The Role of Plasma Fatty Acid Composition in Endogenous Glucose Production in Patients With Type 2 Diabetes Mellitus

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Hepatic insulin resistance and increased endogenous glucose production (EGP) are associated with increased plasma free fatty acids (FFA). However, the contribution of FFA composition to the regulation of EGP is not known. Six obese nondiabetic subjects and 6 patients with type 2 diabetes mellitus (DM2) were studied after an overnight and a 3-day fast. Plasma insulin concentrations after an overnight fast were similar in the DM2 and nondiabetic patients (88.8 ± 26.4 *v* 57.6 ± 12.6 pmol/L, not significant [NS]) despite increased plasma glucose (9.9 ± 1.8 *v* 5.1 ± 0.1 mmol/L, $P < .01$) and EGP (510.3 ± 77.7 *v* 298.3 ± 18.3 $\mu mol \cdot m^{-2} \cdot min^{-1}$, $P < .05$) in the patients with DM2. Absolute rates of gluconeogenesis using the heavy water method were also increased in the patients with DM2 (346.8 ± 74.9 *v* 198.8 ± 16.4 $\mu mol \cdot m^{-2} \cdot min^{-1}$, $P < .05$). No differences were observed in plasma polyunsaturated fatty acids (PUFA) between the diabetic and nondiabetic subjects. However, total saturated fatty acid (SFA) concentrations (350 ± 37.4 *v* 230.9 ± 33.3 μmol/L, $P < .02$) were significantly increased in the diabetic subjects. Rates of EGP were correlated with total plasma FFA concentration ($r = .71$, $P < .01$) and the concentration of SFA ($r = .71$, $P < .01$), but not monounsaturated fatty acids or PUFA. Rates of gluconeogenesis were also correlated with plasma FFA ($r = .64$, $P < .05$) and SFA ($r = .67$, $P < .05$). We observed no relationship between EGP and either total FFA or fatty acid composition after a 3-day fast. We conclude that increases in EGP are associated with concentrations of plasma SFA after an overnight fast.

TYPE 2 DIABETES mellitus (DM2) is characterized, in part, by hepatic insulin resistance,[1] which has been increasingly linked to elevations in plasma free fatty acids (FFA).[2-4] Direct evidence supporting a role for FFA in hepatic insulin resistance has been provided by studies in which pharmacologic increases in plasma FFA during lipid infusion impaired suppression of endogenous glucose production (EGP) in nondiabetic individuals.[5] One mechanism proposed to explain FFA-mediated insulin resistance has focused on gluconeogenesis. Stimulation of gluconeogenesis by FFA in vitro has been well documented, and some,[6,7] but not all,[8] studies in nondiabetic individuals have demonstrated a direct relationship between the proportion of EGP derived from gluconeogenesis and plasma FFA concentrations. However, increases in EGP are not routinely observed with elevations in FFA concentrations. We and others have suggested that the failure of increased gluconeogenesis to increase EGP is the result of reciprocal suppression of glycogenolysis in nondiabetic subjects.[9,10] The recent demonstration that liver glycogen depletion, assessed directly using nuclear magnetic resonance (NMR) spectroscopy during a progressive fast, was decreased by infusion of lipid emulsion and heparin[11] despite an increase in gluconeogenesis[12] is consistent with this hypothesis. These studies suggest an important role for hepatic glycogen storage in the regulation of hepatic insulin sensitivity.

