Nicotinic Acid–Induced Insulin Resistance Is Related to Increased Circulating Fatty Acids and Fat Oxidation But Not Muscle Lipid Content

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Insulin resistance is associated with increased circulating lipids and skeletal muscle lipid content. Chronic nicotinic acid (NA) treatment reduces insulin sensitivity and provides a model of insulin resistance. We hypothesized that the reduction in insulin sensitivity occurs via elevation of circulating nonesterified fatty acids (NEFAs) and an increase in intramyocellular lipid (IMCL). A total of 15 nondiabetic males (mean age 27.4 ± 1.6 years) were treated with NA (500 mg daily for 1 week, 1 g daily for 1 week). Insulin sensitivity (glucose infusion rate [GIR]) was determined pre- and post-NA by euglycemic-hyperinsulinemic clamp. Substrate oxidation was determined by indirect calorimetry. Skeletal muscle lipid was assessed by estimation of long-chain acyl-CoA (LCACoA) and triglyceride (TG) content and by ^{1}H-magnetic resonance spectroscopy quantification of IMCL (n = 11). NA reduced GIR ($P = .03$) and nonoxidative glucose disposal ($P < .01$) and increased fasting NEFAs ($P = .01$). The decrease in GIR related significantly to the increase in fasting NEFAs ($r^2 = .30$, $P = .03$). The intrasubject increase in basal and clamp fat oxidation correlated with the decrease in GIR ($r^2 = .45$, $P < .01$ and $r^2 = .63$, $P < .01$). There were no significant changes in muscle LCACoA, TG, or IMCL content. Therefore, induction of insulin resistance by NA occurs with increased availability of circulating fatty acids to muscle rather than with increased muscle lipid content.

NICOTINIC ACID (NA) is a B group vitamin, which lowers total low-density lipoprotein (LDL) and triglyceride (TG) levels and increases high-density lipoprotein (HDL) levels. Despite its favorable lipid profile, chronic administration of NA has been shown to result in deterioration of glucose tolerance in normal humans and deterioration of glycemic control in people with type 2 diabetes. The cause of this alteration in glucose metabolism remains unknown: net hepatic glucose output is unchanged,[1] and reported changes in insulin secretion[2] have not been confirmed.[3] However insulin resistance, measured by euglycemic-hyperinsulinemic clamp, is induced by 2 weeks administration of NA.[3,4] The mechanism of this increase in insulin resistance may be related to a "rebound" in circulating nonesterified fatty acids (NEFAs), which are acutely suppressed.[5] NA may therefore provide an experimental model of insulin resistance in humans.

Insulin resistance has been associated with increases in both circulating lipids and adipose tissue depots.[6] Parenteral elevation of circulating NEFA over several hours has been shown to reduce insulin sensitivity in both rodents and humans.[7,8] Cross-sectional studies have found that increased skeletal muscle lipid content is associated with reduced insulin sensitivity.[9] Accumulation of lipid in skeletal muscle (measured biochemically) has been demonstrated in rodents infused with intravenous lipid, producing supraphysiologic levels of circulating NEFAs, with an associated decrease in insulin sensitivity.[10] Recent human studies have reproduced these findings with parenteral infusion of lipid leading to acute elevation of skeletal muscle lipid (quantified by ^{1}H-magnetic resonance spectroscopy) and decreased insulin sensitivity.[5,11] Whether accumulation of muscle lipid is also induced in humans by long-term elevation of fatty acids within high physiologic, rather than supraphysiologic, levels is unknown.

