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Postprandial changes in plasma acylcarnitine concentrations as markers of fatty acid flux in overweight and obesity

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ABSTRACT

This study determined whether reductions in postprandial plasma nonesterified fatty acid (FFA) flux would lead to reductions in plasma acylcarnitine (AC) concentrations. Plasma AC was measured by liquid chromatography with tandem mass spectrometry in the fasting state and over 6 hours after a high-fat (50% energy) meal was fed to 16 overweight and obese subjects with a wide range of insulin sensitivities. Body composition was measured by dual-energy x-ray absorptiometry; insulin sensitivity by insulin-modified, frequently sampled intravenous glucose tolerance test; substrate oxidation by indirect calorimetry; blood metabolite and hormone concentrations biochemically; and fatty acid flux by using stable isotope tracers. Lean body mass and fasting fat oxidation correlated positively ($r > 0.522$, $P < .05$), whereas glucose oxidation correlated negatively ($r < –0.551$, $P < .04$), with fasting AC. Postprandially, plasma glucose, insulin, and triglyceride concentrations increased; and FFA concentrations decreased significantly. The responses of plasma AC species depended on chain length and saturation, with C14:0, C16:0, and C18:0 remaining unchanged, and unsaturated species (eg, C14:1, C14:2) falling significantly (21%-46%, $P < .03$). Postmeal nadir AC concentrations were positively associated with lean body mass, postprandial fatty acid flux, and FFA concentrations ($r > 0.515$, $P < .05$). By contrast, nadir AC correlated negatively with insulin sensitivity and spillover of meal-derived fatty acids ($r < –0.528$, $P < .04$). Conditions that impact fatty acid flux contribute to the control of postprandial plasma AC concentrations. These data underscore the need for a better understanding of postprandial fatty acid oxidation and dietary fat delivery in the setting of adipose insulin resistance to determine how postprandial lipemia contributes to chronic disease risk.

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[1] Collected insulin sensitivity data, analyzed all results, performed statistical analysis, and wrote the manuscript.
[2] Assisted with study design, performed sample analysis, and contributed essential interpretation of the data.
[3] Contributed to the conduct of the study, and collected and analyzed dietary data.
[4] Designed the study, analyzed the data, and contributed to manuscript writing.

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1. Introduction

Acylcarnitines (ACs) are carnitine esters of fatty acids that have entered the mitochondria [1], and their quantitation has been used for more than 20 years to diagnose inborn errors of fatty acid metabolism [2]. Recently, plasma ACs have been proposed as biomarkers of insulin resistance and metabolic inflexibility in adults [3-4]. Although the plasma nonesterified fatty acid (FFA) pool is the original precursor of intracellular tissue fatty acids, Kanaley et al [5] determined that the intramyocellular triglyceride (TG) pool is the immediate fatty acid precursor of intramyocellular long-chain AC (LCAC) in fasted humans. With respect to the role of dietary fatty acids, one report by Kien et al [6] demonstrated that a dietary change as brief as 7 days can result in similarities between the dietary fatty acid composition and muscle TG and AC species, again assessed in the fasting state. Thus, both endogenous and dietary fatty acids have the potential to impact the composition of AC species, varying in chain length and saturation.

Fasting plasma AC concentrations have been shown to be elevated in obese individuals with either impaired glucose tolerance or diabetes [3,7], and these findings support incomplete fat oxidation in these subjects. However, AC concentrations increase in the plasma of lean, insulin-sensitive subjects during long-term fasting [8] and relatively healthy overweight subjects during caloric restriction [9]. These observations suggest that the AC increase in obesity may not only be due to an impairment of metabolism but also be a natural response to an excess supply of lipid. Thus, increased production of AC could result from excess fatty acid flux emanating from lipid stored either intracellularly or peripherally. Indeed, plasma AC concentrations are reduced during an oral glucose tolerance test and during a euglycemic-hyperinsulinemic clamp, both procedures that reduce plasma FFA concentrations significantly [4,10]. Yet, under these conditions, obese and diabetic subjects maintained higher AC concentrations than lean controls [4].

Metabolic dysfunction can be uncovered by the challenge of food consumption [11,12]. Given that elevations in postprandial lipemia pose an independent risk for the development of chronic disease [13-15], it is important to understand how the metabolic transitions from fasting to feeding can be reflected in changes in plasma AC. The present study was designed to determine whether AC concentrations would fall when subjects were fed a high-fat, mixed meal, which produced a physiologic pattern of postprandial elevations in glucose and insulin and reductions in FFA, concurrent with significant increases in plasma TG-rich lipoproteins. We sought to (1) compare the dynamic responses of different AC species and (2) identify subject characteristics associated with changes in postmeal concentrations of plasma AC. Our goal was to test the hypothesis that a fall in plasma AC after a standardized mixed meal would be positively related to measures of insulin sensitivity. We hypothesized that the concentrations of all plasma AC would fall after consumption of a meal that induces a significant reduction in adipose fatty acid release and plasma FFA concentration.

