Wang, Wei, Alice Basinger, Richard A. Neese, Barry Shane, Su-a Myong, Mark Christiansen, and Marc K. Hellerstein. Effect of nicotinic acid administration on hepatic very low density lipoprotein-triglyceride production. Am J Physiol Endocrinol Metab 43: E540–E547, 2001.—Our objective was to examine very low density lipoprotein-triglyceride (VLDL-TG) kinetics after chronic and acute administration of nicotinic acid (NA). Incorporation of [1,2,3,4-13C4]palmitate and [2-13C1]glycerol into VLDL-TG was measured in five healthy, normolipidemic women. Each subject was studied twice; the 4-day hospital stays were separated by 1 mo, during which time doses of NA were increased to 2 g/day (500 mg, 4 times/day). During posttreatment study, 500 mg of NA were administered acutely at 0800. Under baseline postabsorptive conditions, incorporation curves from 13C-labeled free fatty acid (FFA) and 13C-labeled glycerol were superimposable, and VLDL-TG kinetics were in agreement (t1/2 = 1.4 ± 0.3 and 1.3 ± 0.3 h, and production rates = 27.2 ± 6.1 and 28.5 ± 5.3 g/day, respectively). In the postabsorptive state after chronic NA therapy, VLDL-TG concentrations and production rates were lower despite a trend toward elevated plasma FFA concentrations and flux after hour 2. Plasma homocysteine concentrations increased 68% (P < 0.001) in the NA phase, consistent with chronic increased transmethylation demand. We conclude that 1) NA acutely and chronically decreases VLDL-TG production rate in normal women; 2) the acute effect on VLDL-TG production is associated with an initial suppression of lipolysis but persists for several hours after the antilipolytic action of NA has abated and is observed in the basal postabsorptive state, when lipolytic rates are not reduced; and 3) the effect of NA on VLDL-TG production, therefore, cannot be completely explained by its antilipolytic actions.

And risk for coronary heart disease (CHD) (2, 7, 24, 27). In earlier studies using multivariate analysis, TGs were not found to be an independent risk factor because of their strong inverse relationship with high-density lipoproteins (HDL). However, recent studies have demonstrated clearly that elevated TGs increase the risk for CHD independently of HDL concentrations (22, 24). A gradient of risk of CHD was found with increasing TG levels within stratified HDL cholesterol levels, including subgroups with high HDL cholesterol levels (24).

Very low density lipoprotein (VLDL) is the major lipoprotein carrier for TG in the postabsorptive state. Reported daily production rates of VLDL-TG in humans have varied (17, 18, 37, 43). By modeling the curve of incorporation of radioactively labeled 14C or 3H glycerol into secreted VLDL-TG, synthesis rates in one study ranged between 806 mg/h, or 19 g/day (43), and 1,250 mg/h, or 30 g/day. Most studies have been performed in male subjects. For example, from the incorporation of [2-13C1]glycerol and [1,2,3,4-13C4]palmitate into VLDL-TG in healthy normolipidemic men, Siler et al. (37) reported a production rate of 14.7 mg·kg⁻¹·h⁻¹, or 25 g/day. There are no previous reports available of VLDL-TG synthesis rates in women, however.

Nicotinic acid (NA) is an effective lipid-lowering agent that causes beneficial changes in serum lipids, i.e., reduced concentrations of VLDL-TG and LDL cholesterol and increases in HDL cholesterol, as well as atherosclerosis regression (26, 31) and decreased incidence and mortality of coronary artery disease (5, 34).

The mechanism by which NA lowers VLDL-TG concentrations is uncertain. One proposal has been that NA exerts an inhibitory effect on lipolysis (9, 33), thereby reducing free fatty acid (FFA) flux from adipose tissue to liver and decreasing hepatic VLDL-TG production. Other authors (11, 38) have suggested that high-dose NA may act as a methyl trap and induce hepatic methyl group deficiency, thereby impairing secretion of lipids from the liver (12). The objective of this

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Methods

Portions of this work have been presented previously as a preliminary communication and a subsequent abstract. Further details of this study and of the previous reports have been described in detail elsewhere (40, 41). In the present communication, we report that these data could be extended to women with a particular type of hyperlipidemia, could be established. The data presented here are derived from a study performed in independent of metabolic alterations specific to any particular type of hyperlipidemia, could be established. Portions of this work have been presented previously (40, 41).