Accurate measurement of skeletal muscle lipid content in humans has been difficult. Muscle TG content has been quantified by biochemical extraction methods, which may be contaminated by subcutaneous fat. The validity of our method, involving freeze-drying and dissection of muscle fibers free from contaminants, was supported by the lack of adipsin expression, demonstrating the lack of adipocyte contamination of muscle biopsy samples treated this way.[12] We have also recently described a method of quantifying muscle content of metabolically active long-chain acyl-CoA esters (LCACoAs), which are TG precursors.[13] In addition, the development of proton magnetic resonance spectroscopic methods has enabled noninvasive measurement of intramyocellular triglyceride.[14,15]

This study was undertaken to examine the hypothesis that chronic administration of NA reduces whole-body insulin sensitivity via elevation of circulating fatty acids (to high physiologic levels) and accumulation of skeletal muscle lipid. We also examined the hypothesis that this reduction in insulin sensitivity is related to alterations in fat and carbohydrate (CHO) oxidation.

MATERIALS AND METHODS

Subject Selection and Experimental Protocol

Fifteen healthy Caucasian male volunteers were recruited from advertisements in the local press. Volunteers were excluded if there was a history of diabetes mellitus, cardiovascular, renal, or other clinically significant disease. The St. Vincent's Hospital Research Ethics Committee approved the protocol, and all subjects gave written informed consent. Subjects received NA 500 mg/d for the first week and 1,000 mg/d for a second week. Blinding of treatment phase was not possible due to side effects, mainly flushing, which occurred in all subjects. Adherence to medication was assessed by pill count at the end of the

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study. Before NA and after 14 days of NA, subjects had the same metabolic assessments performed on a single day. After an overnight fast, subjects had proton magnetic resonance spectroscopy of lower leg muscles soleus and tibialis anterior, followed immediately by biopsy of skeletal muscle vastus lateralis. Subjects then had anthropometric measurements and underwent euglycemic-hyperinsulinemic clamp while remaining fasted. Indirect calorimetry was performed in the resting state, before the clamp and in the final 30 minutes of the clamp. After a light lunch, subjects had whole-body dual-energy x-ray absorptiometry (DXA) to assess body composition. DXA was performed before NA only.

Proton Magnetic Resonance Spectroscopy of the Soleus and Tibialis Anterior Muscles

After an overnight fast, subjects were placed in a 1.5 Tesla medical magnetic resonance scanner (G.E., Milwaukee, WI). The right lower leg was studied using an extremity coil. A voxel was placed in soleus ($2.0 \times 2.0 \times 2.0$ cm) and tibialis anterior ($1.5 \times 1.5 \times 1.5$ cm) muscles at the largest diameter of the muscle. Voxels were placed in homogenous muscle tissue, avoiding visible fascia, fat, and vessels. Spectra were acquired by PRESS sequence with echo time 135 ms and repetition time 1,500ms, and data were stored for later analysis. Proton resonance time-domain quantitation was performed with AMARES (MRUI version 99.2, European Union) using constraints to improve fitting reliability, as described by Rico-Sanz et al.[16] Assigned line shapes were gaussian apart from a lorentzian line shape for the water peak. Pairs of peaks assigned with equal line widths were creatine and carnitine, intramyocellular CH_2(IMCH2) and extramyocellular CH_2(EMCH2), and intramyocellular CH_3(IMCH3) and extramyocellular CH_3(EMCH3). A line width ratio of 0.93 was assigned for IMCH3:IMCH2 and EMCH3:EMCH2. Peak areas were corrected for T1 and T2 times. Intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) content were quantitated as areas of IMCH2 and EMCH2 peaks, respectively, and expressed as a percentage of water peak area. In soleus muscle, the coefficient of variation in measurement of IMCL performed on the same day was 8%. Due to technical difficulties, complete spectroscopy data were not available for 4 of the 15 subjects.

Skeletal Muscle Biopsy

Before and after treatment with NA, all subjects underwent skeletal muscle biopsy immediately after proton magnetic resonance spectroscopy studies. Under local anesthesia, a 6-mm diameter University College Hospital muscle biopsy needle was inserted into the vastus lateralis muscle (15 cm above the patella) yielding samples of approximately 150 mg. Biopsied muscle tissue was immersed in liquid nitrogen and stored at -80°C.

