nicotinic acid on fatty acid kinetics, fuel selection, and pathways of glucose production in women

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Wang, Wei, Alice Basinger, Richard A. Neese, Mark Christiansen, and Marc K. Hellerstein. Effects of nicotinic acid on fatty acid kinetics, fuel selection, and pathways of glucose production in women. *Am J Physiol Endocrinol Metab* 279: E50–E59, 2000.—Chronic nicotinic acid (NA) ingestion effectively lowers lipid levels, but adverse effects on glucose metabolism have been reported. Our goal was to investigate acute and chronic effects of NA on lipolysis and glucose metabolism in women. Healthy normolipidemic volunteers ($n = 5$) were studied twice; four-day hospital stays were separated by 1 mo, during which time subjects took increasing doses of NA to 2 g/day (500 mg, 4 times). In the second study, 500 mg of NA was given at 0800. Rates of appearance (R_a) of free fatty acid (FFA), glycerol, and glucose were determined by isotope dilution (of [1,2,3,4-$^{13}C_4$]-palmitate, [2-$^{13}C_1$]glycerol, and [U-$^{13}C_6$]glucose). Mass isotopomer distribution analysis was used to measure gluconeogenesis and glycogenolysis. Fasting FFA concentrations ([FFA]), R_a FFA, and R_a glycerol were nonsignificantly elevated after 1 mo. Acute NA induced a significant reduction followed by a rebound overshoot of [FFA], R_a FFA, and R_a glycerol. Whole body fat oxidation fell initially and then increased back to basal levels; endogenous glucose production (EGP) increased in parallel with carbohydrate oxidation and then returned to basal values. The increased EGP was due entirely to increased glycogenolysis, not gluconeogenesis. We conclude that chronic effects of NA on FFA metabolism are complex (acute suppression followed by overshoot of R_a FFA and [FFA] on top of a trend toward basal elevations), that responses after NA are consistent with operation of a glucose-fatty acid cycle in peripheral tissues, and that secondary effects on EGP were through changes in glycogenolysis, not gluconeogenesis.

glycogenolysis; gluconeogenesis; Randle cycle; mass isotopomer distribution analysis; lipolysis

THE LIPID-LOWERING EFFECTS of multigram doses of nicotinic acid (NA) were first described in 1955 by Altshul et al. (1). Since that report, large doses (1–3 g/day) of NA have been found to lower total cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL) concentrations and increase high-density lipoprotein (HDL) levels in humans (1, 7, 9, 15, 23). This effect is thought to be mediated via decreases in the release of free fatty acids (FFA) from adipose tissue, thereby decreasing the influx of FFA into the liver (15, 23), the hepatic reesterification of FFA, and the production rate of VLDL-TG. NA has been proved in clinical trials not only to lower lipids but also to improve outcomes; treatment with lipid levels by NA resulted in a significant decrease in mortality after seven years (7).

Whereas most studies have focused on the effects of NA on lipid metabolism, the action of NA on carbohydrate metabolism is less well understood. After acute NA administration, glucose concentrations have been reported to fall (8, 29, 33), rise (33), or not change (2) in rats and humans. Results of glucose tolerance tests after acute NA intake have also been inconsistent (17, 31, 40). Whereas some have reported no effect of NA on glucose tolerance (17), Miettinen et al. (31) showed decreased glucose tolerance 30 min after 200-mg intravenous injection of NA in normal subjects.

Chronic administration of NA has consistently resulted in deterioration of glucose tolerance and elevation of fasting blood glucose concentrations in normal humans (17, 21, 22, 31, 40) and deterioration of glycemic control in type 2 diabetes (21). These effects are contrary to what might be expected on the basis of the glucose-fatty acid cycle of Randle et al. (36). If reduction of lipolysis and FFA availability reduces oxidation of fat in tissues (5, 36) and availability of gluconeogenic precursors in the liver (4, 36), insulin sensitivity should improve and glucose concentrations fall, as observed with inhibitors of mitochondrial fatty acid oxidation (43). Indeed, Chen et al. (10) recently reported that acute administration of NA alters gluconeogenesis and glucose production indirectly by altering serum FFA concentrations. They observed initial suppression of gluconeogenesis when FFA concentrations were low and then an increase in gluconeogenesis when FFA concentrations rebounded, consistent with operation of a glucose-fatty acid cycle in liver. On the other hand,

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recent studies (4, 16) have shown that lowering of FFA concentrations with NA can reduce pancreatic insulin secretion, which could outweigh direct insulin sensitizing effects on tissues.

Our objectives in this study were to establish the effects of NA on FFA availability, whole body fuel selection, and pathways of endogenous glucose production (EGP) in healthy women. Specifically, we addressed the following questions. *1*) Do changes in whole body fuel selection parallel changes in serum FFA concentrations in normal NA-treated overnight-fasted humans? *2*) Does acute lowering of serum FFA concentrations by NA result in lower or higher rates of EGP? *3*) If EGP is altered by administration of NA, is this mediated by changes in gluconeogenesis or glycogenolysis?

METHODS

Human subjects. Five volunteers were recruited by advertisement. Protocols received previous approval from both the University of California at San Francisco and the University of California at Berkeley Committees on Human Research. Subjects gave written informed consent before enrollment. This study was part of a larger study of lipid metabolism in women; because body fat distribution and lipolysis may differ between men and women, enrollment was restricted to females. Subjects had no history of medical illnesses, showed no abnormalities on screening physical examination and laboratory testing, had body mass indexes (BMI) between 20 and 25 kg/m^2 and stable weights over the preceding 6 mo, and had normal serum glucose and lipid concentrations. Smokers, heavy coffee drinkers (>3 cups of coffee/day), and users of oral contraceptives were excluded. The age of the subjects was 26.6 ± 1.4 yr, weight was 57.5 ± 3.0 kg, and BMI was 21.1 ± 0.5 kg/m^2 (means ± SE). Characteristics of the subjects are shown in Table 1.

Study design. The design consisted of two study periods, each lasting 4 wk. Subjects participated in two separate 4-day inpatient metabolic ward studies in the General Clinical Research Center (GCRC) of San Francisco General Hospital. The two GCRC admissions were separated by exactly 1 mo; both were performed in the luteal phase of the menstrual cycle (based on subjects' histories). The first GCRC admission was under basal (control) conditions. Before this first GCRC infusion study, two subjects received placebo calcium lactate tablets 4 times/day for 1 mo, whereas three subjects received no intervention during the preceding 1-mo period at home. Before the second GCRC admission, subjects took crystalline NA obtained from the San Francisco General Hospital pharmacy. The doses were built up to 2 g/day over 2–3 wk; all subjects were taking 2 g/day during the second GCRC admission. Although the subjects were not informed of the sequence of control/NA phases, the symptomatic side effects of NA therapy made true blinding impossible. The sequence of control-then-NA study periods was not randomized because of concerns about carryover effects from the NA period.

