Changes in Phosphatidylcholine Fatty Acid Composition Are Associated With Altered Skeletal Muscle Insulin Responsiveness in Normal Man

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The fatty acid composition of skeletal muscle cell membrane phospholipids (PLs) is known to influence insulin responsiveness in man. We have recently shown that the fatty acid composition of phosphatidylcholine (PC), and not phosphatidylethanolamine (PE), from skeletal muscle membranes is of particular importance in this relationship. Efforts to alter the PL fatty acid composition in animal models have demonstrated induction of insulin resistance. However, it has been more difficult to determine if changes in insulin sensitivity are associated with changes in the skeletal muscle membrane fatty acid composition of PL in man. Using nicotinic acid (NA), an agent known to induce insulin resistance in man, 9 normal subjects were studied before and after treatment for 1 month. Skeletal muscle membrane fatty acid composition of PC and PE from biopsies of vastus lateralis was correlated with insulin responsiveness using a 3-step hyperinsulinemic-euglycemic clamp. Treatment with NA was associated with a 25% increase in the half-maximal insulin concentration ([ED_{50}] 52.0 ± 7.5 to 64.6 ± 9.0 μU/mL, $P < .05$), consistent with decreased peripheral insulin sensitivity. Significant changes in the fatty acid composition of PC, but not PE, were also observed after NA administration. An increase in the percentage of 16:0 (21% ± 0.3% to 21.7% ± 0.4%, $P < .05$) and decreases in 18:0 (6.2% ± 0.5% to 5.1% ± 0.4%, $P = .01$), long-chain n-3 fatty acids (1.7% ± 0.2% to 1.4% ± 0.1%, $P < .01$), and total polyunsaturated fatty acids ([PUFAs] 8.7% ± 0.8% to 8.0% ± 0.8%, $P < .05$) are consistent with a decrease in fatty acid length and unsaturation in PC following NA administration. The change in ED_{50} was significantly correlated with the change in PUFAs ($r = -.65$, $P < .05$). These studies suggest that the induction of insulin resistance with NA is associated with changes in the fatty acid composition of PC in man.

SKELETAL MUSCLE accounts for approximately 85% of insulin-stimulated glucose disposal in nondiabetic individuals.[1-3] Moreover, defects in skeletal muscle glucose disposal account for most of the reduction in insulin sensitivity that is characteristic of obesity and type 2 diabetes mellitus.[4] Recent studies have demonstrated impaired glucose transport[4] and/or phosphorylation[5] in obese diabetic and nondiabetic individuals, which is associated with a reduction in the translocation of the insulin-regulated glucose transporter GLUT4 to the sarcolemma.[6] However, the mechanism(s) responsible for reduced glucose transport in insulin-resistant states is not known.

One potential mechanism for altered insulin sensitivity is the composition of skeletal muscle membranes. Kriketos et al[7] and Pan et al[8] have recently shown an inverse relationship between insulin resistance and the percentage of long-chain polyunsaturated fatty acids (PUFAs) in human muscle membrane phospholipids (PLs). Moreover, increased PUFAs are found in membrane PLs of the more insulin-sensitive and oxidative type I muscle as compared with glycolytic type II muscle,[7,9] suggesting that membrane PL fatty acid composition may play a significant role in the relative insulin responsiveness of muscle fiber types.

These important studies were performed on total membrane PLs. However, plasma membranes contain several distinct PL species,[10] and the fatty acid composition of these PL species may have considerable variation. Of particular interest are the PLs phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which together account for more than 75% of the total plasma membrane PLs in man.[10] In addition, it is known that the outer and inner layers of the plasma membrane bilayer contain a greater proportion of PC and PE, respectively. Thus, differences in the fatty acid composition of these 2 PL species could have independent effects on insulin responsiveness. In support of this hypothesis, we have recently demonstrated that the membrane fatty acid composition of PC (but not PE) in type II rat muscle is characterized by an enrichment of palmitic acid (16:0) relative to stearic acid (18:0), which suggests a defect in fatty acid elongation in the insulin-resistant muscle.[11] In addition, studies performed in our laboratory in normal humans demonstrated for the first time that the skeletal muscle membrane fatty acid composition of PC, but not PE, was related to insulin sensitivity.[12]

The present study was designed to determine if induced changes in insulin sensitivity are associated with changes in membrane PL fatty acid composition in man. Using nicotinic acid (NA), an agent known to decrease insulin sensitivity with long-term administration, we have demonstrated significant changes in the ratio of 16:0 to 18:0 and total PUFAs in PC.

