Impaired Free Fatty Acid Utilization by Skeletal Muscle in Non-Insulin-dependent Diabetes Mellitus

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Abstract

This study was undertaken to assess utilization of FFA by skeletal muscle in patients with non-insulin-dependent diabetes mellitus (NIDDM). 11 NIDDM and 9 nondiabetic subjects were studied using leg balance methods to measure the fractional extraction of [^{3}H]oleate. Limb indirect calorimetry was used to estimate RQ. Percutaneous muscle biopsy samples of vastus lateralis were analyzed for muscle fiber type distribution, capillary density, and metabolic potential as reflected by measurements of the activity of seven muscle enzyme markers of glycolytic and aerobic-oxidative pathways. During postabsorptive conditions, fractional extraction of oleate across the leg was lower in NIDDM subjects (0.31±0.08 vs. 0.43±0.10, $P < 0.01$), and there was reduced oleate uptake across the leg (66±8 vs. 82±13 nmol/min, $P < 0.01$). Postabsorptive leg RQ was increased in NIDDM (0.85±0.03 vs. 0.77±0.02, $P < 0.01$), and rates of lipid oxidation by skeletal muscle were lower while glucose oxidation was increased ($P < 0.05$). In subjects with NIDDM, proportions of type I, IIa, and IIb fibers were 37±2, 37±6, and 26±5%, respectively, which did not differ from nondiabetics; and capillary density, glycolytic, and aerobic-oxidative potentials were similar. During 6 h after ingestion of a mixed meal, arterial FFA remained greater in NIDDM subjects. Therefore, despite persistent reduced fractional extraction of oleate across the leg in NIDDM (0.34±0.04 vs. 0.38±0.03, $P < 0.05$), rates of oleate uptake across the leg were greater in NIDDM (54±7 vs. 45±8 nmol/min, $P < 0.01$). In summary, during postabsorptive conditions there is reduced utilization of FFA by muscle, while during postprandial conditions there is impaired suppression of FFA uptake across the leg in NIDDM. During both fasting and postprandial conditions, NIDDM subjects have reduced rates of lipid oxidation by muscle. (*J. Clin. Invest.* 1994. 94:2349–2356.) Key words: non-insulin-dependent diabetes mellitus • human skeletal muscle • free fatty acid metabolism • muscle fiber type • respiratory quotient

Introduction

Patients with non-insulin-dependent diabetes mellitus (NIDDM)[1] have a number of abnormalities of lipid metabolism which may contribute to the increased morbidity and mortality associated with this syndrome (1, 2). Among the abnormalities of lipid metabolism in NIDDM is increased plasma FFA (3). Increased fasting rates of lipolysis (4), insulin resistance in suppression of lipolysis (3, 5), and impaired clearance of plasma FFA, particularly in relation to hyperglycemia (6), contribute to elevated plasma FFA in NIDDM. During postabsorptive metabolism, lipid oxidation provides approximately three-quarters of energy production in healthy individuals and plasma FFA contribute substantially to lipid oxidation (7). Uptake and oxidation of plasma FFA occur in liver, kidney, skeletal muscle, and myocardium, as well as other tissues, so that the site(s) responsible for impaired systemic clearance of FFA in NIDDM is uncertain. However, it does seem unlikely that hepatic utilization of FFA is reduced in NIDDM, as it is generally considered that hepatic fat oxidation is increased and a key factor in potentiating gluconeogenesis (8, 9). In skeletal muscle, lipid is the predominate oxidative substrate during postabsorptive conditions in nondiabetics, accounting for ~ 80% of oxygen consumption by muscle (10–12). Previously, we found that RQ across the leg during postabsorptive conditions was increased in subjects with NIDDM during conditions of fasting hyperglycemia (13). The elevation of leg RQ suggests that skeletal muscle utilization of plasma FFA could be impaired in NIDDM during postabsorptive conditions. Capaldo et al. (14) found that forearm FFA uptake was similar in NIDDM and nondiabetic subjects, yet the NIDDM subjects in that study were lean and had been rendered euglycemic before measuring muscle FFA uptake. Therefore, the findings of the study by Calpaldo et al., which is the only previous investigation of muscle FFA uptake in NIDDM, may not fully apply to the majority of patients with NIDDM, most of whom are obese, nor may these findings pertain to hyperglycemic conditions.