2. Methods

2.1. Human subjects

Sixteen nondiabetic, nonsmoking, sedentary, and overweight or obese subjects were recruited from health fairs and physician referral and gave their written informed consent. This study was a subproject of a larger postprandial study in which our goal was to recruit Hispanic (H) or African American (AA) men or women (aged 20-70 years) with a wide range of insulin sensitivities, stable body weight, and maintenance of pre-enrollment physical activity. The study was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center (IRB no. 062007-025) and was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Study time line

Subjects were studied over a 3-week period that included 2 inpatient admissions to the Clinical Translational Research Center (CTRC). The first admission included an insulin-modified, frequently sampled intravenous glucose tolerance test (FSIVGTT) performed after a 12-hour overnight fast [16,17] and body composition measurements by dual-energy x-ray absorptiometry (Hologic Discovery W, QDR series, Bedford, MA). Three days before this admission, the subject was placed on a weight-maintaining outpatient diet formulated to resemble his or her food intake pattern as assessed by a food frequency questionnaire, and composed of foods that he or she typically ate, as assessed by a 3-day dietary recall [18]. The ad libitum intake of the subjects (mean ± SD) was as follows: 2148 ± 478 kcal/d total energy; 33.5% ± 5.4% energy from fat (of this, 11.2% ± 9.7% from saturated fatty acids, 12.6% ± 10.2% from monounsaturated fatty acids, and 6.8% ± 1.4% from polyunsaturated fatty acids, with the remaining ~2.9% *trans* and omega-3 fats); 48.0% ± 5.9% energy from carbohydrate; 18.5% ± 3.9% energy from protein; 128.5 ± 64.5 g/d total sugars; and cholesterol intake, 331 ± 114 mg/d. The outpatient diet was prepared by staff in the research kitchen at the University of Texas Southwestern CTRC and delivered to the subject for consumption at home. For 10 days total (3 days before admission 1 and 7 days before admission 2), the subject consumed the prepared diet. Alcohol consumption was prohibited from 3 days before admission 1 and throughout the period through admission 2. The average daily intake of caffeine at home was 76 mg, and subjects abstained from caffeine use during the 30 hours of admission 2. The FSIVGTT values of glucose were analyzed immediately with a bedside analyzer from Yellow Springs Instruments (Yellow Springs, OH), and insulin was analyzed within 4 days by enzyme-linked immunosorbent assay (Millipore, #EZHI-14L, Billerica, MA). As shown in Fig. 1, on day 1 of admission 2, at 6:00 PM, the subject consumed a standardized evening meal. The subject fasted for 18 hours and consumed only water or noncaloric, non–caffeine-containing beverages between the evening meal and lunch the next day. The reason for this extended fast was to bring the subject to a moderate rate of lipolysis, as we have observed

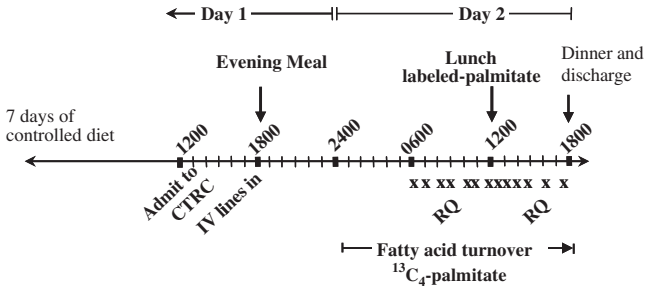


Fig. 1 – Study timeline. During the 7 days before the study, the subject consumed a standardized diet. The X on the figure denotes the timing of blood draws, and RQ denotes the timing of indirect calorimetry.

previously that lipolysis remains relatively low after a 12-hour fast compared with an 18-hour fast (EJP observations between 12 and 18 hours during a 24-hour fast [19]). At midnight, an intravenous (IV) infusion began containing potassium [1,2,3,4-$^{13}C_4$]-palmitate, complexed to albumin, to calculate the rate of appearance of plasma FFA (RaFFA). Indirect calorimetry was performed using a metabolic cart (VMax Encore, Viasys Healthcare, Palm Springs, CA) in the hooded mode, and data were obtained for 30 minutes while subjects were fasting (day 2, 8:00 AM to 8:30 AM) and again 2.5 hours (2:30 PM) after the initiation of the meal (12:00 PM). From 6:00 AM to 6:00 PM on day 2, the subject rested, read, or watched television. Blood was drawn intermittently from an IV line between 6:00 AM and 5:50 PM. Isotopes were purchased from Isotec (Miamisburg, OH), were sterile and pyrogen free, and were prepared using sterile techniques.

2.3. *Meal composition and analysis of blood metabolites*

The noon meal on day 2 consisted of a cocoa-flavored drink (cocoa, corn oil, heavy cream, sucrose, and skim milk), cereal, banana, and skim milk. This meal was formulated to provide 38% of total daily energy needs (Table 1). To prepare the drink, 1 g of a uniformly labeled stable isotope tracer (either d_{31} or $^{13}C_{16}$ palmitate potassium salt) was warmed to homogenize it into the liquid, based on the methodology of Beysen et al [20]. This label was sufficient to quantify the spillover of dietary fatty acids into the plasma FFA pool, and the tracer $^{13}C_4$ IV palmitate delivered at a rate of 7 μg/kg/min was sufficient to measure fatty acid turnover; but both doses were so low so as to not be detectable in the plasma AC pool (see below). The fatty acid compositions of the shake-TG, of plasma-TG, and FFA were analyzed by gas chromatography (GC); and the labeling pattern was analyzed by GC/mass spectrometry (MS) as previously described [21]. All the fat in the meal was found in the liquid drink; and the balance of energy in the meal was made up by the cereal, banana, and milk. The subjects were given 15 minutes to consume the meal. All blood samples were drawn into iced tubes containing EDTA. Plasma was separated immediately by centrifugation (3000 rpm, 1500*g*, 10 minutes) at 4°C, and the samples were kept on ice while preservatives and antioxidants were added. Triglyceride-rich lipoproteins (TRLs) were isolated as described previously [22]. For plasma metabolite concentrations, enzymatic kits were used for plasma-TG in TRL (WAKO, #461-09092, #461-08992, Richmond, VA), glucose (WAKO, #439-90901), and FFA (WAKO, #999-34691, #991-34891, #993-35191, #995-34791). Insulin was measured by enzyme-linked immunosorbent assay (Millipore, #EZHI-14L). Results were read on a Power wave XS microtiter plate reader (Biotek, Winooski, VT).