Euglycemic-Hyperinsulinemic Clamp and Indirect Calorimetry

Before NA (pre-NA) and after 2 weeks of NA consumption (post-NA) height was measured with a stadiometer and weight measured by calibrated scales. Body mass index (BMI) was calculated (kg/m^2). All subjects underwent a euglycemic-hyperinsulinemic clamp for assessment of insulin sensitivity. Clamp studies were started at 8:30 AM after proton magnetic resonance spectroscopy studies and skeletal muscle biopsy. Subjects had fasted overnight. An intravenous catheter was inserted under local anesthesia into an antecubital vein for infusion of human insulin (Actrapid; Novo Nordisk, Denmark) and glucose. A second intravenous catheter in a warmed contralateral forearm vein was used for arterialized blood sampling. Fasting blood samples were obtained for measurement of lipids (LDL and HDL cholesterol, TG, NEFAs), glucose, insulin, C-peptide, and leptin levels. Insulin was infused at 240 $pmol/m^2/min$ for 150 minutes, producing insulin levels in the high physiologic range (~80 mU/L), which has been shown to suppress hepatic glucose output (HGO) in healthy humans.[17] Blood samples were taken every 10 minutes for assessment of glucose, insulin, and NEFA levels. A variable glucose infusion rate was used to maintain subject glucose levels at 5 mmol/L. The steady-state glucose infusion rate (GIR) over the final 40 minutes of the clamp provided an index of whole-body insulin sensitivity and was expressed as micromole per minute per kilogram fat free mass (determined by DXA). Indirect calorimetry (Deltatrac; datex, Helsinki, Finland) was performed for 30 minutes before the clamp and at 120 to 150 minutes during hyperinsulinemia as previously described.[18] Resting metabolic rates and fat and CHO oxidation rates (g/min) were calculated.[19] Nonoxidative glucose disposal was calculated as the difference between GIR and CHO oxidation determined by calorimetry.

Body Composition

Body composition was assessed using whole-body DXA (Lunar DPX; Lunar Radiation, Madison, WI, software version 1.35y).[20] Total and regional tissue compositions of fat, muscle, and bone were measured in grams and as a percentage of tissue. Fat-free mass was calculated as the sum of bone and muscle mass.

Sample Analysis

Plasma glucose was measured by the oxidase method (NOVA 14, Nova Biomedical, Waltham, MA). Serum-free insulin and leptin were assayed by radioimmunoassay (Linco Research, Charles, MO). Serum cholesterol and TG concentrations were determined spectrophotometrically at 490 nm using enzymatic colorimetric kits (Roche Diagnostics, Basel, Switzerland). NEFA levels were determined by enzymatic colorimetry (NEFA C kit, WAKO Pure Chemical Industries, Osaka, Japan). The inter- and intra-assay coefficient of variation for NEFA were 5% and 5% at 500 μmol/L.

Skeletal Muscle TG Content

Approximately 50 mg skeletal muscle was freeze-dried under vacuum for 24 hours. After freeze-drying, the muscle sample was viewed under a microscope (6.3 ×) at room temperature for careful dissection and removal of all traces of adipose tissue, connective tissue, and blood contaminants. This yielded approximately 10 mg dry weight dissected skeletal muscle. The extraction of lipids from freeze-dried and carefully dissected muscle fibers and subsequent estimation of TG content is reproducible in our laboratory with a within-assay variability of approximately 8%.[12]

Skeletal Muscle LCACoA Measurement

LCACoAs were quantified by the methods previously described.[13] The method involved extraction and purification of LCACoA from muscle and quantification by reverse-phase high-performance liquid chromatography (HPLC) (Waters, Milford, MA) using a C18 column (Waters Nova-Pak). LCACoA peaks were detected by photodiode array (Waters 996) and quantitated by comparing sample peak areas with those of LCACoA standards. The amount of individual CoA species from each sample was corrected for loss during extraction by adjusting for the recovery of the internal standard in each individual sample. Within-assay variability was approximately 10%. The proportion of each of palmitoyl (16:0), palmitoleoyl (16:1), linolenoyl (18:3), linoleoyl (18:2), and oleoyl (18:1) long-chain fatty acids in muscle TG was calculated as a percentage of the total. The total LCACoA content was calculated as the sum of the major CoA species, palmitoyl, linoleoyl, and oleoyl, which make up approximately 90% of the fatty acids.