Diet. During the 4-day inpatient stay, diet was eucaloric, self-selected, and of the subject's usual macronutrient composition. GCRC dietitians estimated basal metabolic rate with the Harris-Benedict equation and dietary recalls. The same diet was eaten during both inpatient admissions. Diet was adjusted if necessary (<100 kcal/day of changes) to maintain constant weight. The average composition of the self-selected diets was 30% fat, 55% carbohydrate, and 15% protein.

Metabolic infusion protocol. Infusion studies (Fig. 1) were designed to quantify the following metabolic processes in the postabsorptive state, both before and for several hours after NA administration: adipose lipolysis, plasma FFA flux, EGP, gluconeogenesis, glycogenolysis, whole body fat and carbohydrate oxidation, and energy expenditure. The protocol consisted of intravenous infusions of [U-$^{13}C_6$]glucose (0.02 mg · kg^{-1} · min^{-1}) from 0300 to 1400, plus sodium [2-$^{13}C_1$]glycerol (18 mg · kg lean body mass^{-1} · h^{-1}) and [1,2,3,4-$^{13}C_4$]-palmitate (7 μg · kg^{-1} · min^{-1}) from 0400 to 1400. All labeled substrates were >98% enriched and were sterile and pyrogen free. Indirect calorimetry was performed by use of a Deltatrac Metabolic Cart (Sensor Medix, Yorba Linda, CA) in the hooded mode. Two 30-min measurements were performed in the morning before NA administration (0700–0800), two 30-min measurement periods were shortly after NA administration (0900–1000), and two 30-min measurement periods were in the early afternoon (1300–1400). NA (500 mg) or a placebo was administered at 0800 on *day 4* (Fig. 1).

Subjects remained fasted (other than noncaloric, non-caffeine-containing fluids) from 2200 of the preceding evening (*day 3* of GCRC admission) until the end of the infusion study (1400 of *day 4*). A baseline blood draw was taken the previous evening, and repeat samples were taken from an indwelling blood-drawing line at several time points (Fig. 1).

Clinical laboratory measurements. Serum lipids were measured by standard methods (Clinical Laboratories, San Francisco General Hospital). Serum insulin was measured by RIA and serum glucose by autoanalyzer.

Body composition measurements. Body fat mass and fat free mass (FFM) were measured by bioelectrical impedance analysis (Xitron, model 1990B, Valhalla Scientific, San Diego, CA).

Isolation of metabolites and mass spectrometry. Gas chromatography-mass spectrometry (GC-MS) (model 5971; Hewlett-Packard, Palo Alto, CA) was used for analysis of isotopic enrichments of serum glucose, glycerol, and FFA.

FFAs were extracted from plasma as described previously (24, 39) and separated from cholesterol and phospholipids on silica gel G thin-layer chromatography plates (Analtech, Newark, DE). Derivatization to fatty acid methyl esters (FAME) was as described elsewhere (24). FAME were analyzed by GC-MS with a 12.0-m DB1 fused silica column under selected-ion monitoring with electron impact ionization. Abundances of ions at mass-to-charge ratio (*m/z*) 270–274, representing the M_0-M_4 isotopomers, were quantified, and enrichments [molar excess (ME)] were calculated by comparison with standard curves of [1,2,3,4-$^{13}C_4$]palmitate. Concentrations of individual fatty acids were determined simulta-

Table 1. *Subject characteristics*

Age, yr	Weight, kg	%Body Fat	Fat Mass, kg	LBM, kg	BMI, kg/m^2
26.6 ± 1.4	57.5 ± 3.0	27.3 ± 2.6	15.9 ± 1.9	41.6 ± 2.2	21.1 ± 0.5

Values are means ± SE; n = 5. LBM, lean body mass; BMI, body mass index.

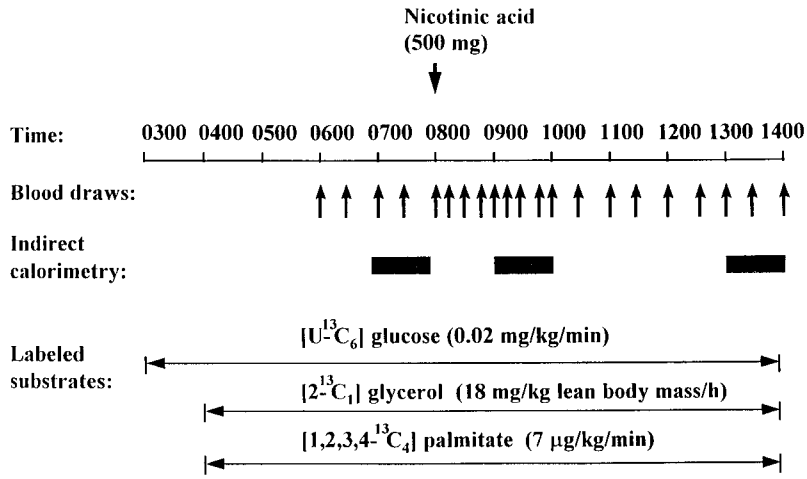


Fig. 1. Metabolic infusion protocol.

neously by use of a splitter that diverted a portion of the GC effluent to a flame ionization detector.

Glucose and glycerol were isolated from 350 μl of plasma and derivatized (24, 38) to glucose pentaacetate and glycerol triacetate, respectively. Samples were dried under N_2 at 60°C, and 800 μl of ethyl acetate were added. A 100-μl aliquot was used for GC-MS analysis of glucose pentaacetate. The remaining 700 μl was used for GC-MS analysis of glycerol triacetate.

A DB-17 fused silica column was used for GC-MS analysis of glucose pentaacetate. For [U-$^{13}C_6$]glucose, the molecular ion minus acetate and its M_6 isotopomer (*m/z* 331 and 337, respectively) were monitored by means of selected-ion monitoring and chemical ionization (24). For calculations of gluconeogenesis by mass isotopomer distribution analysis (MIDA), *m/z* 332 and 333 were also monitored (27, 32). Glycerol triacetate was analyzed with a DB-225 column by chemical ionization (26, 40). Selected-ion monitoring was of *m/z* 159 and 160, representing mass M_0 and M_1, and enrichments were determined by comparison with standard curves of [2-$^{13}C_1$]glycerol.