2.4. *Analysis of plasma ACs by electrospray ionization targeted tandem MS*

To determine whether the tracer isotopes given would appear in the AC pool, more sensitive multiple-reaction monitoring was carried out. This analysis did not identify, nor did altering the types of added internal standards uncover, labeled palmitate or its products (L Sweetman, E Parks, unpublished results). Thus, the analysis of AC in 15 μL of plasma was performed by flow injection analysis electrospray ionization targeted tandem MS as previously described [23]. The internal standards were deuterium-labeled free carnitine, C3, C5, and even-number AC from C2 to C18 in 200 μL of methanol added to the plasma samples. After removal of protein by centrifugation and drying, butyl esters were formed by adding 100 μL of 3 N HCl in butanol and heating at 55°C for 15 minutes, then dried and redissolved in 150 μL 80% acetonitrile:20% water for flow injection analysis tandem MS. The mass spectra of the AC precursors of the common product ion at

Table 1 – The composition of the test meal

	Mean ± SD
Test meal composition [a]	
Energy (kcal)	790.4 ± 118.7
Fat (g)	44.4 ± 9.5
Protein (g)	15.8 ± 8.2
Carbohydrate (g)	90.1 ± 22.7
Total sugars (g)	63.4 ± 12.5
Fiber (g)	4.22 ± 2.11
Cholesterol (mg)	83.3 ± 11.9
Meal-TG fatty acid composition [b]	
Saturated fatty acids (wt%)	
12:0	1.32 ± 0.03
14:0	4.66 ± 1.02
16:0	22.64 ± 2.00
18:0	7.55 ± 1.07
Monounsaturated fatty acids (wt%)	
16:1, n-7	0.78 ± 0.21
18:1, n-7	0.12 ± 0.13
18:1, n-9	27.13 ± 0.62
20:1, n-9	0.87 ± 0.05
Polyunsaturated fatty acids (wt%)	
18:2, n-6	31.89 ± 4.39
18:3, n-3	0.03 ± 0.06

[a] The macronutrient composition of the meal (n = 16) was analyzed using The University of Minnesota Nutrition Data System for Research (2009). All the fat in the meal was carried in the liquid drink.
[b] The fatty acid compositions of the TG from the drinks were analyzed individually by GC.

m/z 85 for all ACs were obtained, and concentrations of ACs were calculated from ratios of intensities to the internal standard closest in *m*/z. Total LCAC species were defined as all saturated, monounsaturated, and polyunsaturated species of 12 to 18 carbon chain length. The AC lower limits of quantitation are 10 to 20 nmol/L [2]. It should be pointed out that a few of the postprandial AC concentrations approached this lower level. The accuracy of these low concentrations is supported by the presence of these data within a postmeal fall in concentrations characteristic of classic biological production and decay kinetics.

2.5. *Calculations and statistical analysis*

Energy expenditure and substrate oxidation were calculated using the equations of Jequier et al [24]. Fasting protein oxidation was calculated from the known grams of protein present in the foods consumed during the previous 24 hours, whereas fed-state protein oxidation was calculated from total urea nitrogen measured in all urine collected from 12:00 PM to 6:00 PM on day 2. This calculation of protein oxidation is based on the assumption that meal protein disposal occurred continuously for 6 hours after the meal [25]. The presence of the dietary fatty acid label in the plasma FFA pool is evidence of meal-derived fatty acids [26]. The fatty acid infusate composition and enrichments were analyzed by GC and GC/MS, and the calculations of the RaFFA as described previously [21,27] using Steele's [28] equation for non–steady-state kinetics. The nadir RaFFA and nadir AC values were determined based on the lowest value achieved during the 6 hours after the meal. Both fatty acid species and AC species are presented and discussed herein. To distinguish the 2 in the text, AC notation will be preceded by a capital letter C (eg, C18:1), whereas fatty acids will not (eg, 18:1). Calculations were performed using Excel (version 2007; Microsoft, Seattle, WA), and statistical analyses were performed using Statview for Windows (version 5.0.1; SAS Institute, Berkeley, CA). A P value of $< .05$ was considered statistically significant. Data for insulin sensitivity (S_I) were transformed using natural logarithm because of their nonnormal distribution.