Table 1. Clinical Characteristics of Subjects

	Mean ± SEM	Range
Age (yr)	27.4 ± 1.6	20-36
BMI (kg/m^2)	24.8 ± 0.8	20.1-29.1
Total fat* (%)	20.3 ± 0.2	10.3-33.3
Leg fat mass (kg)	5.8 ± 0.5	3.2-8.4
Fat-free mass* (kg)	66.3 ± 0.2	54.6-78.0
Fasting plasma glucose (mmol/L)	5.1 ± 0.1	4.3-6.2

NOTE. Results expressed as mean ± SEM.
*Derived from DXA.

Statistical Analyses

Previous studies have shown an approximately 18% change in insulin sensitivity with NA with within-subject variation of 10%.[4] To detect significant changes (at $P = .05$ level) of this order, with power of 80%, using a paired design, required a sample size of n = 6. IMCL in calf muscles has been shown to increase by 50% with high-fat feeding.[11] Assuming a lower level of IMCL change with NA of 25%, and within subject variation of 10%, n = 10 for power of 80% and significance at $P < .05$.

Data were analyzed using StatView 4.51 (Abacus Concepts, Berkely, CA). Subject characteristics were expressed as the mean ± SE. Comparisons between treatment groups were performed by Student's paired *t* test. Relationships between continuous variables were assessed using Pearson's simple correlation analysis. $P < .05$ was considered significant.

RESULTS

Subject clinical characteristics are shown in Table 1. Subjects were young men (mean age ± SEM 27.4 ± 1.6 years) and normal to overweight (BMI, 24.8 ± 0.8kg/m^2). None was obese. There was a broad range of adiposity by DXA (10.3% to 33.3% total fat). The 2 subjects with fasting plasma glucose >5.5 mmol/L had oral glucose tolerance tests, which showed normal glucose tolerance. There were no significant changes in body weight (82.6 ± 2.6 *v* 82.2 ± 2.6 kg, $P = .1$), fasting plasma glucose (5.1 ± 0.1 *v* 5.2 ± 0.2 mmol/L, $P = .7$), insulin (9.0 ± 1.4 *v* 9.7 ± 2.0 mU/L, $P = .7$), leptin (3.7 ± 0.7 *v* 3.8 ± 0.8 ng/mL, $P = .7$), or C-peptide (1.5 ± 0.3 *v* 1.4 ± 0.3 ng/mL, $P = .3$) levels pre-NA versus post-NA treatment.

Two weeks treatment with NA significantly reduced mean insulin sensitivity by approximately 15% (GIR, 49.6 ± 3.9 *v* 42.5 ± 4.1 μmol/L, $P < .01$) (Table 2). Clamp insulin levels were not significantly different post-NA (80.5 ± 5.4 *v* 85.2 ± 4.9 mU/L, $P = .8$). LDL cholesterol decreased significantly (3.1± 0.2 *v* 2.8 ± 0.3 mmol/L, $P < .05$) and HDL increased significantly (1.1 ± 0.1 *v* 1.25 ± 0.1 mmol/L, $P < .05$). There was no change in TG levels (Table 2). Basal and clamp fat and glucose oxidation were not significantly different after NA treatment (post-NA) (Table 2). Nonoxidative glucose disposal during the clamp was reduced by approximately 30% (325 ± 42 *v* 229± 45 mg/min, $P < .01$).