It is important to note that most studies, which have examined the impact of FFA elevation on EGP, have used the infusion of an emulsion made up of essential polyunsaturated fatty acids (PUFA), notably linoleic (18:2) and linolenic (18:3). Because intravenous emulsions of saturated fatty acids (SFA) are not available, the impact of acute changes in saturated fatty acid concentration on EGP is not known. Nevertheless, an association between diets high in saturated fat and hyperglycemia is increasingly appreciated. Links between dietary fat and impaired glucose tolerance have been known for many years, and evidence supporting the long-held contention that fatty acid composition plays an important role in glucose tolerance is now accumulating. Increased serum SFA and decreased PUFA have been observed in patients with DM2 after an overnight fast.[13,14] In addition, the modest impairment in glycosylated hemoglobin (HbA_{1c}) observed during normal pregnancy is associated with a similar increase in circulating SFA and decrease in PUFA.[15] Most recently, Vessby et al[16] have demonstrated that a diet high in SFA is associated with an increased fasting insulin/glucose ratio, suggesting that the diet had induced hepatic insulin resistance in man. Surprisingly, no studies to date have examined the relationship between plasma SFA concentrations and EGP and gluconeogenesis in man. The present study was designed to examine the relationship between plasma FFA composition and rates of EGP in diabetic and nondiabetic subjects. We were also interested to see if prolonged fasting might alter plasma FFA composition and play a role in hepatic glycogen regulation. Our data demonstrating a direct relationship between SFA, but not PUFA, concentration and EGP suggest that FFA composition may play an important role in the regulation of EGP in man. By analogy, the failure of Intralipid infusion to increase EGP may be related to the absence of SFA in the emulsions used.

MATERIALS AND METHODS

Study Design

Six obese subjects with mild DM2 and 6 nondiabetic subjects (11 women, 1 man), (body mass index [BMI], 30.9 ± 1.2 and 36.5 ± 2.9 kg/m^2, not significant [NS]), (age 41.0 ± 6.8 and 35.0 ± 4.4 years, NS) respectively, were enrolled in the present studies. The studies were approved by the Institutional Review Board at Virginia Commonwealth University (VCU), and informed consent was obtained from all sub-

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jects before enrollment. All of the subjects were in good health at the time of their participation, and none had impaired liver or renal function as assessed by routine blood chemistries. The presence or absence of diabetes was based on fasting plasma glucose values[17] and/or a current history of DM2. Oral glucose tolerance tests were not performed before enrollment. However, potential nondiabetic subjects were excluded from the study if there was a positive family history of diabetes mellitus, and none had impaired fasting glucose.[17] In the case of the diabetic subjects (whose duration of diabetes diagnosis ranged from 1 to 7 years), hypoglycemic medication was discontinued for 5 days before the study, and blood glucose was monitored to prevent significant elevations in blood glucose. Three of the subjects were being treated with sulfonylureas and 3 with insulin. None of the subjects had received thiazolidinediones. All subjects were admitted to the General Clinical Research Center for the metabolic studies. After an evening meal (completed by 7 PM), the subjects received only water and/or ice chips until completion of the studies. Beginning at 12 midnight, subjects drank a total of 5.0 mL ^2H_2O/kg total body water to enrich total body water to approximately 0.5%. Total body water was estimated as 50% of body weight for women and 60% for men, corrected for obesity by dividing the total body water by the ratio of actual BMI/25 kg/m^2. The water was ingested in 4 equal portions to minimize dizziness, which has been described in some subjects. At 5 AM, a primed (26.7 μmol/kg fat-free mass [FFM]) continuous (0.33 μmol $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}) infusion of [6,6-^{2}H]glucose was administered via forearm vein for the assessment of overall EGP. Blood samples were obtained from a heated dorsal hand vein at 8:30 AM, 8:40 AM, 8:50 AM, and 9 AM for the measurement of isotopic enrichment and plasma concentrations of intermediary metabolites and glucoregulatory hormones. Thus, blood samples were obtained 3.5 to 4.0 hours after initiation of isotopic infusion. Evidence for isotopic steady state in both groups of subjects after the 4-hour infusion was provided by enrichment coefficients of variation of less than 10% in both the diabetic and nondiabetic subjects over the final 30 minutes of infusion. In addition, indirect calorimetry was performed. Urine was collected for the measurement of urinary nitrogen and urinary water enrichment. After completion of the described studies, subjects continued the fast, ingesting water enriched to 0.5% with ^2H_2O to maintain total body water enrichment. On the third day of the fast, the above described studies were repeated. To assess glycogen stores, a 1.0-mg bolus of glucagon was administered after collection of baseline blood samples and sequential samples were obtained at 15-, 30-, 45-, and 60-minute time points.

