On the suppression of plasma nonesterified fatty acids by insulin during enhanced intravascular lipolysis in humans

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Carpentier, André C., Frédérique Frisch, Denis Cyr, Philippe Généreux, Bruce W. Patterson, Robert Giguère, and Jean-Patrice Baillargeon. On the suppression of plasma nonesterified fatty acids by insulin during enhanced intravascular lipolysis in humans. Am J Physiol Endocrinol Metab 289: E849–E856, 2005. First published June 21, 2005; doi:10.1152/ajpendo.00073.2005.—During the fasting state, insulin reduces nonesterified fatty acid (NEFA) appearance in the systemic circulation mostly by suppressing intracellular lipolysis in the adipose tissue. In the postprandial state, insulin may also control NEFA appearance through enhanced trapping into the adipose tissue of NEFA derived from intravascular triglyceride lipolysis. To determine the contribution of suppression of intracellular lipolysis in the modulation of plasma NEFA metabolism by insulin during enhanced intravascular triglyceride lipolysis, 10 healthy nonobese subjects underwent pancreatic clamps at fasting vs. high physiological insulin level with intravenous infusion of heparin plus Intralipid. Nicotinic acid was administered orally during the last 2 h of each 4-h clamp to inhibit intracellular lipolysis and assess insulin’s effect on plasma NEFA metabolism independently of its effect on intracellular lipolysis. Stable isotope tracers of palmitate, acetate, and glycerol were used to assess plasma NEFA metabolism and total triglyceride lipolysis in each participant. The glycerol appearance rate was similar during fasting vs. high insulin level, but plasma NEFA levels were significantly lowered by insulin. Nicotinic acid significantly blunted the insulin-mediated suppression of plasma palmitate appearance and oxidation rates by ~60 and ~70%, respectively. In contrast, nicotinic acid did not affect the marked stimulation of palmitate clearance by insulin. Thus most of the insulin-mediated reduction of plasma NEFA appearance and oxidation can be explained by suppression of intracellular lipolysis during enhanced intravascular triglyceride lipolysis in healthy humans. Our results also suggest that insulin may affect plasma NEFA clearance independently of the suppression of intracellular lipolysis.

intracellular lipolysis; lipid oxidation; postprandial state; lipid metabolism

ABNORMAL PLASMA NONESTERIFIED FATTY ACID (NEFA) metabolism is felt to play an important role in the development of type 2 diabetes (23). Prolonged experimental elevation of plasma NEFA reduces insulin-mediated glucose utilization in muscle, impairs glucose-mediated insulin secretion, and increases endogenous glucose production in vivo in humans (8, 31, 38). One of the earliest identifiable metabolic defects in patients at high risk of developing type 2 diabetes is elevation of postprandial levels of plasma triglycerides and blunting of early postprandial lowering of plasma NEFA levels (2). Impaired postprandial suppression of plasma NEFA could be implicated in the development of insulin resistance and type 2 diabetes through increased exposure of extra-adipose tissues to NEFA in individuals at high risk of developing type 2 diabetes (23).

In contrast to the fasting state, where insulin reduces extra-adipose tissue exposure to NEFA mainly by suppressing intracellular lipolysis in adipose tissue, the effect of insulin on circulating NEFA metabolism in the postprandial state may be more complex. First, insulin stimulates lipoprotein lipase (LPL)-mediated lipolysis of chylomicrons in the microcirculation of the adipose tissue, a major mechanism by which this hormone can stimulate preferential partitioning of fatty acids in the adipose tissue during the postprandial state (13). Second, insulin suppresses intracellular lipolysis in adipose tissue, thereby reducing plasma NEFA appearance rate (10, 27). Third, insulin may stimulate the esterification of NEFA in the adipose tissue, potentially contributing to the adipose tissue uptake and trapping of plasma NEFA that are generated from intravascular triglyceride lipolysis (10, 12, 15, 27). Insulin may also reduce postprandial lipid oxidation independently of change in plasma NEFA availability possibly by increasing intracellular glucose flux (35). Because of these potential effects of insulin, the relative role of suppression of intracellular lipolysis on in vivo plasma NEFA metabolism in the postprandial state cannot readily be predicted.