3. Results

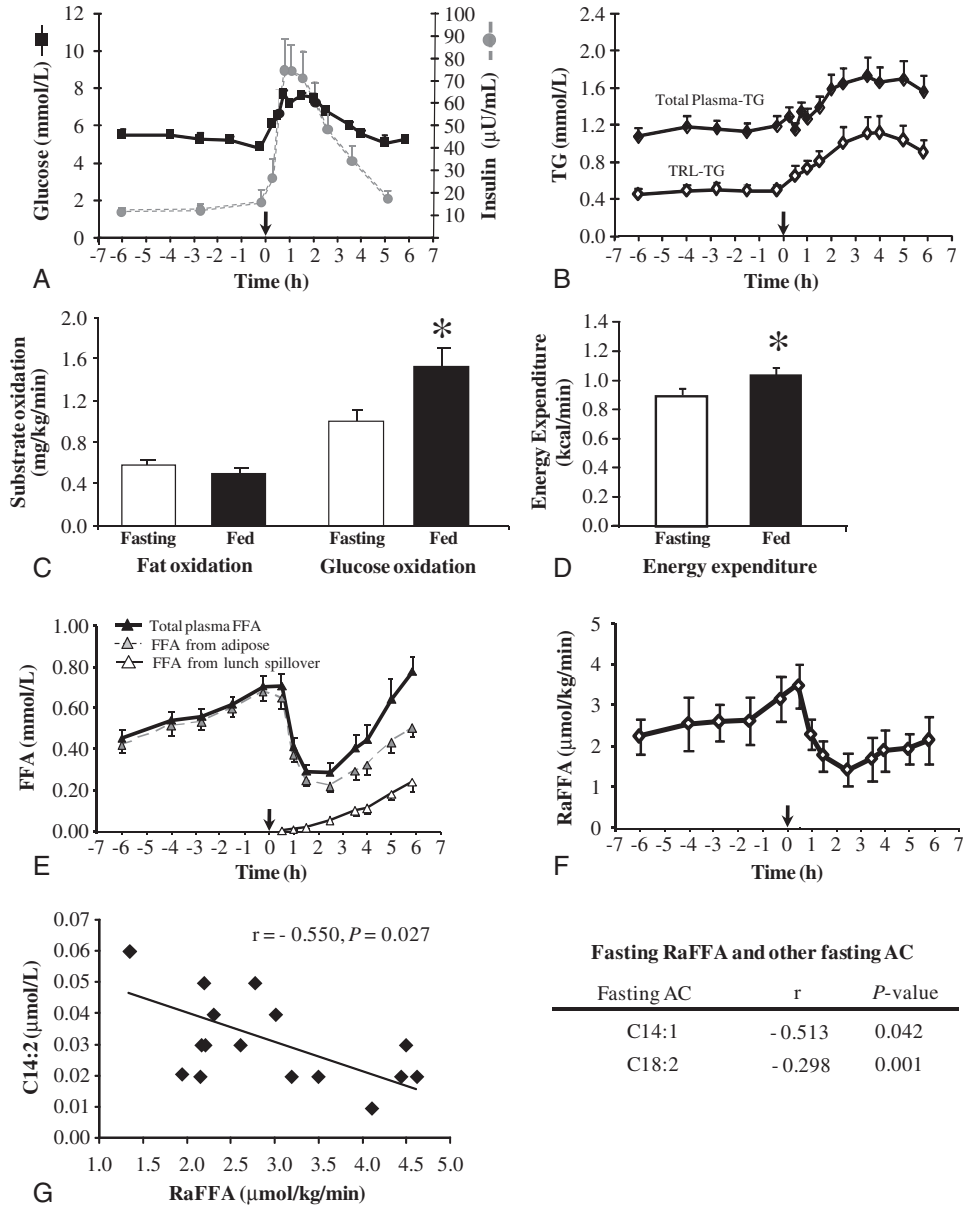
The sex/ethnic distribution was 12 women (7 H and 5 AA) and 4 men (3 H and 1 AA). As part of the project, each subject was screened on one occasion and then was admitted to the CTRC to study fatty acid metabolism (Fig. 1). Clinical and laboratory values obtained during screening procedures revealed that the subjects were overweight to obese (body mass index 35.4 ± 7.4 kg/m^2, mean ± SD); were aged 45.8 ± 8.5 years; and had total body weight of 92.9 ± 22.7 kg, fat mass of 38.6 ± 16.1 kg, lean body mass (LBM) of 51.1 ± 9.8 kg, and waist to hip ratio of 0.92 ± 0.07. Results from the FSIVGTT demonstrated variability in insulin sensitivity, with mean S_I = 2.61 ± 1.47 10^{-4} min^{-1}·μU^{-1} mL^{-1} (range, 0.89-6.25) and a disposition index of 1318 ± 699 (unitless) (range, 175-2510).

3.1. *Concentrations of metabolites and hormones in the fasting and fed states*

The subjects consumed a standardized lunch that was, on average, 50% of energy from fat, 43% from carbohydrate, and 7% from protein (Table 1). Of the major fatty acids in meal-TG, saturated fatty acids made up 36.2 wt% of the total TG-fatty acids, monounsaturates 28.9%, and polyunsaturates 31.9%. The subjects tolerated the meal well, aside from an occasional comment of feeling full. Presented in Fig. 2 are the changes in metabolic parameters during the study. Glucose concentration (Fig. 2A) assessed just before the meal was 5.5 ± 0.1 mmol/L and rose to 7.7 ± 0.4 mmol/L at peak, reflecting the moderate carbohydrate content of the meal (Table 1). Insulin concentration prelunch was 15 ± 6 μU/mL, rose 5-fold postlunch to 74 ± 14 μU/mL, and peaked at 0.75 h (Fig. 2A). The concentration of TRL-TG represented 42.8% ± 20.0% and 63.7% ± 23.2% of the total plasma-TG concentration in the fasting and fed states, respectively (Fig. 2B). Whole-body fat oxidation did not change after the meal, but glucose oxidation rose significantly (77.3% ± 19.9%, P = .005, Fig. 2C), as did energy expenditure (10.5% ± 2.6%, P = .001, Fig. 2D). Fasting FFA concentration (0.46 ± 0.05 mmol/L at 6:00 AM) rose significantly to 0.71 ± 0.05 mmol/L just before lunch (P = .0001). The prelunch FFA concentration was suppressed by 60% at 2 hours to 0.28 ± 0.03 ($P < .001$, Fig. 2E). The RaFFA in the fasting state was 2.91 ± 0.25 μmol/(kg min) just before lunch and fell 59 ± 3.8% to a nadir of 1.15 ± 0.13 μmol/kg/min ($P <$ .0001, Fig. 2F). The fasting RaFFA was negatively associated with the fasting concentration of C14:2 (Fig. 2G) and 2 other fasting ACs (C14:1, C18:2), suggesting either that those subjects with the greatest fatty acid turnover had low levels of fatty acids entering β-oxidation or that those fatty acids that entered were completely oxidized to CO_2. It is likely that the former was true because fasting fatty acid turnover (RaFFA) was not related to fasting whole-body fat oxidation (data not shown). Koutsari and Jensen [29] have shown that 20% of fatty acid flux in the fasting state goes to nonoxidative disposal.