Calculations. Rates of appearance (R_a) of glucose, FFA, and glycerol were calculated by isotope dilution after subtracting the exogenous chemical infusion rate (24, 39).

Because FFA flux was not at a steady state for several hours after NA administration, we used non-steady-state equations (Steele's equation) (44)

$$R_a \text{ palmitate} = \frac{F - pV[(C_2 + C_1)/2][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2} - F'$$

where F = infusion rate of isotopically labeled material (μmol/min), F′ = chemical infusion rate of labeled material (μmol/min), pV = volume of distribution of metabolite (ml), C_2 and C_1 are the concentrations at *times 2* and *1* (μmol/ml), E_2 and E_1 are the enrichments at *times 2* and *1* (ME), and t_2 and t_1 are final and initial times (min)

$$R_a \text{ FFA} = \frac{R_a \text{ palmitate}}{\%\text{ palmitate in total FFA}}$$

Gluconeogenesis was calculated by the MIDA technique, as described in detail elsewhere (25, 27, 32). In brief, the ratio of excess double-labeled to excess single-labeled species, or $\Delta A_2/\Delta A_1$, of glucose reveals the isotopic enrichment of the true precursor (p) for gluconeogenesis by application of probability principles based on the binomial expansion. The fractional contribution from gluconeogenesis to plasma glucose is then calculated from the precursor-product relationship (25, 26). Glycogenolysis was calculated as the nongluconeogenic contribution (i.e., 1.0 minus the fractional contribution from gluconeogenesis). Absolute fluxes into plasma glucose through gluconeogenesis and glycogenolysis were calculated from their fractional contributions to plasma glucose multiplied by EGP.

Nonprotein respiratory quotient (NPRQ), energy expenditure, and whole body oxidation of fat and carbohydrate were calculated by indirect calorimetry according to standard equations (18).

Statistical analyses. Effects of chronic NA treatment on baseline ($t = 0$) values between study periods were determined by paired *t*-test. Interaction between time and study period (i.e., acute effect of NA administration) was determined by paired *t*-test comparing the change from $t = 0$ to 1.5 h (*phase 1*) and $t = 0$ to 3.0 h (*phase 2*) between the two study periods. Significance was set at $P < 0.05$. Results are presented as means ± SE.

RESULTS

Subject characteristics. The 1-mo treatment with NA did not lead to changes in body weight or body fat content. The treatment with NA resulted in significant decreases in total cholesterol (mean, 26.9% reduction; $r = 9.1–48.1\%$), LDL cholesterol (mean, 42.1% reduction; $r = 26.6 - 65.5\%$) and TG (mean, 42.1% reduction; $r = 19.0-{-}59.3\%$). HDL cholesterol did not change significantly (Table 2).

Serum FFA concentrations and lipolytic fluxes. FFA concentrations are shown in response to chronic and acute NA in the treatment and in the control study periods (Fig. 2). FFA concentrations were relatively stable during the control study, with a nonsignificant increase as the fasting continued. After 1-mo treatment with NA, subjects had nonsignificantly elevated fasting FFA concentrations in the basal state (501.9 ± 91.1 vs. 361.4 ± 70.7 nmol/ml, $P = 0.07$). The acute effect of NA on FFA concentrations was biphasic (Fig. 2). After the acute dose of 500 mg NA at 0800 (*hour 0* time point), FFA concentrations dropped to nadir values (151.9 ± 16.5 nmol/ml, $P < 0.05$ vs. *time 0* and control study) at 1.5 h post-NA (*phase 1*). FFA concen-

Table 2. *Lipid measures*

	Cholesterol	Triglyceride	HDL	VLDL	LDL
Pre-NA	153.4 ± 19.7	93.4 ± 31.4	51.4 ± 5.4	13.0 ± 1.2	87.2 ± 15.8
Post-NA	110.4 ± 14.0*	48.4 ± 11.0*	53.8 ± 6.5	10.2 ± 2.9	50.4 ± 11.1*
%Change	−26.9 ± 6.3	−42.1 ± 7.5	+6.8 ± 12.6	−23.5 ± 17.0	−42.1 ± 6.4

Values are means ± SE in mg/dl. NA, nicotinic acid; HDL, high-density lipoprotein; VLDL, very low density lipoprotein; LDL, low-density lipoprotein. *$P < 0.05$ vs. pre-NA values.

trations then rebounded, increasing to 1,338.7 ± 310.4 nmol/ml (about two- to threefold increase, $P < 0.05$, vs. *time 0* and control study) at 3 h post-NA (*phase 2*) and remained elevated above basal values until the end of the study.

Plasma R_a FFA showed parallel changes to FFA concentrations (Fig. 3). The nadir value at 1.5 h (*phase 1*) was 3.4 ± 0.6 μmol · kg^{-1} · min^{-1}, significantly different from *time 0* and from control study; the 3-h (*phase 2*) value was 14.8 ± 2.6 μmol · kg^{-1} · min^{-1}, also significantly greater than *time 0* and control study values.

Plasma R_a glycerol reflects absolute adipose lipolysis (24, 44), because glycerol released from intracellular hydrolysis of TG cannot be reesterified in the adipocyte. The rates of [2-$^{13}C_1$]glycerol infusion required for gluconeogenesis measurement by MIDA represent a substantial nontracer exogenous glycerol load; plasma glycerol enrichments were in the range of 40–50%. Nevertheless, R_a glycerol was relatively constant during the control study (Fig. 4), and basal R_a glycerol was not significantly higher after 1 mo of NA treatment compared with the control study period (3.1 ± 0.3 vs. 2.2 ± 0.3 μmol · kg^{-1} · min^{-1}). R_a glycerol fell during *phase 1* after NA (to 1.3 ± 0.5 μmol · kg^{-1} · min^{-1} at 2 h, $P < 0.05$ vs. *time 0* and control study) and then rebounded in the same manner as R_a FFA values during *phase 2* (maximum 7.8 ± 0.7 at 3 h post-NA, $P < 0.05$ vs. *time 0* and control study) and remained significantly higher than pre-NA values through 4 h and nonsignificantly higher through 6 h.