Sample Analyses

Arterialized venous blood samples obtained at selected time intervals were placed immediately into ice-cold fluoridated tubes for determination of isotopic enrichment of glucose and measurement of plasma hormones and intermediary metabolites. Plasma insulin[18] and glucagon[19] were determined with double antibody radioimmunoassays. Plasma leptin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (Diagnostic Systems Laboratories, Webster, TX). Plasma glucose was measured by the glucose oxidase method. Blood samples for measurement of intermediary metabolites are immediately deproteinized with ice cold 3 mol/L perchloric acid. The supernatant is neutralized with 3 mol/L potassium hydroxide (KOH) and the resulting supernatant assayed for L-lactate, alanine, β-hydroxybutyrate (βOHB) and acetoacetate,[20] and glycerol[21] with microfluorometric assays.

Isotopic Analyses

Aliquots of plasma for the determination of isotopic enrichment were deproteinized with the Somogyi procedure ($Ba(OH_2)$ and $ZnSO_4$) and the neutralized supernatant chromatographed over sequential anion and cation exchange resins and the glucose eluted with H_2O. For the determination of glucose enrichment with 6,6-^{2}H-glucose, the eluate from the ion exchange columns is evaporated and derivatized to the pentacetate derivative with acetic anhydride:pyridine.[22] The sample was analyzed by gas chromatography-mass spectroscopy (GC-MS) in the chemical ionization mode with selective ion monitoring of masses m/z 333 and 331 using a Hewlett Packard (Palo Alto, CA) HP-5985 gas chromatograph-mass spectrometer.

Analysis of glucose carbons enriched with ^{2}H in the C-5 position was performed according to the method of Landau.[23] Briefly, glucose was isolated from deproteinized plasma as described above. Approximately 3 mg glucose was stirred in acetone and H_2SO_4 and precipitated with NaOH. The diacetone glucose was acid hydrolyzed and the monoacetone extracted into chloroform. Carbon 6 was cleaved from the monoacetone with periodic acid, and the monoacetone was reduced with sodium borohydride. The monoacetone xylose was then hydrolyzed to D-xylose with H_2SO_4, and the hydrolysate was chromatographed over sequential anion (AG 1-X8, formate) and cation (AG 50W-X8 hydrogen) exchange columns. The neutral fraction containing xylose was evaporated to dryness. A hexamethylenetetraamine (HMT) derivative of C5 in xylose was performed by the formation of formaldehyde. Oxidation of the xylose was performed by incubation with periodic acid (0.3 mol/L) and sodium bicarbonate (1 N). After 1 hour at room temperature, hydrochloric acid (HCl) (1 N) and sodium arsenite (1.2 N) were added, and the formaldehyde was distilled. The distillate was placed in 7 mol/L NH_4OH and evaporated to dryness. Determination of deuterium enrichment in the HMT was performed by GC-MS in electron ionization mode by monitoring at mass 141 (m+1). Yields with this method were consistently 35% to 40% of the starting glucose. Enrichment of plasma water was determined by GC-MS from plasma samples using the [U-^{13}C]acetone method of Yang et al.[24] Close agreement between results using the deuterium enrichment of carbon 2 of glucose and plasma water has recently been demonstrated.[12]

Phospholipid Fatty Acid Composition

Phospholipid (PL) fatty acid composition was analyzed according to Borkman et al[25] with minor modifications. Total lipids were extracted by the method of Folch et al.[26] The lipid extracts were dried under nitrogen, dissolved in 10 mL of 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 1 N 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 species, free fatty acids, as well as phospholipids, were first separated by thin-layer chromatography on silica gel G plates (Whatman, LK6D; Clifton, NJ) using a solvent system consisting of chloroform-ethanol-triethylamine-water (30:34:30:8, vol/vol) for the first development (SF1) and hexane-dimethyl ether (50:50, vol/vol) for the second development (SF2). The lipids were visualized under ultraviolet (UV) light after spraying the plate with rhodamine G. The separated pots were scraped and placed into glass tubes. Fatty acid methyl esters were prepared as described above by treatment with methanolic acid.