3.2. *Relationships between fasting AC concentrations and subject characteristics*

Because previous studies have observed significant relationships between fasting AC and measures of insulin resistance, we tested the effects of insulin sensitivity and other subject characteristics on fasting AC concentrations in the present population. Surprisingly, fasting glucose concentration, homeostasis model assessment of insulin resistance, insulin, LnS_I, and hemoglobin A_{1c} were not related to the fasting C2 concentrations (Fig. 3A), potentially because of the smaller sample size in the present study compared with previous investigations [4]. However, both LBM (Fig. 3B) and fasting fat oxidation (Fig. 3C) were strongly and positively associated with multiple AC species. Specifically, the association between LBM and fasting plasma AC was significant for several *short-chain* AC (C3, C3-DC, C4, C5, C5:1) and C10:1, whereas fasting fat oxidation was related to the concentration of *long-chain* saturated and unsaturated



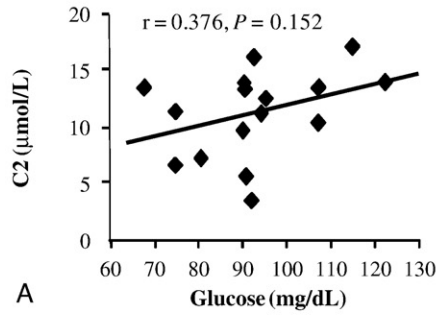
Fasting RaFFA and other fasting AC

Fasting AC	r	*P*-value
C14:1	- 0.513	0.042
C18:2	- 0.298	0.001

Fig. 2 – Metabolite and insulin concentrations, substrate oxidation, energy expenditure, and RaFFA in the fasted and fed states. N = 16; data are mean ± SEM. Subjects had remained fasted from the time of consumption of the previous evening meal at 6:00 PM on day 1. Samples were collected from –6 hours (6:00 AM), and the noon meal is denoted by the arrow at time 0. For panels C and D, fasted values represent data from –4 hours and fed data from +2.5 hours. Fasting and fed RaFFAs (F, units in micromoles of total fatty acids per kilogram of total body weight per minute) were determined using Steele's equation ("Methods"). For panel G, presented on the left is a graph of the relationship between fasting C14:2 and the fasting RaFFA (taken at –2 hours). The table on the right presents the other fasting AC data that also correlated with fasting RaFFA. *P ≤ .05.

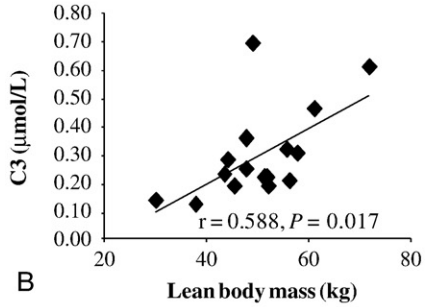
species (C12:1, C14, C14:1, C14:2, C16:1). By contrast, whole-body glucose oxidation was negatively associated with C2 concentration and several LCACs (Fig. 3D). In other words, those subjects with the lowest fasting AC concentrations were those who had the lowest fat oxidation and the highest glucose oxidation in the fasting state. Fat mass was not significantly associated with any fasting AC concentrations (data not shown).

Although studies have tested the impact of glucose utilization on AC concentrations, postprandial changes in plasma AC concentrations in adults have not been investigated in the physiological setting of eating, that is, subjects consuming a mixed meal containing fat and carbohydrate. We hypothesized that if the plasma FFA pool was the original precursor for AC produced in tissues, then when plasma FFA fell postprandially, plasma AC would fall in concert.



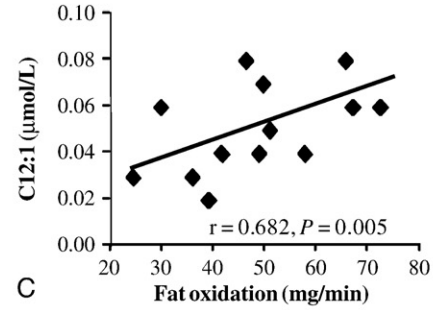
Non-significant correlations between indices of glucose metabolism and C2

Variables	r	*P*-value
HOMA-IR	0.365	0.165
Fasting insulin	0.300	0.258
LnS_I	- 0.236	0.378
HbA1c	0.293	0.271



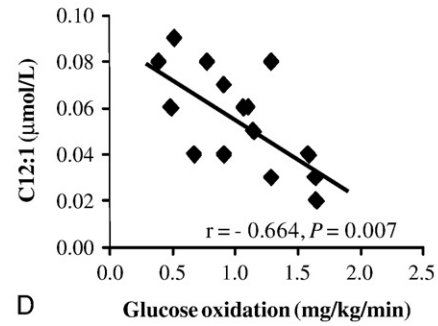
Lean body mass and other fasting AC

Fasting AC	r	*P*-value
C3 DC	0.522	0.038
C4	0.712	0.002
C5	0.522	0.038
C5:1	0.668	0.005
C10:1	0.684	0.004



Fasting fat oxidation and other fasting AC

Fasting AC	r	*P*-value
C14	0.645	0.009
C14:1	0.693	0.004
C14:2	0.543	0.036
C16:1	0.585	0.022
LCAC	0.523	0.046



Fasting glucose oxidation and other fasting AC

Fasting AC	r	*P*-value
C2	- 0.584	0.022
C12	- 0.551	0.033
C14	- 0.605	0.017
C14:1	- 0.604	0.017

Fig. 3 – Correlations between selected variables and fasting AC species. Panel A depicts the lack of association between variables relevant to carbohydrate metabolism (fasting glucose, homeostasis model assessment of insulin resistance, fasting insulin, S_I transformed as the natural logarithm, and hemoglobin A_{1c}) and C2. For panels B to D, presented on the left is a representative graph of the relationship between independent variables (LBM, fat oxidation, and glucose oxidation) and AC species. The table on the right presents the other AC data that also correlated with each independent variable of interest.