The current study was undertaken, therefore, to test the hypothesis that muscle FFA utilization is impaired in NIDDM during fasting hyperglycemia. The leg balance method was used in conjunction with an infusion of labeled oleate to measure uptake of FFA, and limb indirect calorimetry was used to obtain the RQ across the leg. Because the metabolic profile of skeletal muscle and fiber type characteristics can be important determinants of substrate utilization (15), vastus lateralis muscle was obtained by percutaneous biopsy for these characterizations. The leg balance measurements were conducted during postabsorptive conditions, then carried forward for 6 h after ingestion of a fat-enriched meal. The findings indicate that there is impaired oxidation of lipid by skeletal muscle in NIDDM and that uptake of plasma FFA by leg tissue is also reduced during fasting conditions. However, in further contrast with the pattern found among nondiabetic individuals, leg FFA uptake after meal ingestion is poorly suppressed in patients with NIDDM.

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Received for publication 29 March 1994 and in revised form 3 August 1994.

1. *Abbreviations used in this paper:* CK, creatine kinase; COX, cytochrome *c* oxidase; CS, citrate synthase; FFM, fat-free mass; GAPDH, glyceraldehydephosphate dehydrogenase; HADH, 3-hydroxyacyl CoA dehydrogenase; HK, hexokinase; NIDDM, non-insulin-dependent diabetes mellitus; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase.

The Journal of Clinical Investigation, Inc.
Volume 94, December 1994, 2349–2356

Table I. Clinical Characteristics of Nondiabetic and NIDDM Subjects

	Nondiabetic (3F/6M)	NIDDM (3F/8M)	*P*
Age (*yr*)	53±3.2	59±2.0	NS
Weight (*kg*)	79.6±4.9	87.9±5.4	NS
Body mass index (kg/m^2)	27.5±1.6	30.1±1.4	NS
Fat mass (*kg*)	22.6±0.2	25.6±0.1	NS
WHR	0.98±0.03	1.01±0.03	NS
Fasting blood glucose (*mM*)	4.92±0.14	10.2±1.11	0.01
HbA1C (%)	5.35±0.15	8.03±0.57	0.01
Duration (*yr*)	—	4.7±1.1	
Triglyceride (*mM*)	1.39±0.30	2.96±0.46	0.01
Cholesterol (*mM*)	4.97±0.25	5.39±0.27	NS
HDL cholesterol (*mM*)	1.27±0.24	1.05±0.12	NS

Normal range for HbA1C is 4.2–6.4%.

Methods

Subjects. 11 subjects with NIDDM and 9 nondiabetic subjects participated in this study, and their clinical characteristics are shown in Table I. The groups did not differ in age or degree of obesity, as determined by body mass index and fat mass, the latter estimated using bioelectric impedance plethysmography (Bioelectrical Sciences, Inc., La Jolla, CA), as described previously (16). 9 of the 11 NIDDM subjects were treated with sulfonylureas, which were withheld for 2 wk before a study, and the other 2 NIDDM subjects were treated with diet. None of the subjects had a major medical illness (other than NIDDM), none were taking medications known to influence lipid metabolism, and nondiabetic subjects had normal glucose tolerance. Subjects with NIDDM were excluded if they had moderate to severe complications of diabetes mellitus, defined as more than mild peripheral neuropathy, the presence of proliferative retinopathy, > 200 mg/24 h of urinary protein. The protocol was approved by the University of Pittsburgh Institutional Review Board, and each subject gave written informed consent before participation.

Study design. Subjects were admitted to the University of Pittsburgh General Clinical Research Center on the day before a study. Subjects were instructed to ingest a balanced diet containing at least 200 g of carbohydrate for 3 d preceding a study. On the evening of admission, subjects ingested a standard dinner (10 kcal/kg, 50% carbohydrate, 30% fat, and 20% protein) at 6 p.m. and then fasted overnight. At 7 a.m., a radial artery and a femoral vein were cannulated to obtain arteriovenous samples for leg balance measurements (17). A continuous (0.40 $\mu Ci\text{-}min^{-1}$) infusion of [9, 10 H^3]oleate (New England Nuclear Research Products, Boston, MA) was started in a forearm vein, and 1 h was allowed for isotope equilibration. Postabsorptive measurements were conducted for 30 min, starting at ~ 8:30 a.m. At 15-min intervals, three sets of simultaneously drawn arterial and femoral venous samples were obtained for oleate specific activity, plasma FFA, triglyceride and glucose, and arterial samples for insulin. At 5-min intervals, arterial and femoral venous samples were obtained for measurements of blood O_2 and plasma CO_2 content to measure leg gas exchange for limb indirect calorimetry, as described previously (17). Leg blood flow was measured in triplicate, at 15-min intervals, using venous occlusion strain gauge plethysmography (Hokanson, Bellevue, WA). Systemic glucose and lipid oxidation were measured using a canopy indirect calorimetry system (DeltaTrac, Anaheim, CA), placed for 30 min. A 12-h overnight urine collection was made for measurement of urea-nitrogen to estimate postabsorptive protein oxidation. At the completion of postabsorptive measurements a percutaneous muscle biopsy of the left vastus lateralis was performed, using the Bergström method with suction (18). A portion of the muscle sample to be used for measurement of enzyme activity was immediately frozen in liquid nitrogen. The remaining portion, used for muscle fiber type distribution and capillary density, was frozen in isopentane cooled to its freezing point (−160°C) by liquid nitrogen.