Fasting NEFA levels (measured 12 hours after the last dose of NA) showed a significant increase of ~65% compared with pre-NA levels as shown in Fig 1 (304 ± 25 *v* 501 ± 66 μmol/L, $P = .01$). There was a trend to higher clamp NEFA levels after NA treatment (20 ± 5 *v* 48 ± 14 μmol/L, $P = .056$), but no difference in percentage degree of suppression of NEFAs with insulin infusion (89% ± 3% *v* 80% ± 7%, $P = .10$). Muscle TG as measured by multiple methods: ^{1}H-magnetic resonance spectroscopy of soleus and tibialis anterior muscles and biochemical TG in vastus lateralis did not change after NA treatment (Table 3). The increase in total LCACoA in vastus lateralis (Table 3) was not significant, and there were no significant changes in individual LCACoA peaks (data not shown).

Table 2. Metabolic and Lipid Parameters Pre- and Post-nicotinic Acid Treatment

	Pre-NA	Post-NA
Glucose infusion rate (μmol/min/kgFFM)	49.6 ± 3.9	42.5 ± 4.1*
LDL cholesterol (mmol/L)	3.1 ± 0.2	2.8 ± 0.3†
HDL cholesterol (mmol/L)	1.1 ± 0.1	1.3 ± 0.1†
Triglyceride (mmol/L)	1.1 ± 0.11	1.1 ± 0.18 NS
Nonoxidative glucose disposal (mg/min)	325 ± 42	229 ± 45*
Basal fat oxidation (mg/min)	64.1 ± 4.6	68.1 ± 4.2 NS
Clamp fat oxidation (mg/min)	34.0 ± 5.8	23.7 ± 4.9 NS
Basal CHO oxidation (mg/min)	161.8 ± 12.5	156.2 ± 17.1 NS
Clamp CHO oxidation (mg/min)	254.0 ± 21	272.7 ± 24 NS

NOTE. Results expressed as mean ± SEM.
Abbreviations: NA, nicotinic acid, CHO, carbohydrate; NS, not significant.
*$P < .01$.
†$P < .05$.

Figure 2A shows the significant negative correlation between the decrease in insulin sensitivity and the increase in basal NEFA levels after treatment with NA ($r^2 = .3$, $P = .03$). That is, subjects with a greater increase in basal NEFAs had a greater reduction in insulin sensitivity (measured by GIR). Although there was not a significant mean change as a group in basal or clamp fat oxidation, Fig 2B shows intra-subject change in basal fat oxidation correlated negatively with intra-subject decrease in insulin sensitivity post-NA ($r^2 = .45$, $P < .01$). Intra-subject decrease in insulin sensitivity also correlated negatively with intra-subject change in clamp fat oxidation ($r^2 = .63$, $P < .01$)

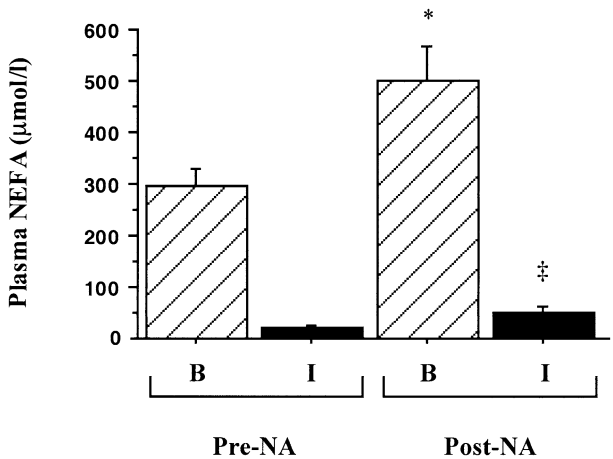

Fig 1. Mean (± SEM) NEFA levels in the (B) basal state and (I) during euglycemic-hyperinsulinemic clamp pre-NA and post-NA administration. *Post-NA basal NEFAs are significantly higher than pre-NA basal NEFAs, $P = .011$. ‡Post-NA insulin-infused NEFAs show a trend to higher levels compared with pre-NA clamp NEFAs, $P = .056$.