SUBJECTS AND METHODS

Eight normal men and 1 woman were enrolled in the present studies (Table 1). Body composition was determined by skinfold measurements[13] and bioimpedance analysis performed after an overnight fast.[14] All of the subjects were in good health with stable body weight at the time of the studies: None had a family history of diabetes mellitus. The woman was studied during the early follicular phase of the menstrual cycle, and she was not using exogenous estrogens or progestins. The studies were approved by the Committee for the Conduct of Human Research at Virginia Commonwealth University, and informed consent was obtained from each subject before enrollment in the protocols.

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Table 1. Demographics of the Subjects Enrolled in Metabolic Studies (N = 9)

Variable	Mean ± SEM	Range
Age (yr)	26.7 ± 2.7	19-43
Body mass index (kg/m²)	24.9 ± 1.4	19.2-30.5
Body fat (%)	19.3 ± 3.3	10.5-37.1

Study Design

Subjects were admitted to the General Clinical Research Center (GCRC) at Virginia Commonwealth University on the evening prior to study and consumed a regular diet containing 300 g carbohydrate, 90 g protein, and 90 g fat while on the unit. The diet was chosen based on surveys indicating the customary diet of our study group. Subjects were instructed to consume a similar diet while at home. On the first morning of the study, a biopsy of the vastus lateralis muscle was obtained. After infiltration of the skin with 1% lidocaine, a 1-in incision was made through the skin and fascia in the midlateral thigh and a muscle sample was obtained by excisional biopsy. A portion of the muscle sample was mounted on Tissue Tek II OCT compound (Lab-Tek Products, Naperville, IL) and frozen in isopentane with liquid nitrogen as a coolant. All samples were then frozen at −70°C.

A 3-step (0.4, 1.0, and 10.0 $mU \cdot kg^{-1} \cdot min^{-1}$) hyperinsulinemic-euglycemic clamp was then performed on each subject after an overnight fast as previously described.[15] The clamp was performed 2 days after the muscle biopsy to minimize the biopsy effect on insulin responsiveness.[16] At 7:00 AM, an intravenous catheter was placed in a forearm vein for infusion of insulin and 20% dextrose supplemented with 20 mol/L potassium phosphate. Another catheter was placed in a retrograde fashion in a hand vein, and the hand was placed in a Plexiglas (Rohm & Haas, Philadelphia, PA) box heated to 60°C to obtain arterialized venous blood samples.[17] Both catheters were kept patent with an infusion of normal saline. Each step was performed for 120 minutes. During the final 30 minutes of each step, indirect calorimetry was performed using a Sensormedics 2000 metabolic cart (Anaheim, CA) equipped with a plastic canopy to measure oxygen consumption and carbon dioxide production (liters per minute).

Following completion of the baseline studies, the subjects received NA daily for 4 weeks. The dosage was titrated over 5 to 7 days to 1,000 mg twice daily. All of the subjects received the maximum dose for at least 3 weeks. Symptoms of flushing were treated with aspirin 30 minutes prior to a dose of NA. Two strategies were used to ensure compliance with the NA protocol. All subjects returned to the GCRC after 2 weeks of therapy for pill counting and review of adverse events (particularly flushing). Subjects then received the remainder of the medication and a repeat pill count was performed when they returned. A study nurse also called each of the subjects weekly to remind them of the importance of taking their medication and to inquire about adverse effects. After the 4-week treatment period, the subjects returned and the above-mentioned studies were repeated. The morning dose of NA was administered at 6:00 AM prior to the second biopsy. The subjects then continued the NA and received their final dose at 6:00 AM on the morning of the insulin clamp.

Blood samples were obtained every 2.5 to 5 minutes during the clamp for measurement of plasma glucose (Yellow Springs Instruments, Yellow Springs, OH). In addition, samples were obtained every 30 minutes for measurement of intermediary metabolites and glucoregulatory hormones. Plasma insulin,[18] C-peptide,[19] and glucagon[20] were determined with double-antibody radioimmunoassays. Plasma free fatty acids (FFAs) were determined by enzymatic methods.[21] Blood samples for measurement of intermediary metabolites were immediately deproteinized with ice-cold 3 mol/L perchloric acid. The supernatant was neutralized with 3 mol/L KOH, and the resulting supernatant was assayed for L-lactate, alanine, β-hydroxybutyrate, acetoacetate (all by Maughn's method[22]), citrate,[23] and glycerol[24] with microfluorometric assays. Urinary nitrogen was determined by the Kjeldahl method.

Muscle Fiber Typing

Muscle fiber types were classified according to their myosin adenosine triphosphate activity at pH 4.3, 4.6, and 10.4 as previously described.[25] Fibers designated as type I were stable at acid pH but labile at pH 10.4, whereas the converse was observed for type II fibers. Type IIa fibers were determined from their lability at pH 4.6 but not pH 4.3. At least 300 fibers were analyzed from each sample.