Fatty acid methyl esters from both the total phospholipid fractions, as well as the individual phospholipid species, were redissolved in 20 μL hexane, separated, and quantitated on a Hewlett-Packard 5890 gas chromatograph equipped with a 30 mm $\times$ 0.2 mm fused silica capillary column (Omega wax 320, Supelco, Bellefonte, PA,) and flame ionization detector. The injection temperature was 250°C and detector temperature was 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/minute, then to 280°C at 5°C/minute. Fatty acids were

Table 1. Glucoregulatory Hormone and Intermediary Metabolite Concentrations in Obese Diabetic (DM2) and Nondiabetic (control) Subjects After an Overnight (14 hours) and a 3-Day (64 hours) Fast

	Control		DM2	
	14 Hours	64 Hours	14 Hours	64 Hours
Glucose (mmol/L)	5.1 ± 0.1	3.5 ± 0.2	9.9 ± 1.8‡	6.3 ± 1.3
Insulin (pmol/L)	57.6 ± 12.6	22.2 ± 3.0*	88.8 ± 26.4	43.8 ± 12.6†
Glucagon (ng/L)	56.2 ± 7.9	75.8 ± 5.1†	88.9 ± 11.2§	103.5 ± 17.0
Leptin (ng/mL)	38.6 ± 9.2	18.9 ± 6.7*	27.9 ± 6.9	11.6 ± 1.3†
FFA (mmol/L)	0.58 ± 0.06	1.14 ± 0.16*	0.83 ± 0.07§	0.98 ± 0.05
β-OHB (mmol/L)	0.18 ± 0.04	3.69 ± 0.94*	0.42 ± 0.13	3.11 ± 0.92†

*$P < .01$ compared with day 1; †$P < .05$ compared with day 1.

‡$P < .01$ compared with nondiabetic subjects; §$P < .05$ compared with nondiabetic subjects.

identified by comparing their retention times with those of authentic standards.

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

Calculations

Baseline rates of glucose appearance (Ra) and disappearance (Rd) were calculated using the steady state equation: Ra = F/IE − F, where F is the isotope infusion rate determined by direct measurement of the infusate and IE is the steady state enrichment (expressed as atom percent excess, APE). The fraction of glucose derived from gluconeogenesis was estimated by the ratio of deuterium enrichments of C-5 to that of plasma water. Rates of gluconeogenesis were determined by the product of Ra × % GNG. Glycogenolysis was defined as Ra – GNG. Calculation of carbohydrate and lipid oxidation was performed using the equations of Frayn.[27] Absolute concentrations of individual FFA species was determined as the product of the percentage of fatty acid composition and total FFA concentration.

Statistical Analysis

The primary endpoints of the study were rates of EGP, gluconeogenesis, and glycogenolysis in diabetic and nondiabetic subjects. Rates are expressed in $\mu mol \cdot m^{-2} \cdot min^{-1}$ and mean values obtained in the 2 study groups were compared by analysis of variance after the overnight and 3-day fast. The response to glucagon after the 3-day fast was analyzed by area under the curve using the trapezoidal rule. Statistical significance was assumed at the $P < .05$ level.

RESULTS

Concentrations of glucose and glucoregulatory hormones are shown in Table 1. Fasting plasma glucose and glucagon concentrations were significantly increased in the patients with DM2 after an overnight fast, whereas no significant difference in plasma insulin concentrations was observed. Despite similar insulin concentrations, plasma FFA was also significantly increased in the diabetic subjects after an overnight fast. Analysis of plasma phospholipid fatty acids, as well as the individual plasma free fatty acids for the 2 groups of subjects after an overnight fast was then performed. The relative percentages of plasma phospholipid fatty acids (Table 2), which reflect dietary

Table 2. Plasma Phospholipid Fatty Acid Composition in Obese Nondiabetic Subjects (control) and in Patients With DM2 After an Overnight (14 hours) and a 3-Day (64 hours) Fast