The aim of the present study was to determine the relative role of the suppression of intracellular lipolysis in the modulation of NEFA metabolism by insulin in the presence of enhanced intravascular triglyceride lipolysis in vivo in humans. To that aim, we propose an experimental paradigm based on the following assumptions: 1) an intravenous heparin + Intralipid infusion maximally stimulates the production of plasma NEFA from intravascular triglyceride lipolysis by activating LPL sitting at the endothelium into the microcirculation of tissues and by supplying chylomicron-like particles in the circulation (9); 2) nicotinic acid given orally in humans is very effective at suppressing intracellular lipolysis in adipose tissues (7), allowing us to determine any effect of insulin on systemic NEFA flux independently of its suppressing effect on intracellular lipolysis. Under these conditions, it would be expected that any non-LPL-mediated effect of insulin in stimulating NEFA esterification (i.e., intracellular trapping) and in limiting the entry of NEFA into the systemic circulation generated from intravascular triglyceride lipolysis in the adi-
pose tissue circulation would become more apparent. This experimental paradigm allowed us to assess the relative importance of suppression of intracellular lipolysis vs. enhanced trapping of plasma NEFA derived from intravascular triglyceride lipolysis in the adipose tissue in the suppression of plasma NEFA appearance and oxidation by insulin. In case of a predominant effect of the suppression of intracellular lipolysis in causing insulin-mediated reduction of plasma NEFA appearance and oxidation, one would expect that any difference observed in these parameters between fasting and high plasma insulin levels would be abolished to a large extent during nicotinic acid intake.

MATERIALS AND METHODS

Subjects. Ten healthy nonobese Caucasian subjects (mean BMI 25.2 ± 0.7 kg/m², 3 females, 7 males) aged 21 to 56 yr (mean 40.9 ± 3.7 yr) participated in the studies. None had diabetes, based on repeated assessment of fasting glucose concentration (1). None were taking any medication, had any current medical condition known to affect lipid levels or insulin sensitivity, or had known cardiovascular disease. The three women were premenopausal, and the studies were conducted during the follicular phase of their menstrual cycle. Informed written consent was obtained from all participants in accordance with the Declaration of Helsinki and the protocol was approved by the Human Ethics Committee of the Centre hospitalier universitaire de Sherbrooke.

Experimental protocols. All subjects participated in four studies 3–4 wk apart, and they avoided change in lifestyle and weight throughout the study. They were provided with dietary instructions to maintain a eucaloric diet and follow a 3-day food diary prescribed by a registered dietician, and compliance was ascertained on the morning of each study. The participants were told to avoid strenuous exercise for 48 h before each study, as described previously (9). The subjects were admitted to our metabolic investigation center on each occasion between 0730 and 0830 after a 12-h overnight fast and remained fasting for the duration of the study. On arrival, body weight and height were measured, and lean body mass was determined by electrical bioimpedance (Hydra ECF/ICF; Xitron Technologies, San de Sherbrooke). An intravenous catheter was placed in one forearm for infusions, and another was placed in a retrograde fashion in the contralateral arm maintained in a heating box (55°C) for blood sampling.

Protocols A and C and B and D (Fig. 1) were designed to produce a similar and sustained elevation of intravascular lipolysis of triglycerides during 4 h by means of an intravenous infusion of heparin (250 U/h) and 20% Intralipid (40 ml/h) (9) and a triglyceride emulsion composed of 50% linoleate, 26.5% olate, 10.5% palmitate, 8.5% linolenate, and 3.5% stearate. In protocols A/C, fasting insulin was maintained by a continuous low (0.05 mU·kg⁻¹·min⁻¹) insulin infusion (Novolin R, Novo Nordisk). In protocols B/D, high insulin was obtained using a primed (0.8 μU/kg) continuous high (1.2 mU·kg⁻¹·min⁻¹) insulin infusion (with 10 ng/ml KCl to avoid insulin-induced hypokalemia). We (9) have previously shown that intravenous Intralipid plus heparin infusion results in a small but significant increase in plasma glucose at fasting insulin level. Therefore, protocol A or C was always performed first to match as precisely as possible the expected small increase in plasma glucose levels between the A/C and B/D studies by using a variable infusion of 20% dextrose adjusted according to plasma glucose level. Octreotide (30 μg/h Sandostatin, Sandoz) and human growth hormone (GH; 3 ng·kg⁻¹·min⁻¹ Nutropin, Roche) were administered in all four protocols (20). Glucagon was not replaced, because it can result in insulin secretion breakthrough at low insulin infusion rate (20) and because it has minimal effect on NEFA metabolism in humans (3, 16, 29).