Methods

Human subjects. Volunteers were recruited by advertisement. Written consent was obtained before enrollment in the study. Study protocols received previous approval from both the University of California at San Francisco and the University of California at Berkeley Committees on Human Research. All subjects were healthy females who had no history of medical illnesses, showed no abnormalities on screening physical examination and laboratory testing, had body mass index (BMI) between 20 and 25 kg/m² and stable weights over the preceding 6 mo, and had normal serum glucose and lipid concentrations. Smokers, heavy coffee drinkers (>3 cups/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/m² (means ± SE). Characteristics of the subjects are shown in Table 1.

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.6 ± 1.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>57.5 ± 3.0</td>
</tr>
<tr>
<td>%Body fat</td>
<td>27.3 ± 2.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.1 ± 0.5</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>153.4 ± 19.7</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>93.4 ± 31.4</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>51.4 ± 5.4</td>
</tr>
<tr>
<td>VLDL, mg/dl</td>
<td>13.0 ± 1.2</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>87.2 ± 15.8</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE; n = 5. HDL, LDL, and VLDL, high-, low-, and very low density lipoprotein, respectively; BMI, body mass index.

The study was to explore the relationship between plasma FFA flux and hepatic VLDL-TG production in response to acute and chronic NA administration in healthy, normolipidemic women. Normolipidemic subjects were chosen so that the basic mechanism of action of NA, independent of metabolic alterations specific to any particular type of hyperlipidemia, could be established. Portions of this work have been presented previously (40, 41).

Study design. Subjects participated in two separate 4-day in-patient metabolic ward studies in the General Clinical Research Center (GCRC) of San Francisco General Hospital. The two GCRC admissions were separated by 1 mo; both were performed in the luteal phase of the menstrual cycle, based on subject recall of the previous two menstrual cycles. The first GCRC admission was under basal (control) conditions. During the month between the first and second GCRC admissions, subjects took increasing doses of 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 (as 500 mg, 4 times/day) at the time of the second GCRC admission. All subjects had received the 2 g/day dose of NA for ≥10 days, and some for a longer period, at the time of the repeat infusion study. Because of the flushing side effects of NA therapy, true blinding was impossible and was not attempted. In addition, the sequence of control followed by NA study periods was not randomized because of concerns about carry-over effects from the NA period.

Metabolic study protocol. During the 4-day in-patient stays, diet was eucaloric, self-selected, and of the subject’s usual macronutrient composition. GCRC dietitians estimated basal metabolic rate by use of the Harris-Benedict equation and dietary recall. The same diet was eaten during both in-patient admissions. Diet was adjusted if necessary (<100 kcal/day incremental changes) to maintain constant weight. The average composition of the self-selected diets was ~30% fat, 55% carbohydrate, and 15% protein. On day 4 of each in-patient stay, metabolic infusion studies were performed (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.

The metabolic infusion protocol consisted of intravenous infusions of [U-13C6]glucose (0.02 mg·kg⁻¹·min⁻¹) from 0300 to 1400, and [2-13C1]glycerol (18 mg·kg lean body mass⁻¹·h⁻¹) and [1,2,3,4-13C4]palmitate (7 μg·kg⁻¹·min⁻¹) from 0400 to 1400. Isotopes were purchased from Isotec (Miamisburg, OH). All labeled substrates were >98% enriched and were sterile and pyrogen free. During the second GCRC admission, NA was given at 500 mg, 4 times a day for the first 3 days, and then a single dose of NA (500 mg) was adminis-

![Fig. 1. Metabolic infusion protocol performed on day 4 of each General Clinical Research Center admission; ± NA, time at which nicotinic acid (NA) was given in NA phase.](http://ajpendo.physiology.org/Downloaded from http://api.embddophysiology.org/ by 10.203.31.1 on May 28, 2017)
tered at 0800 on day 4. Glucose kinetic results from these subjects have been published previously (41); in this paper, only the lipid kinetic results are presented.

**Clinical laboratory measurements.** Serum lipids were measured by standard methods (Clinical Laboratories, San Francisco General Hospital).