Comparison of the fasting and nadir concentrations (Fig. 4) revealed that, in the postprandial state, C2, C3-DC, and C4-DC were reduced 20% to 30%, whereas C3 and C4 tended to increase 15% postmeal ($P = .07$). Only minor changes were observed in the concentrations of the other short- and medium-chain ACs (Fig. 4B). The saturates C14, C16, and C18 did not change postprandially (Fig. 4C); but in each case, the unsaturates of these ACs decreased significantly after the meal (↓21%-46%, $P < .03$, Fig. 4C). The postprandial changes in concentrations of all the individual ACs can be found in supplemental figures 1 and 2. Although all plasma FFA, saturated, and unsaturated species were reduced after the meal (Fig. 2E and suppl. figs. 1 and 2), the responses of the plasma AC depended on chain length and saturation.

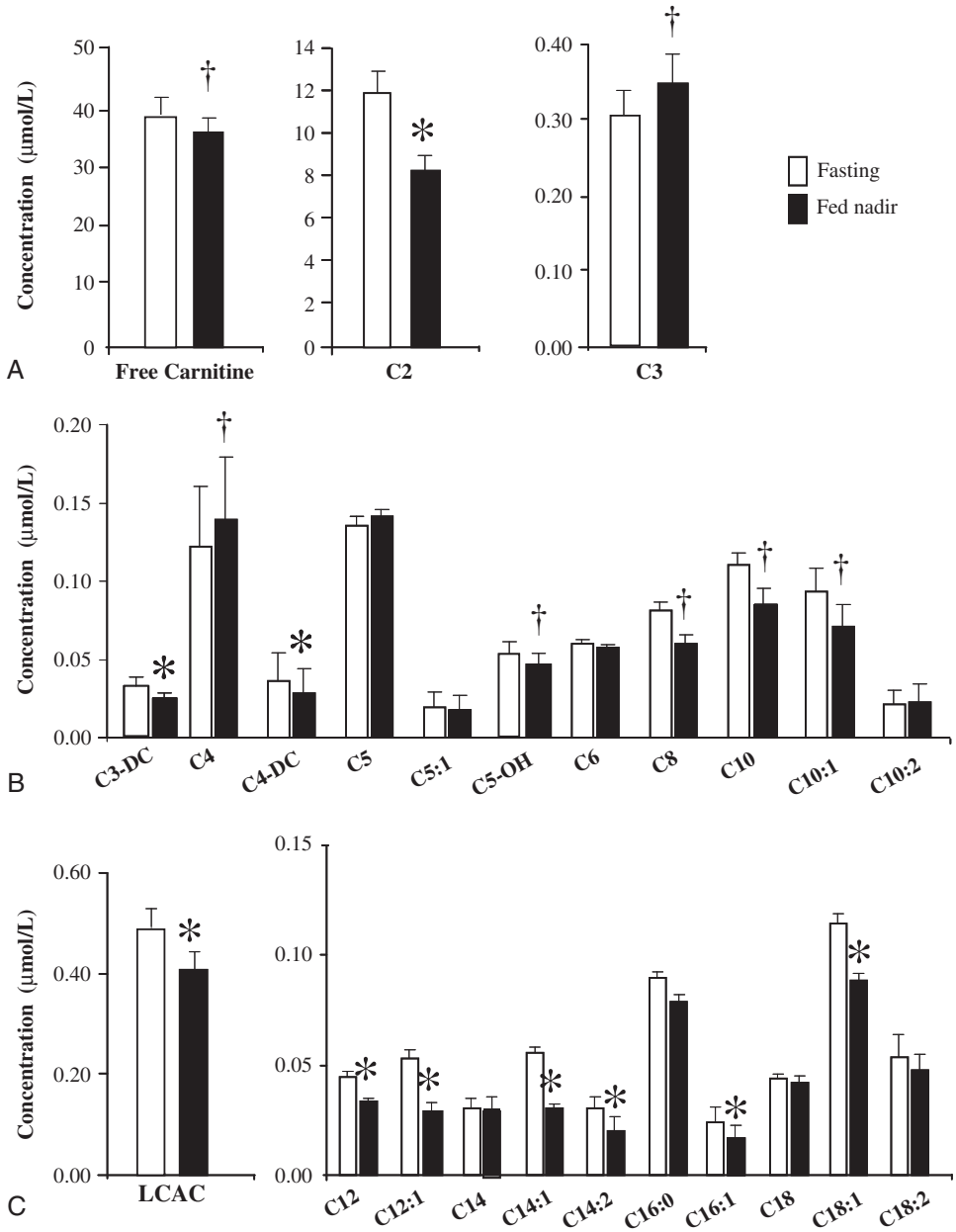
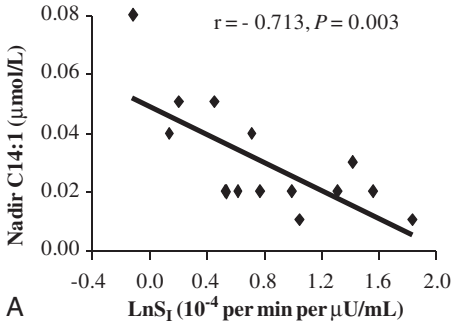


Fig. 4 – Fasting and postmeal nadir concentrations of plasma AC species. Values are mean ± SEM. Fasting data (open bars); fed nadir concentration (solid bars). *P < .05; †P < .09.