	Control		DM2	
Phospholipid FA	14 Hours	64 Hours	14 Hours	64 Hours
14:0	0.8 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
16:0	23.3 ± 0.9	24.2 ± 1.2	24.3 ± 1.0	27.5 ± 0.9*
16:1	3.2 ± 0.3	2.4 ± 0.2	2.9 ± 0.2	2.7 ± 0.2
17:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
18:0	6.1 ± 0.2	5.9 ± 0.2	6.5 ± 0.2	5.9 ± 0.2
18:1	20.6 ± 1.2	20.9 ± 0.4	20.7 ± 1.0	21.6 ± 0.8
18:2	29.5 ± 0.9	26.3 ± 1.0	28.0 ± 1.3	25.5 ± 1.3
18:3	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
20:4	6.0 ± 0.7	8.5 ± 0.7*	6.0 ± 0.6	7.2 ± 0.6*
20:5	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
22:5	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:6	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Σ SFA	30.5 ± 1.0	30.8 ± 1.3	31.6 ± 1.0	34.2 ± 1.0†
Σ PUFA	58.1 ± 1.0	57.7 ± 0.8	56.8 ± 0.9	55.9 ± 0.9

NOTE. Results are expressed as mean ± SEM percentages.

*$P < .01$ compared with 14 hours.

†$P < .05$ compared with 14 hours.

Table 3. Plasma FFA (μmol/L) Composition in Obese Diabetic and Nondiabetic Subjects After an Overnight (14 hours) and Prolonged (64 hours) Fast

	Control		DM2	
Fatty Acid	14 Hours	64 Hours	14 Hours	64 Hours
14:0	13.2 ± 6.8	21.9 ± 3.9	17.9 ± 3.5	20.3 ± 2.6
16:0	163.9 ± 53.2	302.7 ± 42.4*	234.9 ± 58.1†	283.9 ± 42.8
16:1	26.1 ± 11.3	48.2 ± 8.2*	32.2 ± 11.2	40.0 ± 12.3
17:0	3.2 ± 1.2	6.1 ± 0.7‡	5.3 ± 1.6†	5.4 ± 0.8
18:0	50.7 ± 21.1	99.2 ± 11.0‡	92.2 ± 28.1§	95.9 ± 18.1
18:1	160.6 ± 46.7	385.6 ± 62.6‡	253.5 ± 67.3§	315.1 ± 79.9
18:2	73.4 ± 18.4	142.9 ± 27.8*	86.9 ± 22.9	94.7 ± 17.4
18:3	3.9 ± 1.6	9.2 ± 2.1*	4.7 ± 1.6	5.0 ± 1.0
20:4	5.7 ± 3.5	7.8 ± 1.3	6.5 ± 2.2	6.4 ± 2.2
20:5	0.4 ± 0.4	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.2
22:5	0.1 ± 0.2	0.4 ± 0.3	0.3 ± 0.3	0.2 ± 0.2
22:6	0.6 ± 0.7	1.7 ± 0.7*	1.5 ± 0.7	1.5 ± 0.6
Σ SFA	230.9 ± 33.3	430.1 ± 59.8‡	350.2 ± 37.4§	405.5 ± 24.8
Σ PUFA	84.5 ± 8.9	163.1 ± 32.9*	100.7 ± 11.9	108.8 ± 8.9

NOTE. Results are expressed as means ± SEM.

*$P < .05$ compared with 14 hours.

†$P < .05$ compared with nondiabetic subjects.

‡$P < .01$ compared with 14 hours.

§$P < .02$ compared with nondiabetic subjects.

fat,[28] did not differ between the 2 groups. Individual plasma free fatty acid concentrations are shown in Table 3. Palmitic (16:0) (234.9 ± 58.1 *v* 163.9 ± 53.2 μmol/L, $P < .05$), stearic acid (18:0) (92.2 ± 28.1 *v* 50.7 ± 21 μmol/L, $P < .02$) and total SFA concentrations (350 ± 37.4 *v* 230.9 ± 33.3 μmol/L, $P < .02$) were significantly increased in the diabetic subjects. We also observed a significant increase in the concentration of oleic acid (18:1) in diabetic subjects. No differences were observed in plasma PUFA between the diabetic and nondiabetic subjects. Estimates of elongase and desaturase activity were performed based on the product/precursor ratio of plasma fatty acids as previously described.[29] Elongation (18:0/16:00) was not different in the diabetic and nondiabetic subjects (0.4 ± 0.08 *v* 0.31 ± 0.12, NS). Estimates of delta 9 (0.14 ± 0.05 *v* 0.16 ± 0.05), delta 5 (8.8 ± 4.5 *v* 7.9 ± 3.3), and delta 6 (0.01 ± 0.0 *v* 0.01 ± 0.01) desaturase were also not different in the 2 groups. As the fast was continued for a total of 64 hours, plasma glucose and insulin levels decreased in both groups. Plasma glucagon, FFA, and βOHB concentrations increased in the nondiabetic subjects. Only plasma βOHB concentrations increased significantly in the diabetic subjects. Changes in the concentration of specific fatty acids during the 3-day fast are also shown in Tables 2 and 3. Fasting was associated with modest changes in the relative proportion of fatty acid species. As had been observed after an overnight fast, there were no differences in the relative percentages of phospholipid fatty acids with prolonged fasting between the diabetic and nondiabetic subjects. Increases in both saturated and polyunsaturated fatty acids were observed in the nondiabetic subjects with fasting, whereas little or no change was observed in the subjects with DM2.