**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.** Plasma samples were ultracentrifuged twice, at 33,500 rpm for 30 min each in a Beckman 50.3 rotor at 15°C (1.6 × 10⁶ g) to remove chylomicrons, as described previously (19, 37); VLDL was then isolated by ultracentrifugation for 17 h at 40,000 rpm in a 50.3 Beckman rotor (1.3 × 10⁶ g at 12°C). VLDL-TG fatty acids were transsterified to fatty acid methyl esters (FAME) for gas chromatography mass spectrometry (GC-MS) analysis (model 5971 mass spectrometer with a model 5890 GC and autosampler, Hewlett-Packard, Palo Alto, CA). The aqueous component (containing TG-glycerol) was also derivatized as described previously for VLDL-glycerol enrichment (19, 37).

Plasma FFA was extracted with a known quantity of pentadecanoic acid in heptane as internal standard, as described previously (19, 37), and was separated from cholesterol and phospholipids on silica gel G thin-layer chromatography plates (Analtech, Newark, DE). Derivatization to FAME was as described elsewhere (19).

FAME were analyzed by GC-MS with the use of a 12.0-m DB1 fused silica column under selected-ion monitoring with electron impact ionization. Abundances of ions at mass-to-charge ratios (m/z) 270 and 274, representing the M₄ and M₄ isotopeomers, were quantified, and enrichments (molar excess ME) were calculated by comparison to standard curves of known percentages of [1,2,3,4-13C₄]palmitate and natural abundance palmitate (19). Concentrations of individual fatty acids were determined simultaneously by use of a splitter that diverted a portion of the GC effluent to a flame ionization detector and comparison to pentadecanoic acid standard. Glucose and glycerol were isolated from 350 μl of deproteinized plasma by ion exchange chromatography. Derivatization (19, 36) to glucose pentaacetate and glycerol triacetate, respectively, was performed by reaction with acetic anhydride in pyridine (1:1) at 60°C, followed by extraction with ethyl acetate, respectively, was performed by reaction with acetic anhydride in pyridine (1:1) at 60°C, followed by extraction with ethyl acetate.

A DB17 fused silica column was used for GC-MS analysis of glucose pentaacetate. For calculation of precursor pool enrichment (p) by the mass isotopomer distribution analysis (MIDA), the molecular ion minus acetate and its enrichment (p) by the mass isotopomer distribution analysis of glucose pentaacetate. For calculation of precursor pool enrichment (p) by the mass isotopomer distribution analysis of glucose pentaacetate. For calculation of precursor pool enrichment (p) by the mass isotopomer distribution analysis of glucose pentaacetate.

**RESULTS**

**Plasma lipid and lipoprotein concentrations.** Compared with control lipid levels, 1 mo of NA treatment significantly lowered fasting plasma TG (from 93.4 ± 31.4 to 48.4 ± 11.0 mg/dl), total cholesterol (from 153.4 ± 19.7 to 110.4 ± 14.0 mg/dl), and LDL-cholesterol concentrations (from 87.2 ± 15.8 to 50.4 ± 11.1 mg/dl). The reductions in TG (42.1 ± 7.5%) and LDL-cholesterol (42.1 ± 6.4%) were greater than in total cholesterol (26.9 ± 6.3%). There were no significant changes in HDL cholesterol concentrations [from 51.4 ± 5.4 to 53.8 ± 6.5 mg/dl, not significant (NS)].

**Fasting serum concentrations and rate of appearance of FFA.** After 1-mo treatments with NA, postabsorptive FFA concentrations were nonsignificantly elevated (502 ± 91 vs. 361 ± 71 nmol/ml, P = 0.07) compared with the control phase. Basal FFA rate of appearance (Rₒ) also showed a nonsignificant increase compared with the control phase (6.9 ± 1.0 vs. 5.1 ± 1.5 μmol·kg⁻¹·min⁻¹, P = 0.16).