Plasma glycerol concentrations paralleled changes in R_a glycerol. Acute administration of NA resulted in an initial fall [from 19.7 ± 1.3 to 14.0 ± 2.5 mg/dl (mean ± SE, $n = 3$ subjects) from baseline to 1.5 h post-NA], followed by an overshoot and return to baseline (27.0 ± 4.4 and 20.0 ± 2.9 mg/dl at 3 and 6 h post-NA, respectively). In contrast, plasma glycerol concentrations were unchanged over time in the placebo phase (19.3 ± 4.9, 19.0 ± 6.2, 17.3 ± 3.3, and 17.3 ± 3.4 mg/dl at baseline, 1.5, 3.0, and 6.0 h, respectively). The change in glycerol concentrations over time was significantly different between the two phases for baseline to 1.5 h and from 1.5 h to 3.0 h ($P < 0.05$).

EGP, gluconeogenesis, and glycogenolysis. Figure 5 shows EGP before and after NA treatment. EGP values were relatively constant during the control study. Basal values of EGP were not different between NA and control phases. After acute NA administration, EGP increased during *phase 1*, reaching a peak (2.7 ± 0.2 mg · kg^{-1} · min^{-1}, $P < 0.05$ vs. *time 0* and control study) at 1.5 to 2 h post-NA. EGP then gradually declined to basal levels (1.9 ± 0.1 mg · kg^{-1} · min^{-1}) during *hours 3–6* (not significant vs. *time 0* or control study). Enrichments of plasma glucose (excess M_6) ranged from 0.75 ± 0.01% (mean ± SE) at −1.5 h to 0.84 ± 0.03% at +6.0 h (control study) and from 0.80 ±

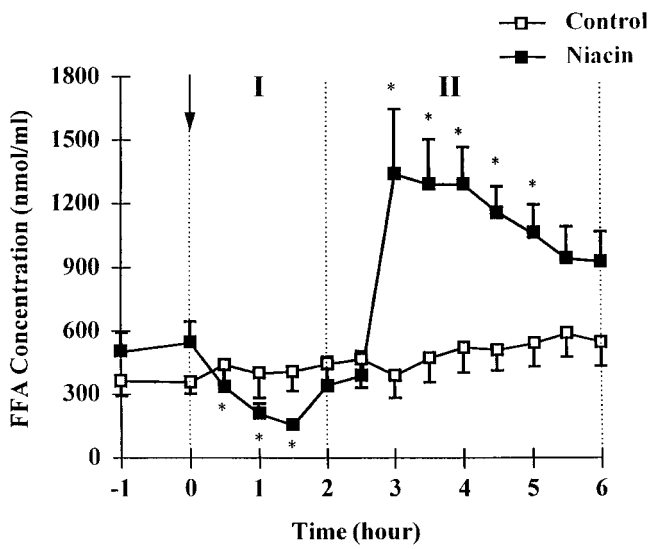


Fig. 2. Plasma free fatty acid (FFA) concentrations in response to chronic and acute nicotinic acid (NA) or control. *$P < 0.05$ vs. control. Arrow denotes time of NA administration.

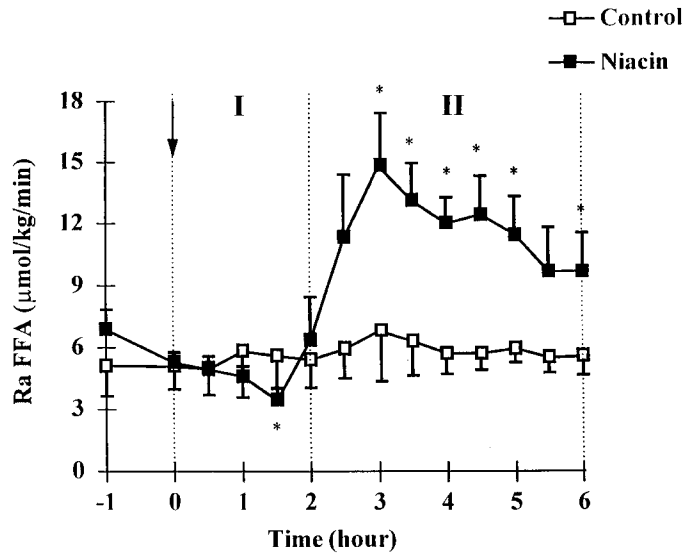


Fig. 3. FFA rate of appearance (R_a FFA) in response to chronic and acute NA or control. *$P < 0.05$ vs. control. Arrow denotes time of NA administration.

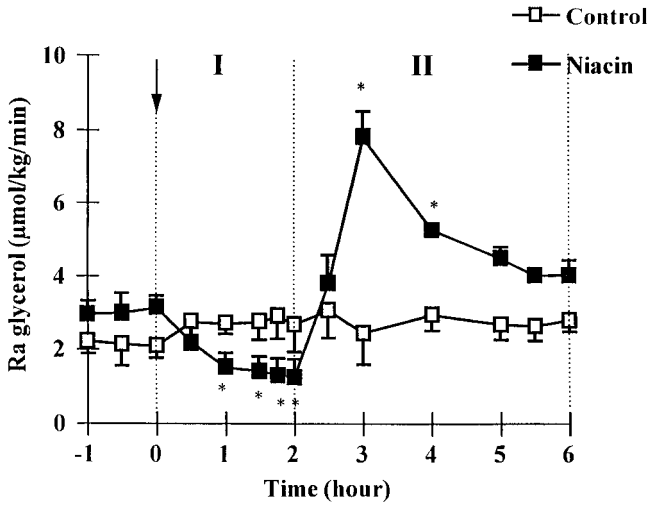


Fig. 4. R_a glycerol in response to chronic and acute NA or control. *$P < 0.05$ vs. control. Arrow denotes time of NA administration.

0.01% at −2.0 h to 0.67 ± 0.05% at 1.5 h and to 0.88 ± 0.06% at 6.0 h (NA study).

The fractional contribution from gluconeogenesis to plasma glucose fell nonsignificantly during *phase 1* after acute NA and then returned to values that were higher than but not significantly different from the control study (Fig. 6*A*). Basal gluconeogenesis was 35.2 ± 3.1% (NA) vs. 27.8 ± 2.5% (control), 28.7 ± 4.1% (NA) vs. 35.4 ± 3.3% (control) at 2 h, and 53.5 ± 3.3% (NA) vs. 41.4 ± 3.5% (control) at 6 h. Absolute gluconeogenesis values also did not change after NA treatment (Fig. 6*B*), although a nonsignificant decrease in absolute gluconeogenesis was observed between 1.5 and 3 h post-NA. The calculated precursor pool (hepatic triose phosphate) enrichments were not different after NA vs. control (Fig. 7). In the control study, enrichments of plasma glucose M_1 and M_2 mass isotopomers ranged from 4.56 ± 0.49% at −1.5 h to 6.58 ± 0.40% (mean ± SE) at 6.0 h for excess M_1 and 1.23 ± 0.20% at −2.0 h to 1.74 ± 0.13% at 6.0 h for excess M_2. In the NA study, excess M_1 enrichments ranged from 4.49 ± 0.99% at 1.5 h to 7.45 ± 0.24% at 6.0 h and excess m_2 enrichment ranged from 1.00 ± 0.26% at 1.5 h to 1.93 ± 0.12% at 6.0 h.