In association with the increase in fasting plasma FFA, rates of EGP were significantly higher in the diabetic subjects compared with the nondiabetic subjects after an overnight fast (510.3 ± 77.7 *v* 298.3 ± 18.3 $\mu mol \cdot m^2 \cdot min^{-1}$; $P < .01$). Prolongation of the fast resulted in expected decreases in rates of EGP in both groups, such that there was no significant difference in EGP after a 3-day fast in the 2 groups (242.2 ± 35.6 *v* 188.6 ± 16.1 $\mu mol \cdot m^{-2} \cdot min^{-1}$, NS.). When rates of EGP were correlated with plasma glucose concentrations, a strong direct relationship was found after an overnight fast ($r = .86$, $P < .001$), which persisted after a 3-day fast ($r = .91$, $P < .001$). We also examined the relationship between plasma FFA and EGP. Rates of EGP after an overnight fast were correlated with total plasma FFA concentration (Fig 1, left panel, $r = .71$, $P < .01$) and the concentration of SFA (Fig 1, right panel, $r = .71$, $P < .01$). When SFA, oleic acid, and PUFA were examined in multivariate analysis, only SFA was retained in the model, suggesting that SFA, but not 18:1 or PUFA, explained most of the association between FFA and EGP. In contrast, we observed no relationship between EGP and either total FFA or fatty acid composition after a 3-day fast.

With respect to gluconeogenesis, the fractional contribution of gluconeogenesis to EGP was virtually identical in the 2 groups of obese subjects after an overnight fast (66.7 ± 4.3 *v* 66.5% ± 4.4%) and increased to near unity after the 3-day fast. There was, therefore, no association between the percent contribution of gluconeogenesis to EGP and FFA. After an overnight fast, absolute rates of gluconeogenesis were significantly increased in the patients with DM2 compared with the nondiabetic subjects (346.8 ± 74.9 *v* 198.8 ± 16.4 $\mu mol \cdot m^{-2} \cdot min^{-1}$, $P < .05$) and were correlated with plasma FFA ($r = .64$, $P < .05$) and SFA ($r = .67$, $P < .05$). As had been observed for EGP, multivariate analysis with rates of gluconeogenesis as the dependent variable and fatty acid composition as independent variables resulted in exclusion of both oleic acid and total PUFA from the model. After a 3-day fast, rates of gluconeogenesis accounted for all of the EGP and were not different in the 2 groups of subjects.