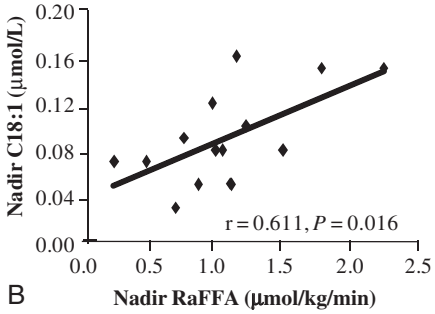
3.3. *Relationships between postprandial AC concentrations and fed state variables*

Because different subjects suppressed their AC concentrations differently after the meal, we sought to determine whether any changes in AC could be predicted by subject characteristics. As shown in Fig. 5A, greater peripheral insulin sensitivity was associated with lower LCAC species in the fed state ($P < .02$). Thus, those subjects with higher insulin-mediated glucose disposal rates exhibited lower nadir concentrations of 3 LCACs (C12:1, C14, and C14:1). Similar to the fasting state, LBM remained positively associated with short-chain AC species (C3, C4, C5, C6; $r = 0.704$ to 0.840; $P < .05$ for all; data not shown), whereas fed-state glucose oxidation again negatively correlated with nadir AC (C12:1, C14:1, C14:2, C16; $r = -0.516$ to -0.644; $P < .05$ for all; data not shown). In the fed state, nadir FFA concentration was significantly and positively associated with nadir RaFFA ($r = 0.612$, $P = .012$, data not shown). Comparing both of these variables with nadir plasma AC revealed significant positive associations (Fig. 5B and 5C). These data show that a lack of suppression of adipose fatty acid release postprandially leads to elevated AC concentrations. Lastly, dietary fatty acids can



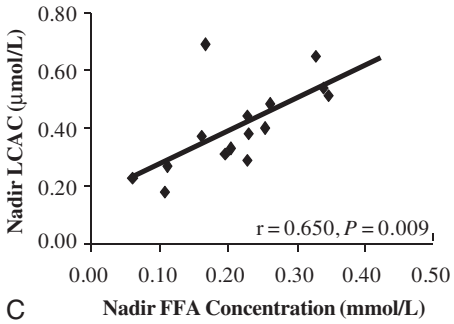
LnS_I and other nadir AC

Nadir AC	r	*P*-value
C14	- 0.728	0.002
C12:1	- 0.601	0.018



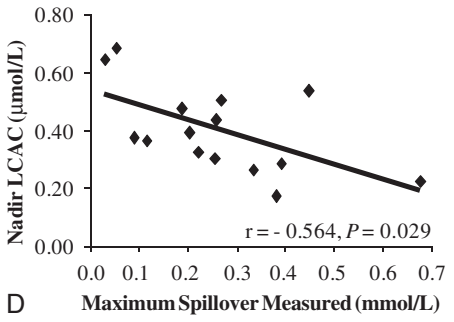
Nadir RaFFA and other nadir AC

Nadir AC	r	*P*-value
C16	0.580	0.024
LCAC	0.441	0.050



Nadir FFA concentration and nadir AC

Nadir AC	r	*P*-value
C14	0.515	0.050
C14:2	0.515	0.049
C16	0.686	0.005
C16:1	0.644	0.010
C18	0.535	0.040
C18:1	0.656	0.008



Maximum spillover measures and nadir AC

Nadir AC	r	*P*-value
C10:1	- 0.564	0.029
C12:1	- 0.528	0.043
C18	- 0.794	0.001

Fig. 5 – Correlations between selected variables and postmeal nadir AC species. The left side of each figure has a representative graph of the relationship between independent variables (LnS_I, nadir RaFFA, nadir FFA concentration, or maximum spillover measured) and nadir AC. The table at the right presents other AC species that also correlated with the variable of interest.

enter the plasma FFA pool through a process known as *spillover* [30], which is thought to occur mainly at adipose [31]. In the present study, the greater the quantity of dietary-derived fatty acids in the FFA pool, the lower the LCAC concentration after the meal (Fig. 5D). These data support the concept that the spillover of chylomicron fatty acids is associated with a lower entry of fatty acids into the mitochondria.

4. Discussion

Acylcarnitines are carnitine esters derived from fatty acids or amino acids transferred into the mitochondria [1]. Elevated AC production can occur when *β*-oxidation rates are in excess of complete oxidation to CO_2 through the tricarboxylic acid cycle [1,23]. Previous studies have

shown that AC concentrations increase with duration of fasting in humans [8,32]. By contrast, glucose-stimulated insulin secretion during an oral glucose tolerance test [10] and hyperinsulinemia during a euglycemic clamp significantly reduce AC concentrations [4,32]. The acute postprandial pattern of AC species was unknown, as past studies were designed to understand the impact of glucose/insulin challenge and not to mimic the physiology of a mixed meal.

As a result of a growing body of data in the literature, plasma AC levels are proposed as biomarkers in subjects with insulin resistance [3-4]. Indeed, AC concentrations are higher in the fasting state in insulin-resistant populations and decrease less during glucose challenge tests [4,10]. This latter observation may be due to a continued release of adipose FFA as a result of adipose insulin resistance or could be due to use of intramyocellular lipid (IMCL) [33]. With respect to the role of dietary fatty acids, very little is known. Acylcarnitine composition has been shown in one study to resemble longer-term, dietary fatty acid composition [6] and also in another study to increase acutely after a single oral bolus of oil [34]. Thus, both endogenous and dietary fatty acids could impact AC composition and concentration. Given the multiple sources of metabolic substrates that could serve as precursors of plasma AC, we sought to determine how AC concentration would change under physiologic conditions that would elicit an insulin response suppressing adipose fatty acid release, while at the same time leading to dietary fat absorption. Our key finding was that the responses of different AC species varied after the meal. The concentrations of the saturates C14:0, C16:0, and C18:0 remained steady, whereas the mono- and polyunsaturates fell significantly. This finding is in line with data supporting greater oxidation of long-chain unsaturated fatty acids (18:1, 18:2) compared with saturates in humans [35,36] and in rodents [37]. Using an isotopic dilution method in vivo, Kanaley et al [5] measured a fatty acid label present in the same AC species. In that study, as in the present project, isotope administration did not result in labeling of downstream AC products—most likely because of administration at tracer amounts used for turnover measurements. The seminal finding of Kanaley et al was that the plasma FFA is an original precursor of muscle AC, but that intramyocellular TG serves as a key intermediate compartment between these 2 pools in fasted individuals [5]. The present results echo these findings, but in the fed state here, by demonstrating a postprandial pattern of reductions in plasma FFA, which precede reductions in long-chain and medium-chain AC concentrations.