Muscle Cell Membrane PL Fatty Acid Composition

PL fatty acid composition was analyzed according to the method of Borkman et al[26] with minor modifications. Muscles (50 to 60 mg) were homogenized and total lipids extracted by the method of Folch et al.[27] The lipid extracts were dried under nitrogen, dissolved in 10 mL hexane, and applied to 3-mL silica gel columns (J.T. Baker, Phillipsburg, NJ). After elution of less polar lipids with 20 mL hexane followed by 10 mL dichlormethane, phospholipids were eluted with 10 mL methanol. The methanol eluates were dried under nitrogen and transmethylated with 1.5 mL 1N methanolic HCl at 80°C overnight. Fatty acid methyl esters were extracted with 6 mL hexane and dried under nitrogen.

To determine the fatty acid composition of individual PL species, PC, PE, sphingomyelin, phosphatidylinositol, cardiolipin, and phosphatidylserine were first separated by thin-layer chromatography (TLC) on silica gel G plates (LK6D; Whatman, Clifton, NJ) using a solvent system consisting of chloroform:ethanol:triethylamine:water (30:34:30:8 vol/vol) for the first development and hexane:dimethyl ether (50:50 v/v) for the second development. PLs were visualized under UV light after spraying the plate with rhodamine G. The separated phospholipid spots were scraped and placed into glass tubes. Fatty acid methyl esters were prepared as described before by treatment with methanolic acid.

Fatty acid methyl esters from both the total PL fractions and individual PL species were redissolved in 20 μL hexane, separated, and quantified on a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph equipped with a 30-mm × 0.2-mm fused silica capillary column (Omega wax 320; Supelco, Bellefonte, PA) and flame ionization detector. The injection temperature was 250°C and detection temperature 300°C. The initial oven temperature was 140°C. After 5 minutes, the oven temperature was increased from 140°C to 200°C at a rate of 20°C/min, and then to 280°C at 5°C/min. Fatty acids were identified by comparing their retention time versus authentic standards.

Most of the gas chromatographic peaks were identified as specific fatty acid methyl esters. In the PC preparations, these fatty acid methyl esters accounted for 82% of the total integrated area, whereas they comprised only 67% of the integrated area in the PE fraction. However, in all samples, there were peaks immediately preceding 16:0 and 18:0, which were suspected to be dimethyl acetal derivatives of fatty aldehydes released from ether PL (plasmalogens). To confirm this identification, PE from bovine brain containing 60% plasmalogens (Sigma, St Louis, MO) was chromatographed before and after mild acid fume hydrolysis. After mild acid fume hydrolysis and separation on TLC plates, the peaks preceding 16:0 and 18:0 suspected of deriving from plasmalogens completely disappeared, essentially confirming their identity.

PL, triglyceride, cholesterol, and fatty acid standards were obtained from Sigma. High-performance precoated silica gel Hp-K plates (10 × 10 cm) were purchased from Whatman. All other reagents and solvents were of analytical grade or high-performance liquid chromatography–grade from Sigma or Fisher (Pittsburgh, PA).

Calculations

The glucose infusion rate during the final 30 minutes of each insulin infusion step was used to determine glucose disposal. The insulin concentration that resulted in half-maximal glucose disposal (ED_{50}) was determined from the best-fit relationship between the plasma insulin concentration and glucose infusion rate during the 3-step clamp. Calculation of carbohydrate and lipid oxidation was performed with the equations of Frayn[28] using the nonprotein respiratory quotient. Protein oxidation was estimated as 6.25 × urinary nitrogen excretion (grams per minute). At the 2 highest insulin infusion rates, it was assumed that the glucose infusion rate equaled total glucose disposal, since hepatic glucose production would be suppressed in these normal subjects. Thus, the rate of nonoxidative glucose disposal was calculated as the difference between the glucose infusion rate and oxidative glucose disposal determined from indirect calorimetry.

Results of PL fatty acid composition were also used to calculate indices of membrane remodeling and unsaturation as previously described.[26]

Statistical Analysis

Comparison of results between treatment groups was made by ANOVA and a permutation test. Comparison within groups was performed by repeated-measures ANOVA with a multiple-comparison test (Dunnett's test). Comparison between groups over time was performed using a mixed-effects ANOVA model. Regression analyses were conducted using SAS Version 6.07; SAS Institute, Cary, NC). Statistical significance was assumed at a P level less than .05. Results are expressed as the mean ± SEM.