![Experimental protocols](image)

**Fig. 1.** Experimental protocols. All of the participants underwent 4 experimental protocols (A, B, C, and D) with IV infusion of heparin + Intralipid during 4 h from time 0 to stimulate intravascular triglyceride lipolysis. After a 30-min baseline period during which blood samples, indirect calorimetry, and breath samples were performed, an iv bolus of [¹³C]NaHCO₃ was injected at time 0 in all 4 protocols. A 4-h euglycemic pancreatic clamp with iv octreotide + growth hormone (GH) and low (protocols A and C) or high (protocols B and D) dose insulin infusions to maintain fasting vs. high plasma insulin levels, respectively, was performed from time 0. Intravenous infusion of 25% dextrose (D25%) and potassium chloride (KCl) were administered during protocols B and D to maintain plasma glucose at fasting level and to avoid a decrease in serum potassium level induced by insulin. In protocols A and B, a primed continuous iv infusion of [1,2,3,3-H⁻³]glycerol and a continuous iv infusion of [U-¹³C]palmitate were administered during 4 h from time 0. In protocols C and D, a continuous iv infusion of [1,2-¹³C]acetate was administered during 4 h from time 0. During the last 2 h of the 4-h clamp in all 4 protocols, participants received nicotinic acid orally every 30 min to suppress adipose tissue intracellular lipolysis. Blood and breath samples and indirect calorimetry were performed between 90 and 120 min and between 210 and 240 min of the clamp.

At time 0 min in protocols A and B, a constant infusion (0.01 mU·kg⁻¹·min⁻¹) of [¹³C]K palmitate (Cambridge Isotope Laboratories, Andover, MA; in 100 ml of 5% human serum albumin) was administered during the 4 h of the study (5), preceded by a bolus infusion of [¹³C]NaHCO₃ (1.2 μmol/kg, Cambridge Isotope Laboratories) to prime the bicarbonate pool (41). The choice of a palmitate tracer in our experimental protocol was based on the following: 1) palmitate, olate, and linoleate, the most prevalent NEFAs in human plasma and in Intralipid, have similar clearance rates in humans (28); and 2) a palmitate tracer had been previously used with success to measure total plasma NEFA turnover in humans after oral fat intake (27). A primed (1.6 μmol/kg) continuous (0.11 mU·kg⁻¹·min⁻¹) infusion of [¹³C]palmitate (Cambridge Isotope Laboratories) was also administered through a 0.22-μm Millipore filter for the duration of the study to quantify plasma glycerol flux as a reflection of whole body lipolysis (22). During protocols C and D, [¹³C]sodium acetate (Cambridge Isotope Laboratories) was infused (0.08 μmol·kg⁻¹·min⁻¹) instead of the palmitate and glycerol tracers to determine the acetate retention factor (5, 33) in conditions identical to those of protocols A and B (see below). All tracers were tested for sterility and pyrogenicity prior to use.

During the last 2 h of the protocols, nicotinic acid was given orally (100 mg at 120 and 200 min, and 150 mg at 150 and 180 min), a protocol previously shown to result in a steady suppression of intracellular lipolysis for up to 4 h (7). The effect of insulin on NEFA metabolism attributable to its inhibitory effect on intracellular lipolysis vs. that attributable to other possible effects, such as enhanced trapping of NEFA derived from intravascular triglyceride lipolysis,
could then be assessed by comparing nicotinic acid-mediated vs. insulin-mediated change in NEFA metabolism.

After a 30-min bed rest, blood samples were taken at 10-min intervals at baseline and during the last 30 min of the first 2 h (without nicotinic acid), and the last 30 min of the 4-h clamp period (with nicotinic acid). Blood was collected in tubes containing Na₂EDTA and Orlistat (30 µg/ml; Roche, Mississauga, ON, Canada) to prevent in vitro triglyceride lipolysis. Urine nitrogen excretion was measured throughout the studies (19). After a 10-min equilibration, oxygen uptake (VO₂) and carbon dioxide production (VCO₂) were measured during a 30-min baseline period and during the last 30 min of the period with and without nicotinic acid to determine total body carbohydrate and lipid oxidation by indirect calorimetry (Vmax29n, Sensormedics) (14). Expiratory gases were collected at baseline and at 10-min intervals into 10-ml exsaters (Labco) throughout these periods to determine 13CO₂ to 12CO₂ ratio by isotope ratio mass spectrometry (IRMS) (34).