At ~ 9:30 a.m., 30 min after the muscle biopsy, subjects ingested a standardized liquid meal containing 50.6 g fat, 50.6 g carbohydrate (48 g as dextrose) (Dextol; Baxter Healthcare Corp., Mundelein, IL), and 19.8 g of protein (ProMod; Ross Laboratories, Columbus, OH), for a total of 737 cal. The fat content was 8.9 g of saturated fat, 23.6 g of monounsaturated fat (olive oil), and 15.3 g of polyunsaturated fat (corn oil). Postprandial measurements were performed for 6 h. Arterial and venous samples were obtained for measurement of oleate specific activity, FFA, and glucose at 30-min intervals, along with arterial samples for plasma insulin, and samples for arterial and venous triglyceride were obtained hourly. Also at hourly intervals, six sets of arterial and venous samples were obtained for blood gas analysis, leg blood flow was measured hourly, and systemic indirect calorimetry was measured, with the canopy in place for 30 min each hour. Urine was collected for 6 h to measure urea-nitrogen. Subjects remained supine, except while ingesting the meal.

Analysis. Arterial and venous glucose was measured using a glucose oxidase method (YSI 23A glucose analyzer; Yellow Springs Instrument Co., Yellow Springs, OH). Samples for blood gas analysis were collected in chilled heparinized syringes, placed in ice, and analyzed within 5 min. Blood O_2 content was measured using a co-oximeter (IL 282; Instrumentation Laboratory, Inc., Lexington, MA), and plasma CO_2 content was determined with a pH/blood gas analyzer (IL 1304 blood gas machine; Instrumentation Laboratory, Inc.), with correction to blood CO_2 based on a regression equation including sample variables of hemoglobin, saturation, and pH (19). Samples for FFA, oleate specific activity, and plasma triglycerides were collected in tubes containing EDTA. Samples for oleate specific activity were kept at −70°C until analysis by HPLC, as previously described (20). Briefly, before HPLC separation, FFA were extracted from plasma using organic solvents (methanol, heptane, chloroform), derivatized with bromoacetophenone, and then separated by reverse-phase isocratic HPLC, collecting the oleate fraction for scintillation counting. An internal standard of D_{31} palmitate was added to each sample to assess extraction recovery. External standards were used to calculate oleate, palmitate, and linoleic acid concentrations from the areas of chromatographic peaks. Total plasma FFA were measured by an enzymatic method (NEFA C; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The sum of the three FFA concentrations measured by HPLC was closely correlated with total FFA concentration measured enzymatically: ($[FFA_{oleic,palm,lino}] = 1.0 \times [FFA_{enz.}] - 241$, $r = 0.80$, $P < 0.01$). Oleic acid comprised 45±2 and 46±2% of plasma FFA determined by HPLC in nondiabetic and NIDDM subjects, respectively, palmitate comprised 31±2 and 32±1%, and linoleic acid comprised 25±2 and 23±2%. The plasma FFA composition did not change during postprandial conditions (data not shown). Plasma triglyceride was measured within 48 h, using an enzymatic assay, correcting for plasma glycerol (Sigma Chemical Co., St. Louis, MO). Plasma insulin was measured using radioimmunoassay (Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden).

Muscle samples were shipped, on dry ice, to the Physical Activity Sciences Laboratory at Laval University for analysis by J.-A. Simoneau, who was blinded as to the clinical status of the subjects. Muscle sections (10-μm-thick) were stained for myofibrillar adenosine triphosphatase according to an established technique (21) to determine the proportion of the different fiber types (I, IIa, and IIb). The average cross-sectional area of 20 randomly selected fibers of each fiber type was measured from myofibrillar adenosine triphosphatase–stained sections. Number of capillaries around different fiber types was obtained from the amylase–periodic acid-Schiff stain technique as described by Andersen (22). Activity levels of seven enzymes were spectrophotometrically determined at 30°C according to previously published techniques (23). These enzymes were creatine kinase (CK, EC 2.7.3.2), hexokinase (HK, EC 2.7.1.1), phosphofructokinase (PFK, EC 2.7.1.11), glyceraldehydephosphate dehydrogenase (GAPDH, EC 1.2.1.12), citrate synthase