Table 3. Muscle Lipid Measurements Pre and Post-NA Treatment

	Pre-NA	Post-NA
Vastus lateralis		
Total LCACoA (nmol/g)	8.0 ± 0.9	9.3 ± 1.2
Vastus lateralis		
Total triglyceride (μmol/g)	31.9 ± 5.7	31.5 ± 9.3
Soleus IMCL		
(% water resonance area)	0.89 ± 0.07	0.81 ± 0.06
Tibialis anterior IMCL		
(% water resonance)	0.61 ± 0.05	0.62 ± 0.01

NOTE. Results expressed as mean ± SEM.

(Fig 2C). The change in basal NEFAs also correlated with the increase in basal fat oxidation ($r^2 = .50$, $P < .01$). There were no relationships between GIR and basal or clamp NEFA either pre-NA or post-NA. Pre-NA assessment of total fat and trunk fat by DXA showed a significant correlation with baseline insulin sensitivity ($r^2 = .34$, $P = .02$; $r^2 = .45$, $P < .01$, respectively), and this relationship was not altered by NA treatment. Pre-NA GIR was negatively related to muscle TG measured biochemically ($r^2 = .47$, $P = .02$), but this was not present post-NA ($r^2 = .29$, $P = .88$). There was no relationship between GIR and LCACoA or IMCL either before or after NA treatment (data not shown).

DISCUSSION

This study examined the hypothesis that chronic administration of NA reduces whole-body insulin sensitivity via elevation of circulating fatty acids (to high physiologic levels) and accumulation of skeletal muscle lipid. We also examined the hypothesis that this reduction in insulin sensitivity is related to alterations in fat and CHO oxidation. While our findings confirm previous reports of a modest reduction in insulin sensitivity with chronic NA,[3,4] we did not demonstrate changes in muscle TG (measured by 2 methods) or muscle LCACoAs. In this experimental model of insulin resistance, we demonstrated a strong relationship between a decrease in insulin sensitivity and an increase in basal NEFAs. We did not show a relationship between changes in insulin sensitivity and changes in skeletal muscle lipid content.

Our study showed both an elevation of basal circulating NEFAs (measured 12 hours after the last dose of NA) and a strong negative correlation between intraindividual increase in basal NEFAs and decline in insulin sensitivity. The 65% increase in basal NEFAs is equivalent to a high physiologic level encountered in obese insulin-resistant people.[21] Wang et al[1] demonstrated that, in young women, NA acutely suppressed NEFAs by approximately 50%, with 2-fold to 3-fold rebound 2 hours after NA administration. NEFAs remained elevated measured out to 6 hours. After 1-month treatment with NA, basal NEFA levels were elevated to a similar degree as in our study, but did not reach statistical significance, probably due to the small numbers of women in that study (n = 5). In our study, with larger numbers of subjects, we have confirmed significantly higher basal levels of NEFAs with chronic administration of NA, as well as demonstrating similar elevations in males as previously demonstrated in females. There was a trend to higher clamp NEFAs suggesting that the lipid perturbation persisted despite high circulating insulin levels.

Both the study by Wang et al and our study used the immediate release formulation of NA. Longer acting NA preparations may not cause a prolonged rebound of NEFAs. A recent 16-week study evaluating the safety of once-daily extended-release NA showed a significant deterioration in glycemic control (measured by glycated hemoglobin [HbA_{1c}]) and increase in antidiabetic medication in subjects with type 2 diabetes.[22] However, free fatty acid levels were not measured. If NEFAs are found not to be elevated with this preparation, future studies may identify whether NA has an effect on insulin sensitivity independent of elevation of circulating fatty acids.