The transition from the fasted to fed states is characterized by changes in the metabolism of long-chain fatty acids that is determined by a balance between adipose fatty acid release, availability of tissue lipid stores, mitochondrial fatty acid transfer, release of dietary fatty acids by intravascular lipolysis, tissue fatty acid uptake, and mitochondrial fatty acid oxidation rates. As shown in the supplementary data, from 0 to 2 hours postprandially, LCACs (C16, C18:1, C18:2) were constant against a background of significant plasma FFA reduction. One possible interpretation of this finding is that from 0 to 2 hours, the source of the AC was IMCL. Concurrently, the intermediate fatty acid β-oxidation byproducts (C8, C10, C10:1, C12, C12:1, C14:1, C14:2, and C16:1) fell from the onset of eating (0-2 hours). This fall in byproducts could have occurred because of their accelerated use or a decline in their production. Our data are consistent with the latter because whole-body fat oxidation remained unchanged. At 4 hours, C16, C18:1, and C18:2 finally reached a nadir and then began to rise in the late postprandial phase when dietary fatty acids were being liberated in the plasma compartment; and at the same time, adipose fatty acid release resumed (Fig. 2E). Altogether, these data suggest that the use of IMCL serves as an approximately 3-hour buffer for the dramatic loss of plasma FFA after the onset of eating. If this interpretation of events is accurate, adipose insulin resistance in the *fed state* could result in significant overload of muscle β-oxidation, leading to negative effects such as inflammation and defects in insulin signaling [38,39].

Our secondary findings include a strong influence of LBM to increase the production of short-chain AC in both the fasted and fed states. Mihalik and colleagues [4] found significant positive correlations between body mass index and multiple fasting AC species. Our data suggest that, for at least the short-chain AC, these relationships were due to a direct provision of substrate (branch-chain amino acids) mediated through elevated LBM. Many of the subjects described in the literature as having the highest fasting AC concentrations were overweight, obese, and diabetic, and had a higher LBM either because of male sex [40], ethnicity (AA [3,41]), or obesity [42]. The composite of increased FFA substrate and availability of muscle mass rich in mitochondria suggests that overweight and obese individuals have both the source and the means to form AC species.

We also observed that those individuals with the lowest LCAC concentrations in both the fasted and fed states were those whose glucose oxidation was the highest at those times. Conversely, positive and significant associations were found between fasting LCAC and fasting fat oxidation. In the fed state, the influence of fatty acid flux was evident by the positive relationships between adipose fatty acid release, postmeal FFA concentrations, and postprandial AC concentrations. Consistent with past observations of higher acetylcarnitine in fasted insulin-resistant subjects [3-5], during the fed state, a high insulin sensitivity index was associated with lower postprandial LCACs, specifically C14 and C14:1. Interestingly, these 2 ACs, frequently used as biomarkers of inborn errors in metabolism [2], were repeatedly predictive of whole-body substrate oxidation in the fasting and fed states. Lastly, dietary fatty acid spillover is found in individuals who have appropriately suppressed fatty acid turnover in the fed state. These are the same people who exhibited high insulin sensitivity, low postprandial nadir FFA concentrations, and a switch to glucose oxidation in the fed state. If this scenario is correct, then dietary fatty acid spillover is a natural result of active adipose liberation of dietary fatty acids through lipolysis but slow turnover of FFA in the blood.

The present study had a number of limitations, including the small sample size of postprandial studies compared with larger fasting metabolomic analyses, the restricted ethnicity of the subjects, and the assessment of AC after only one meal. We were surprised at the very low level of variability between the subjects in their patterns of postprandial AC change at each time point (suppl. figs. 1 and 2). The strong correlations found between fasting (or nadir) AC concentrations and various subject characteristics were present for multiple similar AC species (either long- or short-chain ACs), which provides support for these findings. The rigor of our standardization of the meal protocol, particularly among this group of subjects with a wide range of insulin sensitivities, likely facilitated the testing of our hypothesis. Given the present data, future study designs can now be devised to determine meal labeling parameters that will result in non–steady-state tracking of fatty acids into the mitochondria.

In summary, the present study is the first to demonstrate the temporal pattern of change in plasma ACs in overweight subjects consuming a mixed meal; and we have demonstrated that conditions that impact fatty acid flux contribute to the control of AC concentrations. Elevated adipose fatty acid release postprandially led to increased FFA, which was associated with elevations in products of incomplete β-oxidation. Insulin sensitivity at muscle and adipose can both lead to lower postmeal ACs. Future studies investigating ACs as biomarkers of metabolic function should include a focus on the precursor molecules used for AC production. In subjects with elevated body weights, precursors of short-chain ACs could be derived from muscle stores of branch-chain amino acids and/or from plasma sources (eg, diet). Moreover, medium- and long-chain ACs can be derived from IMCL in the short term and from diet and peripheral stores over the long term. A better definition of the role of ACs during the transition from the fasted to fed states will aid in the understanding of how substrate overload contributes to metabolic dysfunction postprandially.

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Conflict of Interest

No conflicts of interest exist for any of the 4 authors.

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