To determine if hepatic glycogen stores had increased with

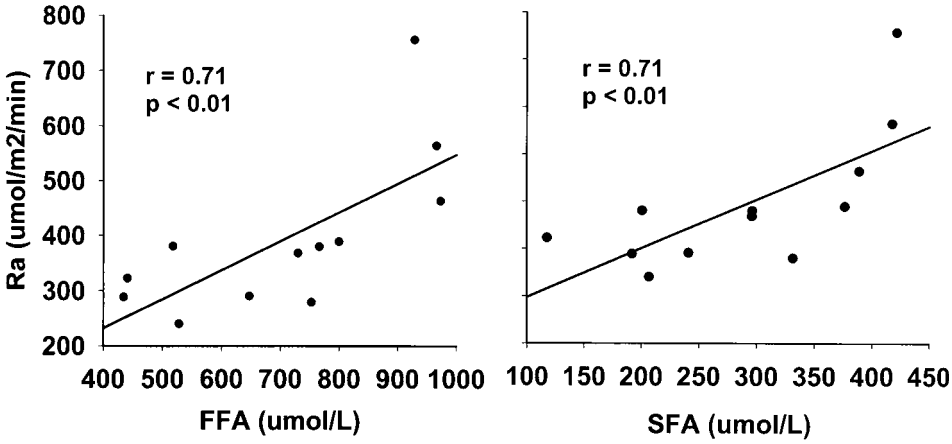

Fig 1. Relationship between rates of endogenous glucose production (Ra, μmol · m^2 · min^{-1}) and (A) total plasma FFA and (B) SFA in obese diabetic and nondiabetic subjects.

fasting in the patients with DM2 and if this increase had been mediated by diversion of newly formed glucose into glycogen, subjects received a 1.0-mg glucagon infusion and blood samples were obtained for 60 minutes thereafter. A significant increase in plasma glucose area under the curve (80.1 ± 22.5 *v* 26.2 ± 10.8 mmol/h/L, $P < .05$) was observed in the diabetic subjects compared with the nondiabetic subjects suggesting that a paradoxical increase in liver glycogen had occurred with fasting in the patients with DM2. No change in the plasma glucose enrichment of C5 was observed after glucagon administration (data not shown).

DISCUSSION

The present studies suggest that elevated concentrations of SFA observed in patients with DM2 are associated with an increase in EGP. Although numerous studies have linked rates of EGP with plasma FFA concentrations, these studies are, to our knowledge, the first to demonstrate a direct relationship between fatty acid composition and EGP. They also appear to suggest that the increase in SFA is closely related to the plasma appearance of newly formed glucose (ie, gluconeogenesis), which is increased in our patients with DM2.

The role of plasma FFA in impaired suppression of EGP by insulin has been recognized for many years. Intravenous infusions of lipid emulsion (predominantly linoleic and linolenic acids) designed either to prevent the decrease in plasma FFA during a hyperinsulinemic clamp or to increase concentrations above baseline have been shown to impair insulin-mediated suppression of EGP in some,[5,30] but not all,[31] studies. More physiologic increases in plasma FFA using these same methods do not appear to induce hepatic insulin resistance.[12] In addition, no correlation is observed between plasma FFA concentration and EGP either at baseline or during a hyperinsulinemic clamp in a large cohort of nondiabetic subjects.[32] Duration of lipid infusion[30] and the contribution of the kidney to EGP[33] appear to be important variables in this response. However, our results suggest that this paradox may also be explained by fatty acid composition.

As indicated in Table 3, the composition of fatty acids delivered to the liver is heterogeneous, with approximately 40% SFA (Table 3). The distribution of plasma fatty acids is clearly influenced by diet. Using plasma phospholipid fatty acid composition as a marker for dietary fat,[28] a direct relationship between the proportion of saturated fat and insulin resistance has been reported in nondiabetic subjects.[16,34,35] Increased saturated fat in plasma phospholipids has also been reported in diabetic subjects in some,[13,14] but not all,[15,35] studies. The present studies (serum phospholipid fatty acids, Table 2) are in agreement with the latter observations and suggest that dietary fat intake was not substantially different in our 2 groups of obese subjects. On the other hand, we have observed a significant difference in the composition of plasma FFA in our diabetic and nondiabetic subjects, with greater SFA and monounsaturated fatty acids in the former group. Differences in turnover and/or utilization of FFA in diabetic individuals may explain this difference.[14] It is also possible that decreased elongation and desaturation of fats may have contributed to the distribution of fatty acids observed.[36] However, estimates of elongase (18:0/16:0) and delta 9, delta 5, and delta 6 desaturase activity (16:1/16:0, 20:4/20:3, and 20:3/18:2, respectively) were not significantly different in the 2 groups of subjects. Finally, dietary carbohydrate intake, which would be expected to increase de novo lipogenesis of palmitic acid,[37] may have been greater in the diabetic subjects. We did not obtain a diet history from our subjects and cannot rule out this possibility.