RESULTS

Administration of NA for 4 weeks was associated with a modest but significant increase in fasting plasma glucose (78.8 ± 2.2 to 83.1 ± 2.3 mg/dL, $P < .05$) and a trend for an increase in fasting plasma insulin (5.4 ± 0.7 to 8.2 ± 1.7 μU/mL, NS), suggesting that insulin sensitivity was altered. The 3-step hyperinsulinemic-euglycemic clamp confirmed the decrease in insulin sensitivity. Plasma insulin increased to 26.5 ± 2.8, 61.5 ± 7.9, and 1,538.7 ± 189.3 μU/mL at 0.4-, 1.0-, and 10-mU · kg^{-1} · min^{-1} insulin infusion, respectively, and was not different before versus after NA administratio n(Table 2). However, NA treatment was associated with a significant decrease in insulin sensitivity (mean ED_{50} increase after NA. 52.0 ± 7.5 to 64.6 ± 9.0 μU/mL, $P < .05$; Fig 1). These changes in insulin sensitivity were observed despite favorable changes in plasma lipids and markers of lipolysis. Fasting plasma glycerol decreased significantly after NA administration (165.6 ± 18.8 to 124.2 ± 26.6 μmol/L, $P < .05$) and, although nonsignificant, plasma FFAs were generally lower after NA (763.1 ± 112.7 *v* 624.4 ± 137.9 μmol/L). In association with the reduction in lipolysis, baseline glucose oxidation increased from 0.96 ± 0.45 to 1.78 ± 0.17 mg · kg^{-1} · min^{-1} ($P < .05$) and lipid oxidation decreased from 1.41 ± 0.24 to 1.00 ± 0.12 mg · kg^{-1} · min^{-1} ($P = .07$) following NA. During the hyperinsulinemic clamp, plasma glycerol and FFA concentrations decreased significantly both before and after NA administration (Table 2). A significant increase of carbohydrate oxidation ($P < .001$) and suppression of lipid oxidation ($P < .001$) were also observed both before and after NA. A comparison of the curves for stimulation or suppression of carbohydrate and lipid oxidation, respectively, before and during the 3-step clamp (Fig 2a and b) by mixed-effects ANOVA suggested greater overall carbohydrate oxidation after NA, but it was not significant ($P = .06$). Thus, the increase in ED_{50}, reflecting decreased insulin sensitivity, was observed despite an increase in carbohydrate oxidation and an apparent reduction in fasting lipid oxidation, conditions that would be expected to mask a decrease in insulin sensitivity.[29,30]

The fatty acid composition of the predominant membrane PLs, PC and PE, obtained from biopsies of the vastus lateralis is shown in Table 3. A striking difference in the fatty acid composition of the 2 PL species is readily apparent. A greater percentage of 16:0, 18:1, and 18:2 was found in the PC fraction compared with the PE fraction, whereas an increased percentage of 18:0, 20:4, and 22:6 was found in PE compared with PC.

Table 2. Plasma Insulin, Glycerol, FFAs, Carbohydrate and Lipid Oxidation, and Glucose Infusion Rate at Baseline and During a Three-Step (0.4, 1.0, and 10.0 mU · kg^{-1} · min^{-1}) Hyperinsulinemic-Euglycemic Clamp Before and After NA Administration in Normal Subjects (N = 9)

Parameter	Baseline	0.4	1.0	10
Insulin (μU/mL)				
C	5.4 ± 0.7	26.5 ± 2.8	61.5 ± 7.9	1,538.7 ± 189.3
NA	8.2 ± 1.7	30.2 ± 3.0	67.9 ± 4.8	1,498.8 ± 160.8
Glucose infusion rate (mg · kg^{-1} · min^{-1})				
C	—	3.04 ± 0.31	7.51 ± 0.60	12.36 ± 0.52
NA	—	2.94 ± 0.32	7.40 ± 0.56	13.33 ± 0.25
Glycerol (μmol/L)				
C	165.6 ± 18.8	57.8 ± 11.3*	50.6 ± 9.4*	53.0 ± 8.1*
NA	124.2 ± 26.6†	68.5 ± 17.3*	49.1 ± 5.2*	54.9 ± 6.2*
FFA (μmol/L)				
C	763.1 ± 112.7	284.6 ± 21.1*	232.1 ± 16.8*	191.4 ± 25.3*
NA	624.4 ± 137.9	198.6 ± 24.9*	194.7 ± 32.8*	132.9 ± 20.5*
CHO oxidation (mg · kg^{-1} · min^{-1})				
C	0.96 ± 0.45	1.91 ± 0.38*	3.11 ± 0.40*	3.00 ± 0.41*
NA	1.78 ± 0.17†	2.73 ± 0.34*	3.93 ± 0.42*	3.81 ± 0.45*
Lipid oxidation (mg · kg^{-1} · min^{-1})				
C	1.41 ± 0.24	0.99 ± 0.25*	0.63 ± 0.19*	0.79 ± 0.19*
NA	1.00 ± 0.12	0.67 ± 0.11*	0.33 ± 0.16*	0.53 ± 0.15*

Abbreviation: C, before NA.