**Laboratory assays.** Glucose was assayed at bedside (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA), Insulin, glucagon, and growth hormone (GH) were measured by specific radioimmunoassays (Linco, St. Charles, MO, and Nichols Institute Diagnostics, Juan San Capistrano, CA). Total plasma NEFA and triglycerides were measured using colorimetric assays (Wako Industries and Thermo DMA, respectively). Plasma glycerol was extracted and derivatized with bis(trimethylsilyl)trifluoroacetamide + 10% trimethylchlororosiliane (Regis Technologies, Morton Grove, IL), and plasma [1,1,2,3,3-2H₅]glycerol enrichment was measured by GC-MS using an Agilent GC model 5890A (Agilent Technologies, Avondale, PA) coupled to an MS detector (model 5971 quadrupole MSD, Agilent) equipped with a Supelco SPB-5 fused silica column (25 m × 0.20 mm, 0.33 μm) and a splitless injector. Electron impact ionization with an electron beam energy of 70 eV was used in selected ion monitoring mode to monitor mass-to-charge ratio (m/z) 117, 205 for glycerol, m/z 120, 208 for [1,1,2,3,3-2H₅]glycerol, and m/z 118, 206 for [1,1,2,3,3-2H₅]glycerol (internal standard). To measure plasma palmitate, linoleate, oleate, and [U-13C]palmitate enrichment, heptadecanoic acid was added as an internal standard to 100 μl of plasma and mixed with 500 μl of methanol. After centrifugation, the supernatant was filtered and injected on a Hyspers ODS column (5 μm, 4.0 × 125 mm) on an LC/MSD series 1100 (Agilent) with monitoring of ions 279 (C18:2), 281 (C18:1), 255 (C16:0), 271 (C16:0 M+16), and 269 (C17, internal standard). Standard curves were generated for C16:0, C18:1, C18:2, and C16:0 M+16 enrichment by use of purified standards of known concentration. The intra- and interassay coefficients of variation were <6.1% for all of these assays. Breath 13CO₂/12CO₂ was determined using a gas isotope ratio mass spectrometer (Delta+ XL; Finnigan, Bremen, Germany).

**Calculations.** The plasma palmitate appearance rate (Ra palmitate) was calculated from the C16:0 M+16 enrichment of plasma palmitate from background and the tracer infusion rate (6):

\[ Ra_{\text{palmitate}} = F / TTR_{\text{palmitate}} \]

where F is the C16:0 M+16 infusion rate determined during each experiment, and TTRpalmitate is the plasma palmitate C16:0 M+16-to-C16:0 M+0 ratio during tracer infusion, corrected for background (which is zero in this case). The total plasma NEFA Ra was determined by multiplying the palmitate Ra by the ratio of concentration of plasma NEFA to plasma palmitate level (21). This approach assumes that the clearance of plasma palmitate is a good estimate of the clearance of the other plasma long-chain free NEFA (except for stearate) (18, 28, 37). To estimate total body triglyceride lipolysis, the plasma glycerol Ra was also determined from plasma glycerol M+5 enrichment from baseline and the tracer infusion rate, as described in (22), corrected for the infusion rate of free glycerol contained in the Intralipid infusion (4). Clearance rates of plasma palmitate and glycerol were determined by dividing their respective Ra by their plasma concentrations.

The fractional plasma palmitate oxidation was determined (6):

\[ F_{\text{ox, palmitate}} = \frac{VCO_2 \times TTR_{CO_2}}{(\text{INF}_{[1,1,1]}^{[U] \text{palmitate}} \times 16 \times k)} \]

where VCO₂ is expressed in micromoles per kilogram of lean body mass (LBM) per minute, TTRCO₂ is the breath 13CO₂ tracer-to-tracee ratio, INF[1,1,1]^{[U]palmitate} is the palmitate tracer infusion rate in micromoles per kilogram LBM per minute, and k is the acetate recovery factor as calculated by the following (5):

\[ k = \frac{(VCO_2 \times TTR_{CO_2})}{(\text{INF}_{[1,2]}^{[1,2]} \text{acetate}} (2) \]

where INF[1,1,1]^{[U]acetate} is the acetate tracer infusion rate in micromoles per kilogram of LBM per minute and VCO₂ and TTRCO₂ were measured during protocol C or D.

**Statistical analyses.** The data at baseline and with and without nicotinic acid intake were averaged and are expressed as means ± SE. All metabolic parameters measured during the high- vs. fasting-insulin experiments without and with nicotinic acid were examined using an ANOVA for repeated measures, and Scheffé’s test for multiple comparisons was performed whenever the P value was significant. To assess the effect of high- vs. fasting-insulin level and whether and to what extent inhibition of intracellular lipolysis contributes to insulin’s effect, the values of all of the parameters measured during the high-insulin study were subtracted from the corresponding value during the fasting insulin study. These differences with and without nicotinic acid intake were compared by a paired t-test. For all analyses, a two-tailed P value of <0.05 was considered significant. All analyses were performed with the SAS software for Windows, version 8.02 (SAS Institute, Cary, NC).