**VLDL-TG kinetics.** The incorporation curves from [1,2,3,4-13C₄]palmitate and [2-13C₁]glycerol are shown (Fig. 2). In the control phase, production rates of VLDL-TG calculated by kinetic modeling from labeled palmitate and glycerol incorporation were 27.2 ± 6.1 and 28.5 ± 5.3 g/day, respectively, with a calculated half-life of 1.43 ± 0.34 and 1.31 ± 0.30 h, respectively (NS for all comparisons between labeled glycerol and palmitate enrichments). Detailed calculations are shown in Table 2. One of the subjects (subject 2) had an abnormal TG level of 217 mg/dl on the day of the infusion study, although screening TG concentrations before the study had been in the normal range.

\[
y = A₀ × \left[1 - e^{-kₐt - c}\right]
\]

where y = VLDL-TG enrichment, A₀ = the plateau value of VLDL-TG enrichment, t = time in hours, and c = lag period before isotope incorporation into secreted VLDL-TG.

The enrichment of the precursor pool for gluconeogenesis, hepatic triose phosphates, was calculated by the MIDA technique, as described in detail elsewhere (20, 21, 30). In brief, the ratio of excess double-labeled to excess single-labeled species ([AₐΔA₁]) of glucose reveals the isotopic enrichment of the true precursor (p) for gluconeogenesis by application of probability principles based on the binomial expansion. Calculated hepatic triose phosphate enrichment was compared with plateau VLDL-TG glycerol enrichment as an index of the fraction of VLDL-TG that was derived from α-glycerol phosphate (i.e., not derived from preformed hepatic acylglycerides in the hepatic storage pool).

**Statistical analyses.** All of the statistical analyses were performed using the SPSS statistical software (SPSS, Chicago, IL), with P < 0.05 considered statistically significant. Two-factor repeated-measures analysis of variance with two trial factors (treatment and time) was performed when the time-by-treatment interaction was found to be statistically significant, differences among the individual means were assessed using Tukey’s Studentized Range Test at a 5% procedurewise error rate. Results are presented as means ± SE.
In the overnight-fasted state, the incorporation curve of labeled glycerol into VLDL-TG glycerol was lower during the NA phase compared with the control phase (final time point pre-NA administration, Fig. 3, \( P < 0.05 \)), although incorporation of \(^{13}C\)palmitate into VLDL-TG palmitate was not significantly different pre-NA (Fig. 4). Immediately after acute administration of 500 mg of NA, there was complete cessation of incorporation of labeled glycerol (Fig. 3) and palmitate (Fig. 4) into VLDL-TG. In parallel, significant reductions of serum FFA concentration (from 502 ± 691 to 152 ± 171 M) and Ra FFA (from 6.9 ± 1.0 to 3.4 ± 0.6 mmol·kg\(^{-1}\)·min\(^{-1}\)) at 1.5 h were observed after acute NA administration (Fig. 5, \( P < 0.05 \), in the NA phase vs. control phase for both measures). However, despite marked rebound overshoot of FFA concentrations and flux (to maximum values of 1,339 ± 310 μM and 14.8 ± 2.6 mmol·kg\(^{-1}\)·min\(^{-1}\)) at 3.0 h; \( P < 0.05 \) for each vs. baseline or control phase, Fig. 5) between hours 2 and 6 after the acute dose of NA, VLDL-TG synthesis remained suppressed (Figs. 3 and 4). The \(^{13}C\)palmitate incorporation curve remained flat for the entire 6-h period after acute NA administration (Fig. 4), whereas the \(^{13}C\)glycerol curve increased significantly during the last 4 h of study (hours 2–6 post-NA, Fig. 3).

The isotopic enrichment of the intrahepatic triose phosphate precursor pool (p) for TG was calculated by the MIDA of plasma glucose. The value of p remained unchanged before and after NA treatment (Fig. 6). Interestingly, the proportion of VLDL-TG glycerol derived from hepatic α-glycerol phosphate reached a plateau at 65 ± 3% (not 100%) in the control phase (Fig. 3). In the post-NA study, the value reached 37 ± 4% but appeared still to be increasing (Fig. 3).