*$P < .01$ *v* baseline.

†$P < .05$ *v* control.

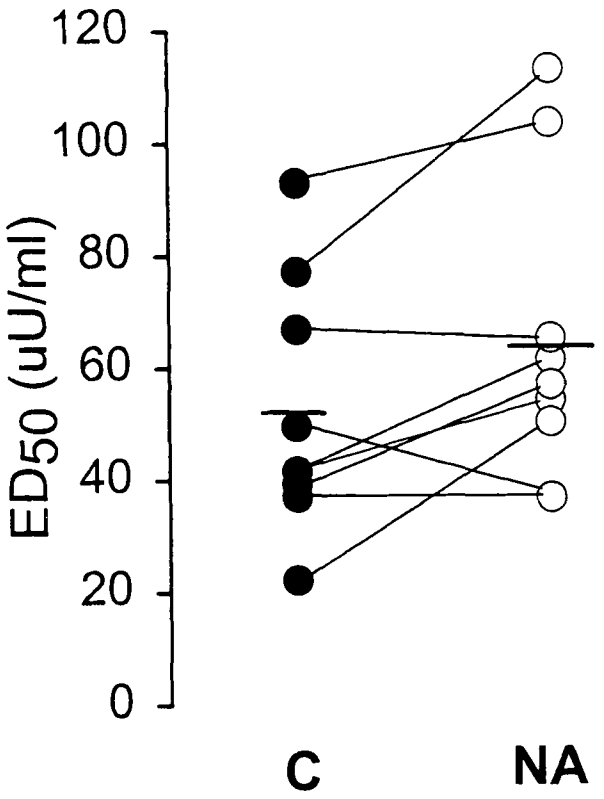

Fig 1. Effect of NA administration on insulin sensitivity (ED_{50}) in 9 normal subjects. Individual results are shown before (C) and after 1 month of NA. Mean ED_{50} is indicated by horizontal lines.

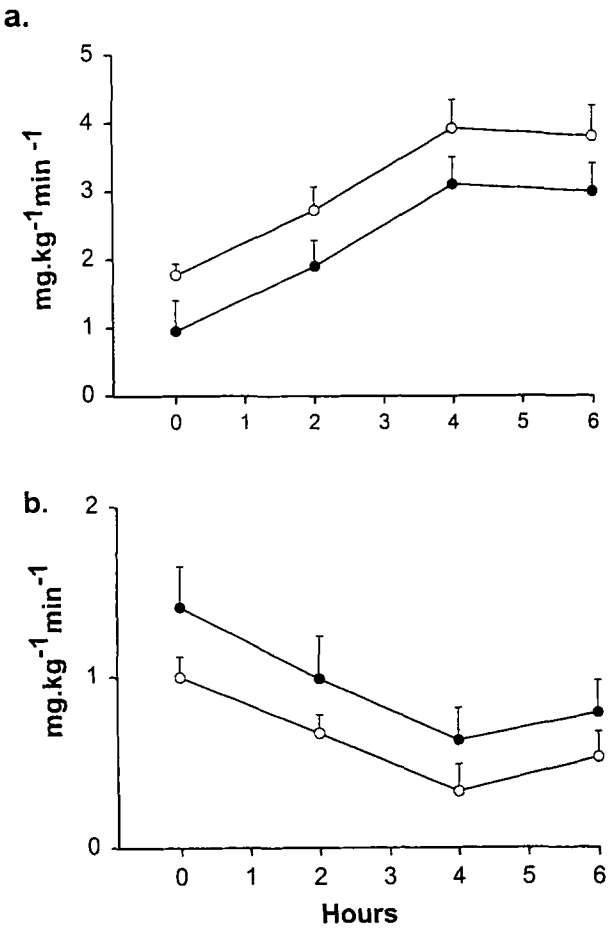

Fig 2. Carbohydrate oxidation (a) and lipid oxidation (b) before and during a 3-step hyperinsulinemic-euglycemic clamp (0.4, 1.0, and 10.0 mU · kg^{-1} · min^{-1}) before (●) and after (○) 1 month of NA administration in normal subjects. Results are the mean ± SEM.