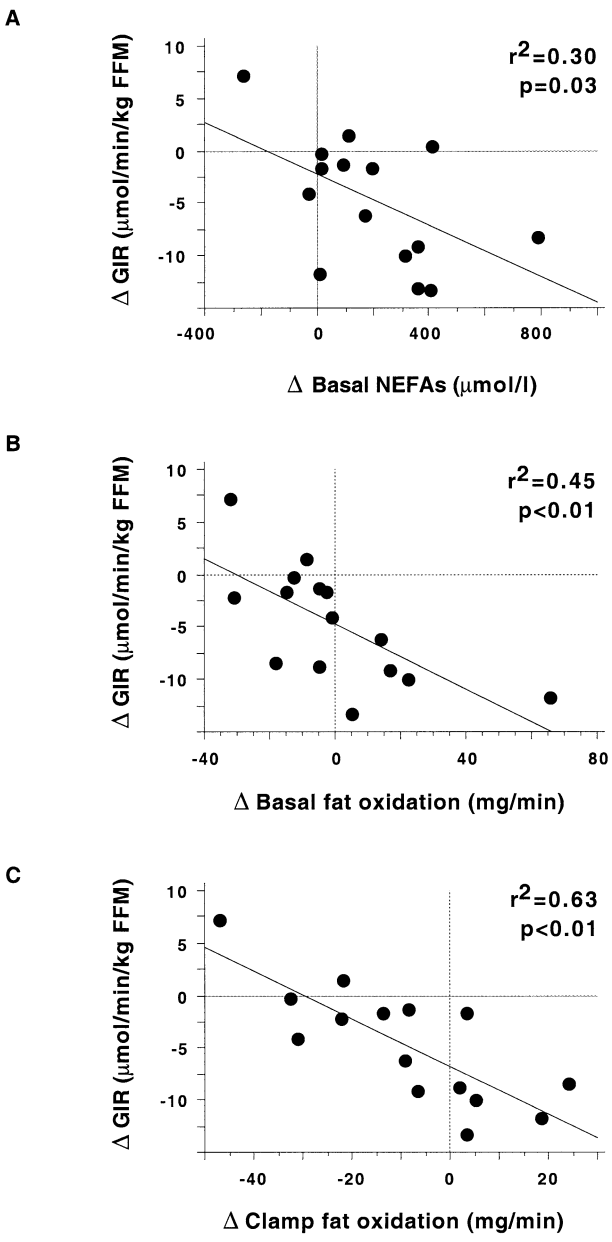

Fig 2. Correlations of change in GIR with (A) change in basal NEFA levels, (B) change in basal fat oxidation, and (C) change in clamp fat oxidation. All Δ are post-NA/pre-NA values.

Although mean fat oxidation rate was not significantly increased by NA treatment, subjects with a greater increase in basal fat oxidation after NA had a greater decrease in insulin sensitivity than those subjects who had no change, or a decrease, in fat oxidation. Conversely, a greater decrease in glucose oxidation was associated with a greater decrease in insulin sensitivity. The associations between declining insulin sensitivity, increased basal NEFAs, and increased basal fat oxidation are in keeping with the Randle cycle (fatty acids competing with glucose as source of fuel oxidation).[23] However, the decrease in insulin sensitivity is unlikely to be due primarily to a Randle effect, as nonoxidative glucose disposal was significantly reduced by NA, whereas the decrease in basal glucose oxidation was not statistically significant. In rodents, elevation of circulating fatty acids has been shown to reduce nonoxidative glucose disposal with inhibition of insulin signalling via protein kinase C pathways and reduced insulin-stimulated insulin receptor substrate tyrosine phosphorylation.[7] Decreased glycogen formation has also been reported.[10] Murine muscle cells incubated with fatty acids had increased intracellular ceramides and showed decreased insulin-stimulated glycogen synthesis.[24] In humans, defects in glucose transport and phosphorylation have been associated with intravenous elevation of fatty acids.[25] The level of hyperinsulinemia in the clamp has been shown to suppress HGO in healthy humans[17]; however, it is uncertain whether HGO is completely suppressed in clamps after NA treatment. Impairment of suppression of HGO may contribute to the defect in insulin action demonstrated after NA.