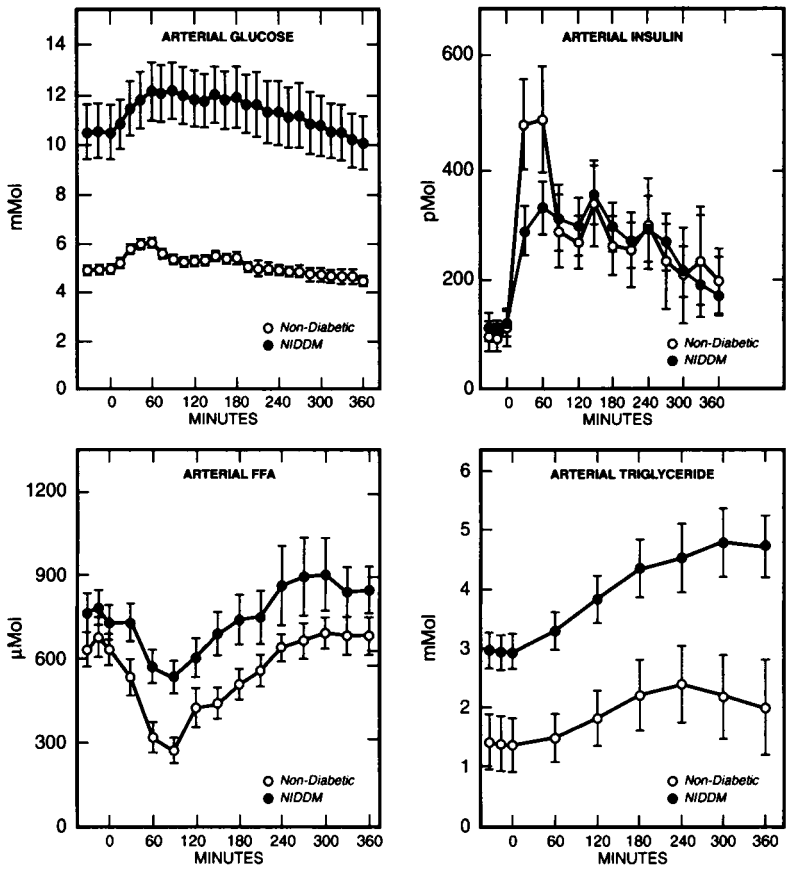

Figure 1. Four panels display, respectively, arterial glucose, insulin, FFA, and triglycerides in nondiabetic and NIDDM subjects during postabsorptive conditions and for 6 h after ingestion of a meal.

(CS, EC 4.1.3.7), cytochrome *c* oxidase (COX, EC 1.9.3.1), and 3-hydroxyacyl CoA dehydrogenase (HADH, EC 1.1.1.35). Values of the enzyme activities were expressed in units (micromoles per minute) per gram of wet weight muscle. The intra-individual reproducibility for these measurements has been established (23, 24).

Calculations. Net balance across the leg was calculated as the product of the arterio-venous difference (nanomoles per milliliter) and blood flow. Blood flow is expressed as ml/min-100 ml leg tissue, which is the convention with venous occlusion plethysmography; so that units for leg balance are nmol/min-100 ml leg tissue. The fractional extraction (F_{ex}) of oleate was calculated as the arterio-venous difference in oleate radioactivity divided by arterial oleate radioactivity. Leg oleate uptake was calculated as the product of oleate $F_{ex} \times [\text{oleate}]_{art} \times$ leg plasma flow (12). The respiratory quotient across the leg (leg RQ) was calculated as the quotient of leg CO_2 production and leg O_2 consumption (10). The tables of Lusk (25) were used to estimate the contributions of fat and glucose oxidation to oxygen consumption, and estimation of the limb oxygen consumption needed for oxidation of FFA uptake was calculated using the equation of Deganais et al. (11). Rates of systemic glucose and lipid oxidation were calculated using the equations of Frayn (26), with protein oxidation estimated from measurement of urine urea-nitrogen.

Statitical analysis. Data are presented as mean±SEM. Repeated measures analysis of variance was used to investigate between group differences and the interaction of time and group for each variable of interest. Where appropriate, Sheffe's post-hoc comparison procedure was used. All statistical assumptions for these tests were met.