These differences likely reflect the fatty acid preferences for enzymes involved in the synthesis of PC and PE, and are in close agreement with previous findings.[10] An analysis of the membrane fatty acid composition of PC and PE before and after NA is also shown in Table 3. Significant changes in the fatty acid composition of PC were observed after 1 month of NA administration, with increases in 16:0, 18:2, and the ratio of 16:0 to 18:0 and decreases in 18:0, 22:5 n-3, and total PUFAs. A significant decrease in the sum of 22:5 n-3 and 22:6 n-3 was also found after treatment with NA (1.7% ± 0.2% *v* 1.4% ± 0.1%, $P < .01$). The sum of n-6 fatty acids in PC also increased modestly but significantly following NA (43.0% ± 0.6% to 43.9% ± 0.6%, $P < .05$). As a result of the increase in n-6 fatty acids and the decrease in n-3 fatty acids, the unsaturation index for fatty acids in skeletal muscle membrane PC did not change after NA (121.6 ± 2.2 *v* 121.0 ± 2.1). No change in the ratio of 20:4 to 20:3 (Δ^5 desaturase) and 20:3 to 18:2 (Δ^6 desaturase) was detected. No significant changes in the fatty acid composition of PE were observed.

To determine the relationship between the changes in fatty acid composition and insulin sensitivity, we analyzed individual fatty acids, total PUFAs, and the ratios of fatty acids that reflect the activity of enzymes of fatty acid elongation and Δ^5 and Δ^6 desaturases. A strong inverse relationship was observed ($r = -.65$, $P < .01$) between ED_{50} values and PUFA content before and after NA. The relationship between the change in ED_{50} and the change in PUFA was also statistically significant ($P < .05$). No correlation between the change in ED_{50} and the change in the 16:18 ratio was found. No association between any of these values and ED_{50} was observed for the fatty acid composition of PE. No change in muscle fiber composition was observed during the 4 weeks of treatment (not shown).

Table 3. Fatty Acid Composition (relative percentage) in PC and PE From Vastus Lateralis in Nondiabetic Men and Women Before and After NA (mean ± SEM, N = 9)

	Control		NA	
Fatty Acid	PC	PE	PC	PE
16:0	21.03 ± 0.28	1.81 ± 0.16	21.75 ± 0.45*	2.02 ± 0.10
18:0	6.22 ± 0.52	17.84 ± 0.83	5.13 ± 0.42†	16.75 ± 0.41
18:1 n-9	11.83 ± 0.66	6.38 ± 0.92	12.05 ± 0.76	7.47 ± 0.72
18:2 n-6	35.78 ± 0.69	11.51 ± 1.19	36.93 ± 0.80*	11.84 ± 1.15
18:3 n-6	0.24 ± 0.02	<0.2	0.33 ± 0.04	<0.2
20:4 n-6	6.95 ± 0.73	27.65 ± 1.56	6.64 ± 0.67	26.23 ± 1.14
22:5 n-3	0.84 ± 0.08	<0.2	0.70 ± 0.08†	<0.2
22:6 n-3	0.87 ± 0.11	2.58 ± 0.35	0.66 ± 0.08	2.90 ± 0.35
16:18 ratio	3.62 ± 0.36	0.11 ± 0.01	4.56 ± 0.50*	0.12 ± 0.01
Σ C20-22	8.74 ± 0.81	30.34 ± 1.47	8.04 ± 0.78*	29.19 ± 1.15

NOTE. Fatty acids that are <0.2% of the total for both PC and PE are not shown.

*$P < .05$, †$P < .01$ *v* control.

As expected, NA administration led to significant improvements in fasting plasma lipid concentrations. Plasma triglycerides (101.4 ± 16.9 to 65.6 ± 10.0 mg/dL, $P < .01$), cholesterol (153.7 ± 12.5 to 137.6 ± 12.4 mg/dL, $P < .05$), and low-density lipoprotein (101.4 ± 17.0 to 71.1 ± 11.3 mg/dL, $P < .01$) decreased and high-density lipoprotein increased (40.3 ± 2.7 to 45.3 ± 3.8 mg/dL, $P < .05$). NA treatment was also associated with changes in serum PL fatty acids. The percentage of 16:0 (17.5% ± 0.8% to 19.7% ± 0.9%, $P < .01$), 22:6 n-3 (2.2% ± 0.4% to 2.5% ± 0.4%, $P < .01$), and total PUFAs (13.9% ± 0.8% to 15.4% ± 0.8%, $P < .05$) increased and 18:1 decreased (22.7% ± 1.3% to 19.2% ± 0.8%, $P < .05$). These latter findings suggest that the changes we observed in skeletal muscle membrane fatty acids are not simply the result of changes in serum PL fatty acids.