Glycogenolysis initially accounted for the major contribution (Fig. 6*C*): 1.5 ± 0.1 and 1.7 ± 0.1 mg · kg^{-1} · min^{-1} (NA and control study periods, respectively). The contribution from glycogenolysis declined during progressive fasting in the control study to 1.2 ± 0.1 mg · kg^{-1} · min^{-1} (58.6 ± 3.9% EGP) at 6 h. After acute NA, the glycogenolytic contribution increased significantly ($P < 0.05$ vs. *time 0* and control study) to 1.9 ± 0.2 mg · kg^{-1} · min^{-1} (71.3 ± 4.1%) concurrently with the decrease in FFA concentration during the first 2 h post-NA (Fig. 6*C*) and then fell during *hours 2–6* by 0.9 ± 0.1 mg · kg^{-1} · min^{-1} (to 46.5 ± 3.3%). After 3 h, the changes were not statistically significant vs. *time 0* or the control study.

Serum glucose and insulin concentrations. Basal serum glucose concentrations after overnight fasting in control and NA study periods were not different (88.4 ± 2.8 mg/dl and 88.7 ± 4.1 mg/dl, respectively). Glucose concentrations did not change significantly post-NA (not shown).

Plasma insulin concentrations were stable during the control study. Fasting insulin concentrations were not different between studies (6.4 ± 1.7 μU/ml in NA study and 5.2 ± 1.0 μU/ml in control study). Plasma insulin concentrations were not significantly different after control vs. NA administration, although there was a nonsignificant fall to 2.8 ± 0.1 μU/ml at 1.5 h and then a rise to 9.0 ± 2.5 μU/ml at 3.5 h after acute NA administration.

Plasma glucose clearance. The clearance of glucose from plasma (R_a glucose divided by plasma glucose concentration) was not significantly different at *time 0* between study periods (0.026 ± 0.001 dl · kg^{-1} · min^{-1} in the control study and 0.024 ± 0.000 dl · kg^{-1} · min^{-1} in the NA study). Glucose clearance increased significantly during *phase 1* after acute NA (0.026 ± 0.002 dl · kg^{-1} · min^{-1} at 1.5 h in the control study vs. 0.029 ± 0.002 dl · kg^{-1} · min^{-1} in the NA study, $P < 0.05$ between studies and from *time 0* for NA study) and then returned to baseline values (0.023 ± 0.001 dl · kg^{-1} · min^{-1} in control and 0.021 ± 0.000 dl · kg^{-1} · min^{-1} in NA study) at 6 h.

Fuel selection and energy expenditure. Basal measurements of NPRQ (0.80 ± 0.01 vs. 0.76 ± 0.03), whole body fat oxidation, (3.79 ± 0.28 vs. 4.72 ± 0.45 μmol · kg^{-1} · min^{-1}), whole body glucose oxidation (1.37 ± 0.03 vs. 0.90 ± 0.38 mg · kg^{-1} · min^{-1}), and resting energy expenditure (1,467 ± 60 vs. 1,519 ± 38 kcal/24 h) were not significantly different at *time 0* between NA and control study periods.

NPRQ increased significantly, to 0.90 ± 0.03 between 1 and 2 h post-NA and then decreased back to basal values (0.73 ± 0.01) during *hours 5* and *6* of the

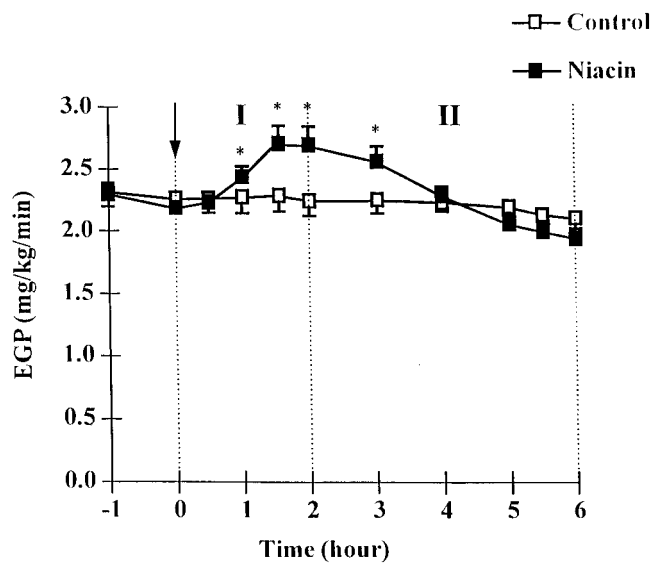
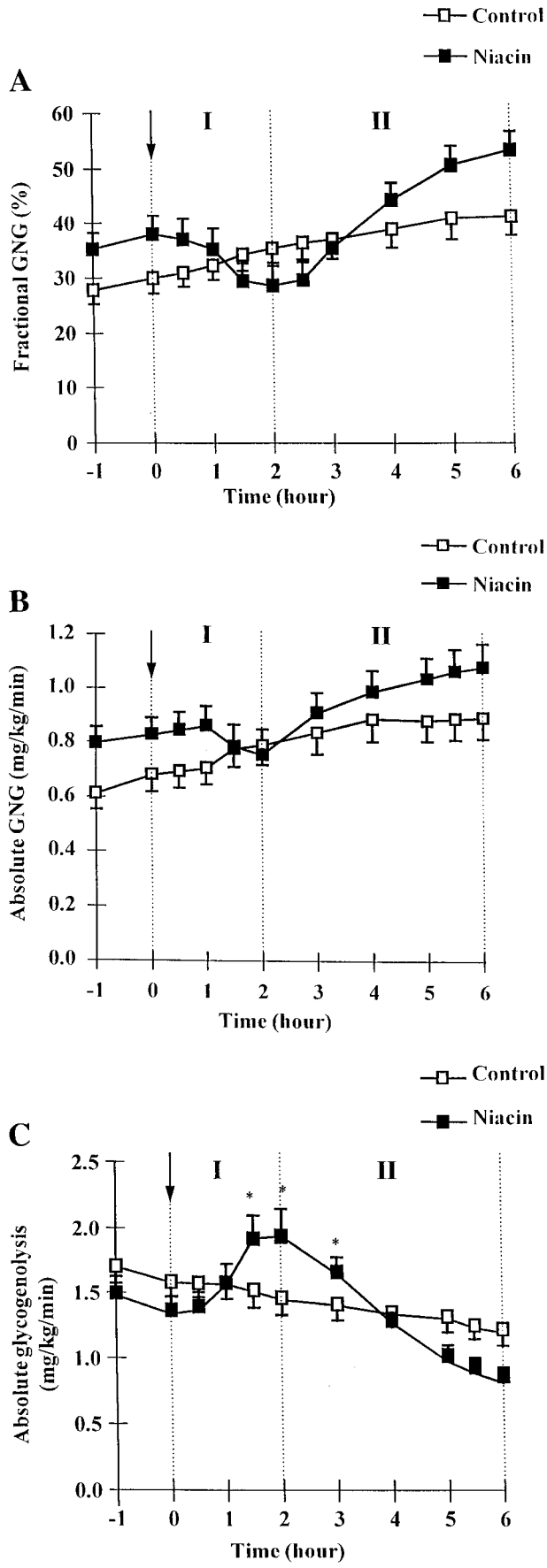