Regardless of etiology, the observation that only SFA concentration was found to correlate with rates of EGP suggests that fatty acid composition may play an important role in the regulation of EGP. The present studies were not designed to examine mechanism, but there is accumulating evidence for an adverse effect of intrahepatic fat in general and SFA in particular on hepatic glucose handling. We have recently completed studies, which demonstrate hepatic insulin resistance in patients with documented fatty liver,[38] or after carbohydrate overfeeding, which may also result in increased SFA accumulation.[39] On the other hand, diets enriched in n-3 fatty acids[40] or infusions of Intralipid[6,7] have been shown to increase gluconeogenesis without an increase in EGP. A hierarchy for fatty acid oxidation is now recognized, with PUFA preferred over SFA.[41,42] This preference is thought to be mediated by changes in activity of the peroxisome proliferator activated receptor alpha (PPARα), which is increased by PUFA.[43] Diminished

activation of fatty acid oxidation due to increased availability of SFA would then be expected to result in increased intrahepatic SFA. Recent studies have suggested that SFA impair insulin-stimulated glycogen storage in skeletal muscle.[44] Although the mechanisms for this inhibition are not completely understood, inhibition of protein kinase B/Akt by ceramides derived from SFA has been implicated.[44] A similar mechanism in liver would therefore result in lower liver glycogen stores[45] and impaired regulation of EGP, as was seen in the present studies. It is also possible that the increase in EGP is mediated through glucose-6-phosphatase (G6Pase) activity, which is increased in patients with DM2.[46] Stimulation of G6Pase activity appears to be directly related to FFA concentration and inversely related to fatty acid chain length and degree of unsaturation.[47-49]

The ^2H_2O method used in the present studies examines the distribution of carbons labeled with deuterium during gluconeogenesis from all sources.[23] Using this method, 2 groups of investigators have recently reported that rates of gluconeogenesis are increased in patients with DM2 after an overnight fast.[8,50] The present studies are in agreement with this conclusion. Our studies are also in agreement with those of Gastadelli et al[8] who recently demonstrated that the fractional contribution of gluconeogenesis to EGP is increased in obese compared with lean nondiabetic subjects, but that no further increase in fractional gluconeogenesis is observed in obese, diabetic compared with obese, nondiabetic subjects. Nevertheless, we have observed a significant relationship between both total FFA concentration and SFA concentrations and absolute rates of gluconeogenesis in our obese subjects. As noted above, a defect in glycogen storage induced by SFA would be expected to increase the appearance of glucose derived from the indirect (gluconeogenic) pathway in the plasma glucose pool. In contrast, graded infusions of PUFA suppress glycogen release despite an increase in gluconeogenesis.[11]

With prolongation of a fast from 14 to 64 hours, our studies suggest that the release of newly formed glucose increases in nondiabetic subjects in association with an increase in plasma FFA (and SFA), but not in patients with DM2. Based on the brisk glucose response to glucagon in the patients with DM2 and analysis of isotope distribution after glucagon stimulation, our data suggest a diversion of newly formed glucose into glycogen in the diabetic subjects as the fast is prolonged. The paradoxical increase in glucose in response to glucagon after a 3-day fast has been demonstrated previously.[51,52] However, a mechanism for this phenomenon has been elusive. A role for leptin in hepatic glycogen storage has recently been proposed. Studies in normal rats demonstrating suppressive effects of leptin on hepatic glycogenolysis in association with increased FFA oxidation (mediated by increased activity of PPARα) and gluconeogenesis[53-55] suggest that a similar effect might be responsible for the increase in liver glycogen in our subjects. However, as shown in Table 1, plasma leptin concentrations were similar in our obese diabetic and nondiabetic subjects and, as expected, decreased in both groups. In fact, leptin concentrations in the patients with DM2 after a 3-day fast were lower than in the nondiabetic control subjects. Whether putative downstream effects of leptin on hepatic glycogen metabolism are altered in patients with DM2 after an overnight fast, but enhanced with prolonged fasting is clearly speculative.

In conclusion, the present studies demonstrate a strong direct relationship between SFA concentration and EGP. Future studies designed to acutely alter plasma FFA composition will be needed to confirm this association. The studies also suggest that the proportion of EGP derived from gluconeogenesis is related to the availability of FFA, but the disposition of the newly formed glucose is dependent on fatty acid composition.

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