DISCUSSION

The present study was designed to determine if a change in insulin sensitivity is associated with a change in skeletal muscle PL fatty acid composition in humans. Potential models for the induction of insulin resistance in man include overfeeding,[31,32] short-term glucocorticoid administration,[33,34] or NA treatment.[35] To our knowledge, no data are available on the rate of turnover of skeletal muscle PL fatty acids in humans. However, based on a study of diet-induced changes in human cheek-cell PL fatty acids,[36] we estimated that 4 weeks of therapy would be required to demonstrate a significant change in skeletal muscle PL fatty acids. Significant weight gain and/or suppression of the hypothalamic-pituitary-adrenal axis induced by overfeeding or glucocorticoid therapy, respectively, for 4 weeks were believed to be unnecessary risks for our normal subjects. On the other hand, NA is well known to induce insulin resistance with minimal side effects when administered over 2 to 4 weeks in healthy subjects.[37] Using the half-maximal insulin concentration (ED_{50}) as a measure of insulin resistance, our studies have shown a significant increase in ED_{50} following NA administration for 1 month in normal subjects. The present studies have also demonstrated a significant change in the membrane fatty acid composition of PC in skeletal muscle biopsies obtained after 1 month of NA administration versus pretreatment (Table 3). In particular, we have shown a significant reduction in the total PUFA content of PC that is related to the change in ED_{50} in our normal subjects.

The mechanism(s) by which long-term NA administration induces insulin resistance is poorly understood. Changes in counterregulatory hormone secretion or insulin secretory capacity do not appear to explain the effect.[37] Recent studies in rats demonstrating an inverse relationship between tissue triglyceride content and insulin sensitivity[38] suggest that changes in tissue lipids might be involved. However, total tissue triglyceride concentrations are reduced in muscle biopsy samples from hyperlipidemic subjects following 4 to 8 weeks of NA therapy.[39] Alternatively, it has been suggested that a rebound increase in FFAs after NA-mediated suppression of lipolysis may increase FFA oxidation and decrease peripheral glucose disposal. Froberg et al.[35] demonstrated that the overnight profile of plasma FFAs was increased by approximately 30% following 10 days of NA administration. More modest effects of long-term NA therapy are generally observed on fasting plasma FFA concentrations.[37,40,41] Using indirect calorimetry to assess whole-body carbohydrate and lipid oxidation, the present studies provide indirect support for the hypothesis that FFA modulation is at least part of the explanation for NA-induced insulin resistance. It is generally accepted that increased concentrations of plasma FFAs are associated with decreased insulin-mediated glucose disposal. Decreases in both insulin-stimulated glucose oxidation via the glucose–fatty acid cycle[29] and nonoxidative glucose disposal (ie, glycogen storage)[30] have been shown when plasma FFAs are increased. Our study design, in which the insulin clamp studies were performed 2 hours after the last dose of NA (6:00 AM), was deliberately chosen to exclude the potential contribution of increased FFA oxidation to insulin sensitivity. The serum concentrations of FFAs and glycerol and indirect calorimetry data indicating increased carbohydrate oxidation and reduced lipid oxidation after NA suggest that the design was successful in this regard. Thus, we believe that the increase in ED_{50} observed in the present study is independent of the glucose-FFA cycle. Our data suggest that this independent effect of NA may be mediated by alterations in the PL fatty acid composition of PC. On the other hand, it is likely that studies demonstrating a greater decrement in insulin sensitivity following long-term NA treatment are influenced by increased FFA oxidation, as well as changes in PL fatty acid composition.

The mean distribution of fatty acids in PC and PE from vastus lateralis samples in our normal subjects is shown in Table 3. Our results are similar to previously reported values in human pectoral muscle samples[10] and demonstrate marked heterogeneity between the 2 PLs. In particular, increased incorporation of 16:0, 18:1, and 18:2 is found in PC, whereas a greater percentage of 18:0, 20:4, and 22:6 is found in PE. The increased proportion of 16:0 and 18:0 in PC and PE, respectively, reflects fatty acid preferences in the *sn1* position, with the other fatty acids occupying the *sn2* position, and is consistent with previous reports. Other PLs that contribute to overall PL fatty acid composition include phosphatidylinositol, phosphatidylserine, and cardiolipin. Together, these PLs comprise about 13% of the total in human skeletal muscle membrane.[10] Because of the limited tissue samples obtained and the relatively small contribution of each to the total, we have not determined the fatty acid composition of these PLs.