Table 2. VLDL synthesis rates calculated from VLDL-glycerol and VLDL-FFA incorporation curves (control phase)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, mg/dl</td>
<td>81</td>
<td>217</td>
<td>47</td>
<td>69</td>
<td>74</td>
<td>98 ± 30</td>
</tr>
<tr>
<td>Plasma volume, dl</td>
<td>28.9</td>
<td>28.4</td>
<td>27.4</td>
<td>34.1</td>
<td>25.0</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Pool size, mg</td>
<td>2,341</td>
<td>6,163</td>
<td>1,286</td>
<td>2,353</td>
<td>1,850</td>
<td>2,798 ± 864</td>
</tr>
<tr>
<td>VLDL-TG-glycerol ( A_e ) ( h^{-1} )</td>
<td>0.075</td>
<td>0.087</td>
<td>0.100</td>
<td>0.086</td>
<td>0.071</td>
<td>0.084 ± 0.005</td>
</tr>
<tr>
<td>( k_{s, h} ), mmol·kg(^{-1})·min(^{-1})</td>
<td>0.86</td>
<td>0.20</td>
<td>0.74</td>
<td>0.35</td>
<td>0.49</td>
<td>0.53 ± 0.12</td>
</tr>
<tr>
<td>VLDL synthesis rate, g/day</td>
<td>48.3</td>
<td>30.2</td>
<td>22.7</td>
<td>19.8</td>
<td>21.6</td>
<td>28.5 ± 5.3</td>
</tr>
<tr>
<td>VLDL-TG-palmitate ( A_e ) ( h^{-1} )</td>
<td>1.086</td>
<td>0.007</td>
<td>0.013</td>
<td>1.080</td>
<td>0.012</td>
<td>0.439 ± 0.263</td>
</tr>
<tr>
<td>( k_{s, h} ), mmol·kg(^{-1})·min(^{-1})</td>
<td>0.89</td>
<td>0.20</td>
<td>0.54</td>
<td>0.34</td>
<td>0.45</td>
<td>0.49 ± 0.12</td>
</tr>
<tr>
<td>VLDL synthesis rate, g/day</td>
<td>49.9</td>
<td>30.2</td>
<td>16.8</td>
<td>19.4</td>
<td>19.8</td>
<td>27.2 ± 6.1</td>
</tr>
</tbody>
</table>

FFA, free fatty acid; TG, triglyceride; \( A_e \), plateau value of VLDL-TG enrichment; \( k_{s, h} \), fractional replacement rate constant.
concentrations were measured on hourly samples from 0600 to 1400. No changes were observed between 0600 and 1400 in any subject in either phase. The mean values for the three subjects in control vs. NA phases were 5.4 ± 0.7 vs. 8.8 ± 0.9, 4.6 ± 0.4 vs. 8.1 ± 0.8, and 5.4 ± 0.3 vs. 9.0 ± 1.5 μM. The difference between NA and control phases was highly significant for each subject (P < 0.0001) and for the group as a whole (5.1 ± 0.5 vs. 8.7 ± 0.5 μM, P < 0.001). The mean increase in homocysteine levels was 68 ± 7% in the NA phase. As a control for sulfur amino acid status, serum cysteine concentrations were also measured. Cysteine levels were not affected by NA treatment (208 ± 10 vs. 217 ± 3 μM in control and NA phases, respectively; P = 0.34).

**DISCUSSION**

VLDL-TG production in normolipidemic women was calculated to be 27.2 ± 6.1 g/day (range 16.8–49.8 g/day) with labeled palmitate and 28.5 ± 5.3 g/day (range 19.7–48.3 g/day) with labeled glycerol. The VLDL synthesis rates calculated from glycerol incorporation were similar to those calculated from palmitate incorporation. These results are within the general range of, although perhaps a bit higher than, daily production values previously reported for men (17, 18, 37, 43).

Perhaps the most interesting results of this study are in the relationship between lipolysis and VLDL-TG production after NA administration. In the chronic NA phase, after an overnight fast but before the morning dose of NA, VLDL-TG production was significantly...
The rebound overshoot of plasma FFA fluxes and concentrations, compared with the control phase after overnight fasting. Moreover, after the acute morning dose of NA, new VLDL-TG synthesis was nearly completely halted in the NA phase and their apparent dissociation from VLDL-TG production deserve further discussion.