**RESULTS**

**Plasma glucose, NEFA, triglycerides, and hormone levels at fasting and high insulin levels.** By design, plasma glucose levels were matched between the fasting and high-insulin conditions. As expected, there was a small but not significant (NS) increase in plasma glucose level from baseline to the end of the 4-h infusion of heparin-Intralipid at fasting insulin (Table 1). Plasma glucose levels were similar between fasting and high-insulin studies with or without nicotinic acid. Also by design, plasma insulin levels were significantly increased from baseline in the high-insulin study but did not change in the fasting-insulin study. Nicotinic acid did not affect plasma insulin levels during the clamps. As expected, total plasma NEFA levels were significantly increased more than twofold at fasting insulin, although high insulin resulted in stabilization of NEFA at their baseline level. Total plasma NEFA levels did not change significantly with nicotinic acid intake at fasting and at high insulin levels. Plasma triglyceride levels were also significantly increased during Intralipid-heparin infusion vs. baseline but were not affected by insulin or nicotinic acid. Plasma glucagon levels were reduced from baseline to similar levels during fasting and high-insulin clamps and were not changed by nicotinic acid intake. Plasma GH levels were not affected by insulin level or nicotinic acid intake.

**Plasma concentration of individual fatty acids, glycerol, and tracer enrichment at fasting and high insulin levels.** Mean plasma palmitate levels were significantly increased by ~70% from baseline at fasting insulin (P < 0.05) and tended to be reduced by ~25% from baseline (NS) at high insulin (Table 2). Nicotinic acid intake reduced the plasma palmitate concentration by ~20% vs. without nicotinic acid at fasting (P < 0.05) but not at high insulin. Mean plasma M+16 palmitate TTR was not significantly affected by insulin or nicotinic acid intake. Plasma oleate concentrations were elevated by ~50% from
baseline at fasting insulin ($P < 0.05$) and tended to be reduced by ~30% from baseline at high insulin, but this difference was not significant. Nicotinic acid resulted in a significant reduction of plasma oleate toward baseline levels at fasting insulin ($P < 0.05$ vs. without nicotinic acid) but did not affect plasma oleate levels at high insulin. Plasma lineolate levels were increased more than fourfold from baseline at fasting ($P < 0.05$) but significantly less so at high insulin level (~2.2-fold elevation, $P < 0.05$ vs. fasting insulin). Plasma lineolate levels were not significantly affected by nicotinic acid intake. Plasma glycerol levels were similarly and significantly increased from baseline at fasting and at high insulin ($P < 0.05$) but were unaffected by nicotinic acid. Plasma glycerol M$+5$ TTR was not significantly affected by insulin level or nicotinic acid.

**Plasma glycerol and NEFA metabolism at fasting and high insulin levels.** Plasma glycerol appearance and clearance rates were unaffected by change in plasma insulin level or by nicotinic acid intake (Table 3). Plasma palmitate $R_p$ was significantly reduced at high vs. fasting insulin without nicotinic acid intake ($P < 0.05$). However, during nicotinic acid intake this difference was reduced and not significant anymore. Total plasma NEFA appearance was not significantly affected by insulin or nicotinic acid intake. High insulin level was associated with a significant increase of plasma palmitate clearance rate by >40% ($P < 0.05$) compared with fasting insulin. Nicotinic acid intake did not affect plasma palmitate clearance rate. Fractional palmitate oxidation was not significantly affected by plasma insulin level or nicotinic acid intake [32.7 ± 4.4 vs. 31.9 ± 3.6 at high vs. fasting insulin, respectively, without nicotinic acid intake; and 29.8 ± 4.0 vs. 25.7 ± 1.7% at high vs. fasting insulin, respectively, with nicotinic acid intake ($P = NS$)]. In contrast, plasma palmitate oxidation rate was significantly reduced at high vs. fasting insulin without nicotinic acid intake ($P < 0.05$), but this difference was not significant anymore during nicotinic acid intake. The acetate recovery factor was not significantly different at high vs. fasting insulin (0.173 ± 0.005 vs. 0.163 ± 0.005%, respectively) and was significantly higher during nicotinic acid intake (0.304 ± 0.009 and 0.290 ± 0.009% at high and fasting insulin, respectively, $P < 0.05$ vs. no nicotinic acid intake).

**Indirect calorimetry and total body carbohydrate and lipid oxidation at fasting and high insulin levels.** There was no significant change from baseline of $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory quotient (RQ) during intravenous heparin-Intralipid infusion at fasting insulin (Table 4). Therefore, there was no significant change in total body carbohydrate and lipid oxidation rates associated with heparin-Intralipid infusion at fasting insulin. $\dot{V}CO_2$, RQ, and, consequently, total body carbohydrate oxidation.