In agreement with the findings of Borkman et al,[26] who related total PL fatty acids to insulin sensitivity, we have recently reported a strong positive relationship between the proportion of skeletal muscle membrane polyunsaturated fatty acids in PC and insulin sensitivity.[12] In contrast, the content of polyunsaturated long-chain fatty acids in PE, although 4-fold greater than PC, was not related to insulin sensitivity. We have also previously reported that insulin sensitivity is inversely related to the ratio of 16:0 to 18:0 (a surrogate marker for fatty acid elongase activity) in membrane PC from both rat[11] and human[12] skeletal muscle. These findings are consistent with those of Vessby et al,[42] who have shown an increase in 16:0 in skeletal muscle samples from older more insulin-resistant individuals. This same group recently described a reduction in the content of 16:0 and an increase in 18:0 in skeletal muscle PL after 6 weeks of exercise in association with a 29% increase in insulin sensitivity.[43] Although the methods chosen to alter insulin sensitivity are different, it is interesting to note that the present studies have demonstrated a significant increase in the 16:18 ratio after a 25% reduction in insulin sensitivity. However, our studies differ in the response of membrane PUFAs to

changes in insulin sensitivity. Whereas a program of exercise sufficient to improve insulin sensitivity had no effect on total PL PUFA,[43] treatment with NA significantly reduced the PUFA content of PC in the present studies. When the PUFA content of PE was combined with that of PC (to approximate the PUFA content of total PL), the significant reduction observed in PC following NA was obscured (39.1 ± 2.1 *v* 37.2 ± 1.9, NS). Coupled with our previous finding that insulin sensitivity is related to the PUFA content in PC but not in PE,[12] the present observations strongly suggest that the fatty acid composition of the outer portion of the lipid bilayer conveys much of the insulin sensitivity related to PL fatty acids.

There are several potential mechanisms by which changes in the skeletal muscle membrane fatty acid composition of PC might alter insulin sensitivity. Greater insulin sensitivity in membranes with an increased content of PUFAs[44,45] may be related to increased membrane fluidity and/or GLUT4 translocation following insulin stimulation. Increased membrane unsaturation has been shown to increase membrane fluidity, insulin receptor number and binding,[46] and insulin receptor kinase activation.[47] In addition, an increase in membrane unsaturation is associated with an increase in insulin-stimulated glucose transport in rat adipocytes.[48] Whether an increase in membrane unsaturation is also associated with an increase in GLUT4 translocation is not known.[49] However, diets high in n-3 fatty acids increase skeletal muscle membrane PUFAs and skeletal muscle GLUT4 gene expression in rats.[50] In the present studies, no change in the unsaturation index in either PC or PE was observed. It is also possible that changes in PL fatty acids may affect insulin sensitivity through alterations in insulin signaling. Insulin-stimulated hydrolysis of PC via phospholipase D has recently been shown to activate protein kinase C (PKC), leading to PKC translocation to the plasma membrane and increased glucose transport.[51] Increased unsaturation of membrane PLs is known to modulate PKC activity,[52] suggesting that the reductions in insulin sensitivity observed in the present study may be related to impaired insulin-mediated activation of PKC. Clearly, further investigation in this area is warranted.

The present studies were not designed to determine the mechanism by which NA might alter membrane fatty acid composition. However, activation of the peroxisome proliferator–activated receptor (PPAR) by NA derivatives has been demonstrated,[53] and agents known to activate PPAR have been shown to reduce fatty acid elongation.[54] Thus, it is possible that the increase in the 16:18 ratio following NA may be mediated by the activation of PPAR. Alterations in the activity of desaturase enzymes also may have contributed to our findings. Unfortunately, we were unable to detect a change in the ratio of 20:4 to 20:3 (Δ^5 desaturase) or 20:3 to 18:2 (Δ^6 desaturase) in the present study. However, it should be noted that the content of 20:3 in PC is consistently less than 0.2%, and thus decreases in desaturase activity may have occurred which are below the level of detection with the methods used in our study. An alternative mechanism for NA-induced alterations in PL fatty acids is via cyclic changes in FFA availability and oxidation. The increase in 16:0, 18:1, and 18:2 in PC following NA administration is reminiscent of the preferred fatty acids for PC synthesis already outlined. As noted earlier, the fatty acid composition of plasma PLs does not explain the changes in muscle membrane PL fatty acids observed. On the other hand, decreased FFA oxidation, mediated by increased malonyl coenzyme A, might be expected to increase the incorporation of the same fatty acids that would otherwise have been preferred substrates for oxidation.[55]

In conclusion, the present study demonstrates for the first time a significant change in the fatty acid composition of PC in muscle membranes that is significantly correlated with a change in insulin sensitivity following NA administration in nondiabetic subjects. Our data also suggest that both an alteration in FFA availability for lipid oxidation and a change in the PL fatty acid composition are involved in the induction of insulin resistance by NA. Whether the changes in PL fatty acids are the cause or the result of changes in insulin sensitivity is not yet clear.

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