Acute rebound overshoot of plasma FFA concentrations has been reported previously (6, 13, 33). The rebound is believed not to have a pharmacokinetic explanation, because it is observed even with long-acting NA analogs such as acipimox when plasma concentrations of the drug remain constant (15, 35, 39). Could the FFA rebound reflect changes in serum insulin or glucose concentrations? We have previously reported on the effects of chronic and acute NA on insulin and glucose kinetics in healthy women (41). In the initial 2 h after acute NA administration, serum insulin concentrations tended to fall (from 4.2 ± 0.9 to 2.8 ± 0.1 μU/ml), whereas FFA concentrations and fluxes were markedly suppressed; during the 2- to 6-h period after acute NA administration, serum insulin concentrations exhibited a trend upward (to 4.3 ± 0.5 μU/ml, on FFA concentrations) when FFA concentrations and reduced VLDL-TG production rates were allowed to fall and only a 32% suppression of VLDL-TG production when FFA concentrations were prevented from falling (by infusion of heparin plus intralipid). No dose-response curve for the effect of insulin on VLDL-TG production was reported, so it is difficult to assess the potential impact of slightly elevated serum insulin concentrations, in the range of 4–9 μU/ml, on VLDL-TG production. The effect is likely to be modest, however, and insufficient to explain the nearly complete inhibition of VLDL-TG secretion observed for the 6 h post-NA (Figs. 3 and 4). The same uncertainty applies to the possible effects of altered hepatic glucose metabolism (41) on VLDL-TG assembly and secretion. Perhaps most useful would be information on the effects of sequentially lowering and then raising FFA concentrations (in the absence of NA) on VLDL-TG secretion, but no data of this type are available.

Another possibility is that the effects on VLDL synthesis are a direct pharmacological effect of NA, independent of FFA metabolism. One long-standing but poorly documented suggestion regarding NA (11, 38) relates to its possible effects on 1-carbon metabolism. NA administration at 2 g/day (~16 mmol/day) is ~50% recovered as methylated derivatives (11), thereby requiring ~8 mmol of methyl groups/day. The daily excretion of methyl groups in healthy human subjects has been estimated by Mudd et al. (29) to be ~10 mmol/day, mostly in the form of creatinine. NA administration at 2 g/day, therefore, results in almost a doubling of the whole body methyl-group demand. Because animals fed methyl group-deficient diets (i.e., methionine/choleine-deficient diets) develop fatty livers (12), impaired capacity to export lipid from the liver has been proposed as a mechanism by which NA might reduce serum lipids. Our finding of a 68% increase in plasma homocysteine levels in the NA phase indicates that tissue S-adenosyl-homocysteine concentrations are elevated (42), consistent with reduced hepatic remethylation capacity (i.e., reduced methyl donor availability). Any link to assembly or secretion of VLDL particles by

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**Table 3. Plasma homocysteine concentrations in control and NA phases**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control Phase</th>
<th>NA Phase</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.4 ± 0.7</td>
<td>8.8 ± 0.9*</td>
<td>+63</td>
</tr>
<tr>
<td>2</td>
<td>4.6 ± 0.4</td>
<td>8.1 ± 0.8*</td>
<td>+76</td>
</tr>
<tr>
<td>3</td>
<td>5.4 ± 0.3</td>
<td>9.0 ± 1.5*</td>
<td>+66</td>
</tr>
<tr>
<td>Group mean ± SD</td>
<td>5.1 ± 0.5</td>
<td>8.7 ± 0.5*</td>
<td>+68 ± 7</td>
</tr>
</tbody>
</table>

Values in each phase represent the mean ± SD from >9 plasma measurements. Concentrations are in μM. NA, nicotinic acid (niacin). Group mean ± SD is based on the mean from each subject. *P < 0.0001 vs. control phase; †P < 0.001 vs. control phase.
the liver remains speculative, however. This area warrants further investigation. Alternatively, profound though transient suppressive effects on lipolysis and FFA delivery to the liver may exert a long-lasting inhibitory effect on VLDL-TG secretion or assembly.