Fig. 5. Endogenous glucose production (EGP) in response to chronic and acute NA or control. *$P < 0.05$ vs. control. Arrow denotes time of NA administration.



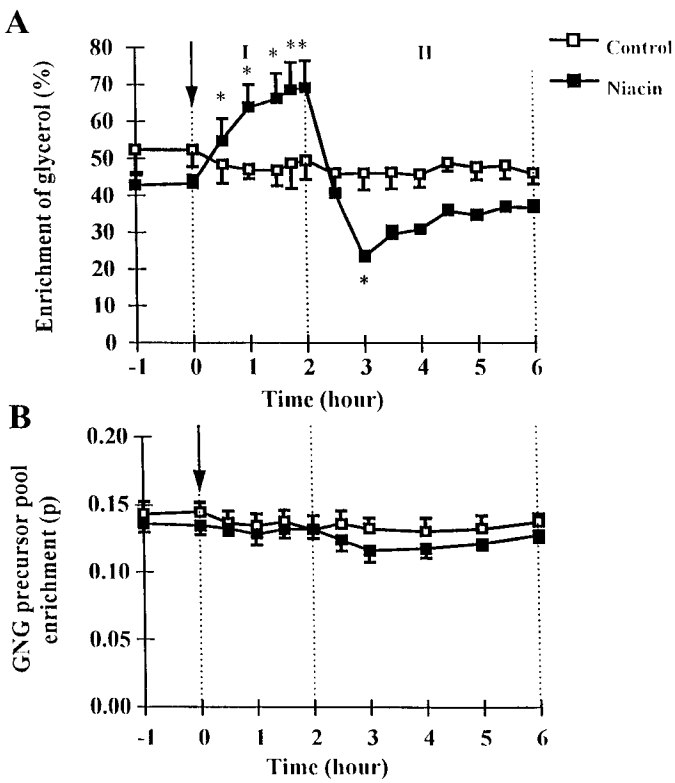


Fig. 7. Plasma glycerol enrichments (*A*) and GNG precursor pool enrichments (p) (*B*) in response to NA or control. Arrow denotes time of NA administration.

study in association with changes in serum FFA concentrations, R_a FFA, and EGP. In the control study, NPRQ fell nonsignificantly to 0.76 ± 0.01 at 5–6 h.

Whole body carbohydrate oxidation was significantly increased, and fat oxidation was significantly decreased (Fig. 8) at 1–2 h post-NA compared with the control study period. The values at 1–2 h post-NA were significantly different from those at 1–2 h in the control study and those at 5–6 h in the NA study.

Total energy expenditure was not significantly different between NA and control study periods or at any time point after NA.

DISCUSSION

Chronic treatment followed by acute NA administration resulted in a complex pattern for serum FFA concentrations in the postabsorptive state; overnight fasting FFA concentrations and R_a FFA tended to be higher (but nonsignificantly so) after chronic NA compared with control periods and then showed a significant decrease after an acute dose that was followed by a significant rebound overshoot. Biphasic changes in serum FFA concentrations after acute NA have been reported previously (34). The consequences of these

Fig. 6. Gluconeogenesis (GNG) and glycogenolysis in response to chronic and acute NA or control. Arrow denotes time of NA administration. *A*: fractional contribution from GNG to plasma glucose; *B*: absolute GNG flux into plasma glucose (mg · kg^{-1} · min^{-1}); *C*: absolute glycogenolysis. *$P < 0.05$ vs. control

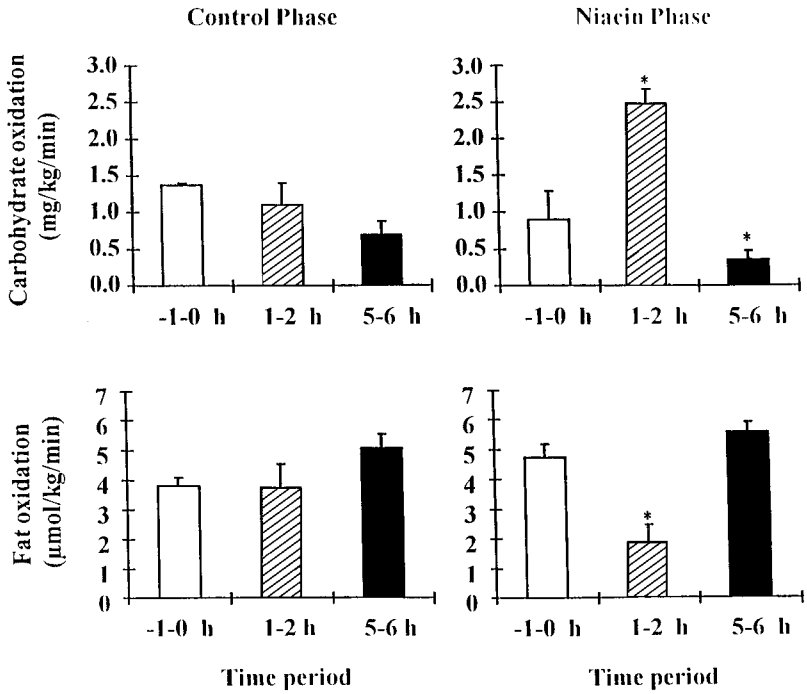


Fig. 8. Fuel selection in response to NA vs. control. Whole body carbohydrate oxidation (*top*); fat oxidation (*bottom*). $*P < 0.05$ vs. control

complex changes in FFA availability on in vivo fuel selection and glucose metabolism were not, however, entirely predictable from studies performed in isolated tissue and cell systems (5, 6). Of particular interest was our demonstration for the first time that increases in glucose production, utilization, and glycogen-to-glucose flux clearly occur in glycogen-replete postabsorptive humans when systemic FFA availability is reduced. Also of interest was the observation of asymmetric effects of sub- vs. supraphysiological FFA availability on whole body fuel selection.