### Table 1. Plasma glucose, NEFA, triglycerides, and hormone levels

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Phase</th>
<th>Base</th>
<th>No NA</th>
<th>NA</th>
<th>$P$, ANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>Fasting insulin</td>
<td>5.5</td>
<td>5.6</td>
<td>6.0</td>
<td>0.24</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>High insulin</td>
<td>5.6</td>
<td>5.5</td>
<td>5.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>Fasting insulin</td>
<td>67</td>
<td>56</td>
<td>60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>High insulin</td>
<td>79</td>
<td>469</td>
<td>446</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>Fasting insulin</td>
<td>73</td>
<td>35</td>
<td>35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GH, μg/l</td>
<td>High insulin</td>
<td>74</td>
<td>33</td>
<td>29</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

Data are means ± SE. GH, growth hormone; NA, nicotinic acid; NEFA, nonesterified fatty acids; TG, triglycerides. $*P$ values are from ANOVA for repeated measures with a post hoc Scheffe’s test performed whenever the $P$ value from the model was significant. †Scheffe’s test $P < 0.05$ vs. baseline. ‡Scheffe’s test $P < 0.05$ vs. fasting insulin.

### Table 2. Plasma concentration of glycerol, individual fatty acids, and palmitate M$+16$ and glycerol M$+5$ TTR

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Phase</th>
<th>Base</th>
<th>No NA</th>
<th>NA</th>
<th>$P$, ANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate, μmol/l</td>
<td>Fasting insulin</td>
<td>99</td>
<td>175</td>
<td>139</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oleate, μmol/l</td>
<td>Fasting insulin</td>
<td>200</td>
<td>301</td>
<td>221</td>
<td>&lt;0.0001</td>
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<tr>
<td>Linoleate, μmol/l</td>
<td>Fasting insulin</td>
<td>84</td>
<td>437</td>
<td>426</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycerol, μmol/l</td>
<td>Fasting insulin</td>
<td>62</td>
<td>168</td>
<td>169</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SE. TTR, tracer-to-tracer ratio. $*P$ values are from ANOVA for repeated measures with a post hoc Scheffe’s test performed whenever the $P$ value from the model was significant. †Scheffe’s test $P < 0.05$ vs. baseline. ‡Scheffe’s test $P < 0.05$ vs. fasting insulin. §Scheffe’s test $P < 0.05$ vs. no NA.
Table 3. Plasma glycerol and NEFA metabolism

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Phase</th>
<th>R&lt;sub&gt;a&lt;/sub&gt; glycerol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fasting insulin</th>
<th>9.0±2.2</th>
<th>10.3±2.7</th>
<th>0.34</th>
<th>ANOVA&lt;sup&gt;*&lt;/sup&gt;</th>
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<tr>
<td></td>
<td></td>
<td>High insulin</td>
<td></td>
<td>8.5±1.8</td>
<td>12.8±3.7</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>R&lt;sub&gt;a&lt;/sub&gt; palmitate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Fasting insulin</td>
<td>54.2±12.5</td>
<td>60.0±16.1</td>
<td>0.26</td>
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<td>High insulin</td>
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<td>57.7±12.4</td>
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<tr>
<td>R&lt;sub&gt;a&lt;/sub&gt; NEFA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Fasting insulin</td>
<td>2.4±0.4</td>
<td>2.2±0.3</td>
<td>0.08</td>
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<tr>
<td></td>
<td></td>
<td>High insulin</td>
<td></td>
<td>1.5±0.35</td>
<td>1.8±0.3</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;2&lt;/sub&gt; palmitate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fasting insulin</td>
<td>15.9±2.6</td>
<td>14.1±2.0</td>
<td>0.16</td>
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<td></td>
<td>High insulin</td>
<td></td>
<td>12.1±2.3</td>
<td>13.4±2.2</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;2&lt;/sub&gt; NEFA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fasting insulin</td>
<td>13.4±1.7</td>
<td>15.6±2.2</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High insulin</td>
<td></td>
<td>23.0±3.5§</td>
<td>24.0±3.6§</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Ox&lt;sub&gt;palmitate&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fasting insulin</td>
<td>0.71±0.06</td>
<td>0.53±0.04</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High insulin</td>
<td></td>
<td>0.43±0.05‡</td>
<td>0.44±0.05</td>
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Data are means ± SE. Cl: clearance; Ox: oxidation; R<sub>a</sub>: appearance rate. *Expressed in μmol·kg LBM⁻¹·min⁻¹. †Expressed in ml·kg LBM⁻¹·min⁻¹. ‡P values from ANOVA for repeated measures with a post hoc Scheffé’s test performed whenever the P value from the model was significant. §Scheffé’s test P < 0.05 vs. fasting insulin.