We also addressed the potential contribution to VLDL-TG from preexisting hepatic acyl-glycerides [i.e., the hepatic cytosolic storage pool of TG (14, 16)]. In the liver, glycerol is phosphorylated to α-glycerol phosphate and then converted to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. If it is assumed that these three triose phosphate metabolites are in isotopic equilibrium, one can calculate the proportion of secreted VLDL-TG that derived from hepatic α-glycerol phosphate. This is possible by use of the MIDA technique: the plasma glucose labeling pattern is used to calculate the isotopic enrichment (p) of the hepatic triose phosphate pool. Absence of fluctuations in the isotopic enrichment of p (Fig. 6) indicates continued uptake of [13C]glucose by the liver and incorporation into the hepatic triose phosphate precursor pool. By this index, we observed a persistent, ~35% dilution between plateau VLDL-TG-glycerol enrichments and calculated hepatic triose phosphate enrichment (Fig. 3). This suggests either that the hepatic triose phosphate pools are not in isotopic equilibrium or that roughly one-third of VLDL-TG are assembled from preexisting acyl-glycerides.

The much greater dilution of VLDL-TG-glycerol after NA administration could also have two explanations. One possibility is that the label incorporation curve, which was increasing again by hours 4–6 (Fig. 3), would have reached the plateau attained in the control phase. An alternative possibility is that the lower VLDL-TG-glycerol enrichment reflects dilution from preformed hepatic TG deposited from chylomicrons or other unlabeled sources of fat. There is evidence that NA treatment can affect chylomicron metabolism (6, 23). Oral administration of NA to groups of rats immediately after a standard oral dose of olive oil resulted in a reduction in chylomicron concentrations present in the serum at 4 h. Greater decreases were observed as the dose of NA increased (23). Thus NA might have an impact on the pathway from dietary fat to hepatic TG. It would be of interest to explore the effects of NA on TG assembly pathways other than from FFA. Because NA is usually ingested with meals, the major pathway that NA influences to lower total TG levels remains an open question.

NA treatment in hypertriglyceridemic subjects typically increases HDL cholesterol concentrations (18, 26, 31). The absence here of significant changes in HDL concentrations may be due to the relatively short period of NA treatment (minimum of 10 days at 2 g/day). Alternatively, baseline hypertriglyceridemia may be required for NA treatment to increase HDL cholesterol. It will be of interest to explore this question in future studies.

It should be recognized that the hypolipidemic actions of NA that we investigated here were in baseline normolipidemic rather than hyperlipidemic subjects. It is, therefore, possible that the actions of NA observed in this study, e.g., the dissociation between lipolysis and VLDL-TG production, would not apply in hyperlipidemic individuals, although we consider this unlikely. In any event, NA did exhibit hypolipidemic effects in these normolipidemic subjects, and these actions are of interest in their own right.

Finally, the study design used here does not rigorously allow us to exclude a time effect as the explanation for changes observed in the NA phase, because the sequence of control/NA phases was not randomized (the NA phase was performed second, to avoid possible carry-over effects of NA treatment). We do not consider a time effect (i.e., acclimation effects of a second infusion study, contact with a research team, etc.) to be a serious alternate explanation, however, for several reasons. The normal variability of repeat serum lipid measurements in an individual is known to be <10% (28). In the present study, serum TG concentrations fell by close to 50% (from 93 to 48 mg/dl) and LDL cholesterol by >40% (from 87 to 50 mg/dl). These changes are well outside the range of normal variation. Also, we have previously carried out placebo-controlled studies of these measures over time. Repeat measures of Rn, FFA and FFA concentrations after 4–6 wk of a control intervention are reproducible to ±10–15% (19). A more complex, randomized study design (e.g., baseline and then two study phases, in randomized order) might have definitively excluded a time effect to explain our findings, but we consider this a priori an unlikely explanation.

In summary, we have quantified VLDL-TG synthesis in lean, healthy women by use of palmitate and glycerol tracers under fasting conditions. The relationship of VLDL-TG production to FFA availability was changed after 1 mo of NA treatment. The fasting VLDL-TG synthesis rate was lowered despite slightly elevated serum FFA concentrations and fluxes. After acute NA administration, VLDL-TG synthesis was markedly decreased, initially in association with reduced serum FFA concentrations and fluxes, but the effect persisted for ≥4 h after the anti-lipolytic action had abated. Inhibition of lipolysis, therefore, is not a sufficient explanation for the hypolipidemic actions of NA.

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