With regard to fuel selection, whole body oxidation of fat decreased by 60% (from 4.7 to 1.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) within 1–2 h after acute NA administration with a reciprocal threefold increase in carbohydrate oxidation (from 0.9 to 2.5 $mg \cdot kg^{-1} \cdot min^{-1}$). This striking change in fuel selection, reflected by a change in NPRQ from 0.76 to 0.90, paralleled the reduced serum FFA concentrations and R_a FFA. Thus, the rate of delivery of circulating FFA to tissues, perhaps in combination with changes in serum hormone levels, appears to regulate fat oxidation in the range below the usual postabsorptive FFA values. In contrast, in the period of supranormal FFA concentrations and fluxes associated with NA administration (the *phase 2* rebound hyperlipolytic period), whole body fat oxidation was not higher than normal. Basal rates of fat oxidation were restored but were not exceeded in the face of elevated serum FFA availability. Thus serum FFA concentrations and the rate of their delivery to tissues was no longer rate controlling for tissue oxidation in the range above the usual postabsorptive FFA levels, consistent with the previous conclusions (24, 28, 45) that serum FFA are present in excess of oxidative needs under normal postabsorptive conditions. Agents that increase FFA mobilization (e.g., nicotine, caffeine, and sympathomimetic agents) cannot, therefore, be expected to increase whole body fat oxidation by this mechanism alone; instead, higher rates of FFA reesterification and VLDL-TG production result (24).

What is the explanation for our clear and reproducible finding of increased glucose production, utilization, and glycogenolysis in *phase 1* after NA (Figs. 5, 6, and 8)? The striking increase in EGP during the first 2 h after acute NA intake in the face of reduced FFA availability is not what would have been predicted from the direct operation of the Randle cycle on fasted liver in isolation. Instead, reduced FFA availability would have been expected to lower gluconeogenesis and reduce glucose production by the liver (6, 29).

These changes in glucose metabolism might best be explained by secondary changes in glucoregulatory hormones induced by the reduction in serum FFA. Lowering of serum FFA concentrations may reduce serum insulin concentrations by two mechanisms. First, serum FFA themselves are secretagogues for insulin release in the β-cell (4, 16), particularly in the postabsorptive state. Second, if lowering of serum FFA allows increased glucose oxidation and utilization by peripheral tissues, as we observed (Fig. 8), the effect will be to reduce plasma glucose concentrations and secondarily to reduce insulin secretion. Subtle changes in portal or systemic insulin and glucagon concentrations in animal models result in marked changes in hepatic glucose production, acting primarily through modulation of glycogenolysis (11, 13, 30). Although our study was not designed to detect subtle changes in portal concentrations of glucoregulatory hormones, we did observe a trend toward reduction in systemic insu-

lin concentrations. Changes in glucoregulatory hormones might, therefore, explain our results.

The observation that plasma glucose clearance was elevated in *phase 1* after NA administration in the absence of higher serum insulin concentrations rules out a primary effect of NA on the liver (e.g., stimulation of glycogenolysis). If NA itself acutely increased EGP, serum insulin concentrations should have been elevated after NA. Instead, we observed lower serum insulin concentrations, as have others (4, 16), after acute NA administration in the postabsorptive state.

Other observations also indirectly support the model that the observed changes in EGP were a secondary effect on liver (or kidney) mediated by glucoregulatory hormones or other systemic signals, rather than a direct effect of FFA on the liver. EGP peaked at an almost identical value ($2.40\ mg \cdot kg^{-1} \cdot min^{-1}$) as whole body carbohydrate oxidation between 1.5 and 2.0 h after NA, consistent with a compensatory increase in glucose production that was modulated to match the peripheral stimulation of glucose oxidation. Also, the increase in EGP being attributable to glycogenolysis rather than to gluconeogenesis is consistent with the acute actions of a variety of glucoregulatory factors on hepatic glucose metabolism, including insulin, glucagon, diet, and exercise, as reported in previous studies in dogs (11, 13, 30). In contrast, most formulations regarding a direct glucose-fatty acid cycle in liver (36) invoke changes in gluconeogenesis.

It is of interest to compare these results with the recent report of Chen et al. (10). These workers studied the effect of a first administration of NA (1,100 mg given over 4 h) during the 16th to the 24th h of a fast, using ^2H_2O incorporation to measure gluconeogenesis. They reported no change in EGP during the period of reduced plasma FFA concentrations, so that the increase in absolute glycogenolytic flux into glucose that they also observed was associated with a fall in absolute gluconeogenesis. Also, Chen et al. observed no change in whole body carbohydrate or fat oxidation and reported that fractional and absolute gluconeogenesis increased to suprabasal levels when FFA concentrations rebounded. They concluded that serum FFA have a primary effect on hepatic gluconeogenesis after NA with reciprocal changes in glycogenolysis that serve to maintain constant EGP. Our results suggest a different model: a primary effect of FFA on glucose utilization by peripheral tissues with the hepatic effect being mainly a secondary alteration in glycogenolysis likely modulated by changes in hormone levels.

Reasons for the differences between these two studies are uncertain. The use of different techniques to measure gluconeogenesis does not explain the results, because the major differences between the studies were for EGP (initially increased after acute NA administration in our study but unchanged in theirs) and whole body fuel selection (altered after NA administration in our study but unchanged in theirs), whereas fractional gluconeogenesis was similar in the two studies (fall then rise). The subjects of Chen et al. differed from those in the present study in that they were studied after 20–24 h of fasting. The duration of previous fasting may affect glycogenolysis or dependence of pancreatic β-cell insulin secretion on serum FFA (16, 19). Also, our subjects were all women, whereas theirs were both men and women. These factors may alter tissue fuel selection or substrate metabolism. Finally, we gave NA chronically before studying its acute effects, to be more representative of the steady-state clinical situation, whereas the subjects in the Chen et al. study had not received previous treatment with NA. It is possible that counterregulatory responses to NA administration (10) are enhanced after chronic NA treatment and contribute to the increased EGP, as well as to the lack of a fall in gluconeogenesis that we observed. In the design of future studies, it will be important to consider these physiological and pharmacological factors.