Unlike some other rodent and human studies,[5,10] we did not detect increased skeletal muscle lipid with elevation of circulating NEFAs, and changes in muscle lipid were not associated with changes in insulin sensitivity. These previous studies used intravenous lipid and insulin infusions and produced NEFA levels unlikely to occur physiologically in humans. Additionally, in the studies by Bachmann et al[11] and Krebs et al,[25] intravenous lipid alone did not increase IMCL. This occurred only when there was a concomitant high-dose insulin infusion. In our study, the 65% increase in NEFAs was in a high physiologic range, and the muscle lipid levels were assessed before high-dose exogenous insulin. The relatively short duration of the study may have influenced the lack of demonstrably increased IMCL. However, the intravenous lipid studies have shown IMCL changes at 6 hours, and in our study, NEFAs were elevated 12 hours after the last dose of NA.

It remains possible that small changes in muscle lipid occurred, which were unable to be detected within the sensitivity of the methods used. The number of subjects studied was sufficient to detect an approximately 15% change in IMCL, but larger numbers may be needed to detect smaller changes. Our study was powered to (and did) detect a modest change in insulin resistance, similar to that related to high-fat feeding.[11] It is possible that only larger reductions in insulin sensitivity consistently produce detectable increases in IMCL. Alternatively, increases in other lipid metabolites may have occurred, which the methods used do not detect. In rodent studies, a nonmetabolisable fatty acid tracer, 3H-(R)-2-bromopalmitate, has shown enhancement of fatty-acid clearance into muscle with elevation of circulating lipids.[26] This tracer may be useful in future human studies to confirm that elevation of circulating NEFAs is associated with increased fatty acid uptake by muscle.

Prior to NA, insulin sensitivity was related to both total and trunk fat as we have shown previously.[27] This relationship persisted after NA treatment. The relationship of muscle TG with insulin sensitivity, which was strong before NA treatment, was absent after 2 weeks of NA, suggesting that circulating factors were the strongest temporal determinants of insulin sensitivity post-NA. Body fat, particularly central fat, is a strong and major predictor of insulin sensitivity,[6] hence the relationship persisted in the face of alteration in circulating lipid supply when body fat depot size had not changed.

There has been a growing complexity in the data relating to the cross-sectional association of muscle lipid and insulin resistance. In our group of untrained young men, muscle TG content, but not LCACoA or IMCL measured by NMR, predicted insulin sensitivity prior to NA. This may be due to the low intra-subject variability of the TG assay in our laboratory, as well as the nature of our subject group. Reports of skeletal muscle LCACoA content as an independent predictor of insulin resistance involved middle-aged sedentary men.[13] An association between IMCL (measured by NMRS) and insulin sensitivity has been demonstrated in lean European, but not in more adipose South East Asian men.[28] Our subjects had a wide range of adiposity as assessed by DXA, which may have affected the strength of the association. LCACoA and TG were measured in the same muscle (vastus lateralis) and IMCL by NMRS in soleus and tibialis anterior. Differing muscle fiber type may influence the different relationships seen with baseline insulin resistance and muscle lipid content measured by different modalities.

The nexus between circulating fatty acids, body fat depots, and muscle lipid is pivotal to our understanding of insulin resistance. We have demonstrated that insulin resistance may be induced without increased levels of skeletal muscle lipid. Induction of insulin resistance with NA is related to elevated circulating fatty acids, intra-subject changes in fat oxidation, and decreased nonoxidative glucose disposal, but not increased muscle lipid measured by multiple modalities. This study suggests that when circulating fatty acids are elevated to high physiologic rather than supraphysiologic levels, insulin resistance is related more to circulating free fatty acid availability to muscle than to muscle lipid content.

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