The effect of insulin on NEFA metabolism attributable to suppression of intracellular lipolysis. Suppression of intracellular lipolysis with nicotinic acid resulted in a significant blunting of the insulin-mediated suppression of plasma palmitate (−68 ± 8 vs. −107 ± 9 μmol/l with vs. without nicotinic acid, respectively, P < 0.05; Fig. 2A), oleate (−102 ± 16 vs. −190 ± 17 μmol/l with vs. without nicotinic acid respectively, P < 0.05), and total NEFA levels (−348 ± 58 vs. −629 ± 79 μmol/l with vs. without nicotinic acid respectively, P < 0.05; Fig. 2B), but not linoleate level (−189 ± 27 vs. −237 ± 35 μmol/l with vs. without nicotinic acid respectively, P = NS). Nicotinic acid intake also resulted in a significant ~60% blunting of the reduction of palmitate appearance induced by insulin (−0.4 ± 0.3 vs. −0.9 ± 0.3 μmol·kg LBM⁻¹·min⁻¹ with vs. without nicotinic acid, respectively, P < 0.05; Fig. 2C), but only tended to blunt insulin-mediated reduction of total plasma NEFA appearance (−1.1 ± 1.7 vs. −4.0 ± 2.4 μmol·kg LBM⁻¹·min⁻¹ with vs. without nicotinic acid, respectively, P = NS; Fig. 2D).

DISCUSSION

Our experimental protocol using intravenous infusion of heparin plus Intralipid resulted in a steady and similar increase in intravascular triglyceride lipolysis at fasting and high insulin levels, supported by no significant change in glycerol level and appearance rate at fasting vs. high insulin levels. This allowed us to determine the effect of insulin on plasma NEFA appearance independently of insulin-mediated change in LPL activity in the adipose tissue. In this condition, it is expected that any non-LPL-mediated stimulating effect of insulin on the esterification of NEFA originating from intravascular triglyceride lipolysis in the adipose tissue would become more apparent. In the present study, the reduction of plasma palmitate appearance and oxidation by insulin during enhanced intravascular triglyceride lipolysis was only ~40%, in contrast with the profound (~80%) suppression of plasma NEFA appearance by insulin observed in the postabsorptive state in humans (28). Thus, as
expected during Intralipid-heparin intravenous infusion, a large fraction of plasma NEFA available to the systemic circulation was derived from intravascular triglyceride lipolysis. This was also suggested by the absence of significant modulation of plasma linoleate levels by nicotinic acid intake, since a large fraction of plasma linoleate is expected to derive from intravascular lipolysis of Intralipid. Nevertheless, we found that hyperinsulinemia reduced plasma palmitate appearance and oxidation rates mostly through inhibition of intracellular lipolysis, as nicotinic acid reduced by at least 60 and 70%, respectively, the suppression of plasma palmitate appearance and oxidation by insulin. It has been previously demonstrated that some fraction of NEFA derived from intravascular triglyceride lipolysis in vivo in humans is available in the systemic circulation during the fasting (26) and the postprandial state (32). According to our results, insulin does not predominantly regulate the systemic NEFA flux originating from intravascular triglyceride lipolysis through a non-LPL-mediated stimulation of trapping of NEFA into the adipose tissue in healthy individuals, although a defect in this mechanism in pathophysiological states cannot be excluded. Thus, as during fasting, suppression of intracellular lipolysis appears to be the most important mechanism by which insulin suppresses systemic NEFA flux during enhanced intravascular triglyceride lipolysis.

Our data also suggest that insulin increases plasma palmitate clearance independently of inhibition of intracellular lipolysis. Interestingly, hyperinsulinemia was also associated with enhanced plasma NEFA clearance in previous human studies during the postabsorptive (28) and the postprandial state (27). However, the relative contribution of NEFA appearance vs. clearance and of the reduction of intracellular lipolysis in the modulation of postprandial plasma NEFA levels by insulin was not addressed in previous studies. A direct acute effect of insulin on transport of NEFA into tissues has been demonstrated in some (11, 24, 30) but not all (42) ex vivo and in vivo studies in animals. No in vivo study in humans, including the present study, reported the effect of insulin on NEFA clearance independent of insulin-mediated reduction in NEFA levels. To our knowledge, whether plasma NEFA clearance can be saturated at high vs. low physiological NEFA levels has not been formally established in vivo in humans. Thus caution should be exerted before concluding that insulin per se and not reduction of NEFA levels by insulin through inhibition of intracellular lipolysis was the cause of the enhanced NEFA clearance during the hyperinsulinemic clamp in the present study.