Comparison with the studies of Fulcher et al. (20) further supports the importance of feeding state and duration of previous NA therapy for the glucose metabolic response to acute NA administration. These investigators reported that a single dose of acipimox, a long-acting NA analog, did not reduce fasting glucose production or concentrations acutely but did so after overnight administration in diabetic subjects. Their explanation for the delayed reduction in EGP was that compensatory increases in glycogenolysis initially overcame reduced rates of gluconeogenesis until glycogen stores were depleted. In rats, initial increases in glycogenolysis and EGP after NA are followed by glycogen depletion and the subsequent development of hypoglycemia (3). Because our subjects were studied between *hours 10* and *16* of overnight fasting, hepatic glycogen stores had not been depleted. If our studies had been performed under conditions of prolonged fasting with depletion of liver glycogen stores, acute administration of NA might have had very different effects on EGP.

Debate continues regarding the source of EGP in postabsorptive humans (27, 29, 32, 37) and the validity of using MIDA to measure gluconeogenesis and glycogenolysis (14, 27, 32, 33, 35, 42). The results presented here are relevant to the latter question. The extremely stable values calculated for p, despite very large changes in serum glycerol concentrations, fluxes, and enrichments (Fig. 7), argues against the criticism postulated by Previs et al. (35) of the MIDA technique for measuring gluconeogenesis from labeled glycerol. These authors proposed that a gradient exists for glycerol across the hepatic lobule that is exacerbated by lower serum glycerol concentrations and results in uneven uptake of label, thereby distorting the combinatorial model (high enrichments in some hepatocytes at the beginning of the lobule and no label in others at the end of the lobule). The postulated consequence is an artifactual overestimation of p (and underestimation of fractional gluconeogenesis). These predictions are not consistent with observations in the present study, however. Both p and fractional gluconeogenesis were constant after acute administration of NA, despite initially reduced and then increased systemic concentrations and delivery of cold glycerol and the op-

posite effects on serum glycerol enrichments. If the glycerol gradient model applied, lowering the flux and concentration of plasma glycerol while increasing its enrichment (during *phase 1* after acute NA administration) should have worsened the isotopic gradient across the liver and resulted in higher values of p (and lower values of fractional gluconeogenesis, all else being equal). The opposite effects should have been apparent during the rebound hyperlipolytic phase after NA: "flooding" the liver with glycerol would be predicted to result in higher fractional gluconeogenesis and lower values of p (35). There was no sign, however, of either of these changes during either phase after NA (Fig. 7). The ~2-h duration of perturbed glycerol metabolism (Fig. 7) is sufficient for changes to have become apparent in plasma glucose labeling ($t_{1/2}$ of plasma glucose pool being ~30 min), if these in fact had occurred.

In contrast, if the postulated labeling gradient from glycerol does not exist, constant values of p would be expected. The rate of delivery of labeled glycerol to the liver should not change, regardless of dilution by endogenous glycerol, because the great majority of glycerol utilization is hepatic. Moreover, the contribution from endogenous plasma glycerol to gluconeogenesis and the triose phosphate precursor pool is relatively small (~10–15%), as we have observed here and previously (27, 32). Even a 50% reduction in endogenous glycerol flux would therefore not be expected to change significantly the substrate load into the gluconeogenic precursor pool. Accordingly, the reproducible observation of constant values for p, despite marked changes in endogenous glycerol flux (Fig. 7), is consistent with the postulated MIDA model rather than with the existence of a labeling gradient. Because of the potentially complex physiological changes involved after administration, these results do not provide conclusive evidence against a labeling gradient. It should be noted, however, that a labeling gradient from [^{13}C]glycerol across the hepatic lobule (as contrasted with uptake across the organ) has never been shown experimentally but has been only postulated (35). Moreover, previous results from our laboratory, as well as those from other laboratories, have not been consistent with the existence of a significant labeling gradient. Fractional gluconeogenesis values have been appropriately high with the technique in fasted animals (32, 33), perfused livers from fasted animals (33), and fasted humans (14, 27, 42). The reason that Previs et al. (35) have observed discordantly low values of gluconeogenesis, unlike other investigators (14, 27, 32, 33, 42), remains uncertain.

Finally, do these findings advance our understanding of how NA can worsen glycemic control or bring out latent diabetes? We did not study diabetic or insulin-resistant subjects, so definitive statements cannot be made in this regard. It is difficult to propose a scenario by which the immediate effects observed post-NA (i.e., increased glucose oxidation and utilization by tissues with a compensatory increase in EGP and glycogenolysis) could worsen glycemia. The *phase 2* rebound (*hours 2–6*) post-NA or the trend toward overnight increases in lipolysis and FFA concentrations might result in impaired glucose utilization or increased EGP, however, in insulin-resistant or deficient states. Whereas the normal subjects studied here did not exhibit a parallel increase in fat oxidation or a decrease in glucose utilization, and glycogenolysis and EGP returned to basal levels, in the face of supraphysiological FFA concentrations, individuals with impaired glucose homeostasis might not be able to adapt as effectively to rapid changes in FFA availability. Another possibility is if pancreatic insulin secretion were more dependent on FFA concentrations in type II diabetic or insulin-resistant subjects than in normal subjects (4, 16), resulting in inadequate insulin secretion after NA to maintain normoglycemia. Future studies will need to examine in detail the metabolic response to the various phases associated with NA treatment in clinical settings of impaired glucose homeostasis.

In summary, a model of the consequences of altered FFA availability on glucose utilization and production in postabsorptive subjects can be proposed based on these results. Under postabsorptive conditions, acute administration of NA initially reduces FFA release into the circulation and affects tissue fuel utilization, with whole body fat oxidation falling and carbohydrate oxidation rising proportionately. In response to increased whole body glucose utilization, presumably mediated by changes in secretion of insulin or other hormones, EGP increases; this is due exclusively to increased glycogenolytic flux without changes in gluconeogenesis. A rebound hyperlipolytic phase then follows but without increases above basal for fat oxidation, EGP, or glycogenolysis; instead, basal rates are restored. We conclude that the glucose-fatty acid cycle occurs under physiological conditions in postabsorptive healthy women but is tissue specific (periphery dominating over liver) and asymmetric (operative in response to lower but not higher FFA concentrations) and that compensatory increases in EGP in this setting operate through stimulation of glycogenolysis, not gluconeogenesis.

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