**Fig. 2.** Insulin-mediated change of plasma palmitate level (A), total plasma nonesterified fatty acid (NEFA or FFA) level (B), plasma palmitate appearance rate (Ra) (C), total plasma NEFA Ra (D), plasma palmitate clearance (E), and plasma palmitate oxidation rate (F) without (filled bars) and with nicotinic acid intake (open bars). In other words, open bars illustrate the effect of insulin independent of its suppressive effect on intracellular lipolysis. *P < 0.05 vs. without nicotinic acid intake. P values are from paired t-tests. LBM, lean body mass; Data are means ± SE.
vivo human studies are underway in our laboratory to address this issue.

Plasma NEFA appearance rate is elevated in the postprandial state in individuals with visceral obesity (17) and in obese patients with type 2 diabetes compared with younger and leaner healthy individuals (27). The precise mechanism for enhanced postprandial plasma NEFA flux in these individuals remains to be established, but a defective control of NEFA flux by insulin is an obvious target. Whether impaired insulin-mediated suppression of intracellular lipolysis or impaired insulin-mediated trapping of fatty acids derived from intravascular lipolysis during the postprandial state could play a role in the increased availability of NEFA to extra-adipose tissues in obesity and type 2 diabetes remains to be established. Our results also indicate that impaired insulin-stimulated clearance of NEFA is another potential mechanism for increased postprandial plasma NEFA levels in insulin-resistant states. Interestingly, a defect in postprandial plasma NEFA clearance has been demonstrated in patients with type 2 diabetes (25, 39).

Whether a defect in insulin-stimulated clearance of NEFA occurs in vivo in humans with type 2 diabetes remains to be determined.

In the present study, we used intravenous heparin to maximally stimulate LPL activity at both fasting and high plasma insulin levels. However, we cannot totally exclude that hyperinsulinemia did not result in a small increase in adipose tissue LPL activity vs. fasting insulin conditions despite the use of heparin. If this had occurred, it would have resulted in enhanced LPL-mediated NEFA appearance into the adipose tissue during hyperinsulinemia, making more apparent any insulin-mediated increase in post-LPL adipose tissue NEFA trapping in limiting systemic NEFA flux. In other words, this would have reduced the apparent role of inhibition of intracellular lipolysis in the reduction of systemic NEFA flux by insulin. Therefore, this possible limitation would nevertheless strengthen our conclusion that insulin reduces NEFA appearance during enhanced intravascular lipolysis mostly by inhibition of adipose tissue intracellular lipolysis.

Nicotinic acid intake significantly blunted the insulin-mediated reduction of plasma palmitate oxidation rate but did not affect insulin-mediated reduction of total lipid oxidation. An increase in intracellular lipolysis in muscle could offer at least part of the explanation for this observation, since this phenomenon has been shown to maintain lipid oxidation during reduction of plasma NEFA by nicotinic acid intake in vivo in humans (40). The lack of effect of nicotinic acid on total lipid oxidation was in contrast to the significant reduction induced by insulin. Thus, during enhanced intravascular triglyceride lipolysis, the inhibitory effect of insulin on total lipid oxidation was not solely dependent on the reduction of plasma NEFA by nicotinic acid intake in vivo in humans. The effect of nicotinic acid on total lipid oxidation was in contrast to the significant reduction induced by insulin. Thus, during enhanced intravascular triglyceride lipolysis, the inhibitory effect of insulin on total lipid oxidation was not solely dependent on the reduction of plasma NEFA by nicotinic acid intake in vivo in humans. This would have resulted in underestimation of the insulin-mediated suppression of plasma palmitate appearance attributed to inhibition of intracellular lipolysis. Therefore, this limitation would not affect our conclusion that most of the insulin-mediated suppression of plasma NEFA appearance and oxidation during enhanced intravascular triglyceride lipolysis in healthy humans is due to inhibition of intracellular lipolysis.

We conclude that, in healthy humans, suppression of intracellular lipolysis is the major mechanism by which insulin reduces the plasma NEFA appearance and oxidation rates during enhanced intravascular triglyceride lipolysis. Insulin increases the apparent clearance of plasma NEFA independently of the suppression of intracellular lipolysis, but the precise mechanism for this effect needs to be further investigated in humans.

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