Results

Arterial hormone and substrate concentrations (Fig. 1). Arterial glucose, FFA, and triglyceride concentrations, basally and during postprandial conditions, were significantly higher in subjects with NIDDM ($P < 0.05$). Basal and mean postprandial arterial insulin were similar in NIDDM and nondiabetic subjects, although the initial rise of postprandial insulin was greater in nondiabetics.

Postabsorptive FFA and glucose metabolism by leg tissue (Figs. 2 and 3 and Table II). Fractional extraction of oleate across the leg during postabsorptive conditions was significantly lower in subjects with NIDDM (0.31±0.08 vs. 0.43±0.10, $P < 0.001$). Fasting arterial oleate concentration (213±22 vs. 225±27 μM, NS) and leg plasma flow (1.07±0.13 vs. 0.91±0.07 ml/min-100 ml leg tissue, NS) were similar in NIDDM and nondiabetic subjects, respectively. Due to the lower fractional extraction, leg oleate uptake was reduced in subjects with NIDDM (66±8 vs. 82±13 nmol/min, $P < 0.01$), as shown in Fig. 2. Release of oleate across the leg was not significantly different in NIDDM compared with nondiabetic subjects (231±36 vs. 213±24 nmol/min, NS), as also shown in Fig. 2. Based on fractional extraction across the leg determined for oleate, postabsorptive rates of leg FFA uptake were lower in NIDDM subjects (217±23 vs. 257±27 nmol/min, $P < 0.05$), while release of FFA across the leg was similar in NIDDM and nondiabetic subjects (145±24 vs. 139±28 nmol/min, NS). During postabsorptive conditions, a clear arterio-venous difference for plasma triglyceride was not detectable in either group, and the net balance across the leg was not different between NIDDM and nondiabetic subjects (−12±6 vs. 15±13 nmol/min). Rates of postabsorptive leg glucose uptake were similar in NIDDM and controls (270±36 vs. 241±48 nmol/min, NS). Net balances of lactate and alanine were negative, indicative of net release, and were not significantly different (−288±37 vs. −256±84 nmol/min; expressed in glucose equivalents).

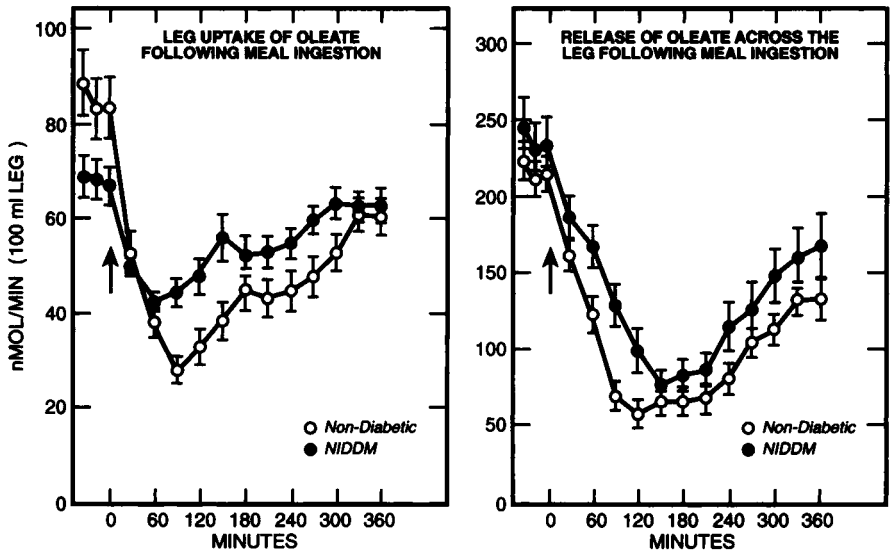

Figure 2. The left panel displays rates of leg oleate uptake, and the right panel displays rates of release of oleate across the leg in NIDDM and nondiabetic subjects during postabsorptive conditions and for 6 h after ingestion of a meal.

Limb indirect calorimetry was performed to estimate the oxidative metabolism of lipid and carbohydrate, and values are shown in Table II. During postabsorptive conditions, the RQ across the leg was significantly higher in NIDDM subjects (0.85±0.03 vs. 0.77±0.02, $P < 0.01$). The higher RQ in NIDDM reflected lower fasting skeletal muscle lipid oxidation (81±13 vs. 143±16 nmol/min) and higher rates of glucose oxidation (339±64 vs. 155±46 nmol/min); both $P < 0.05$. In nondiabetic subjects, lipid oxidation accounted for 77±6% of basal leg oxygen consumption, but significantly less ($P < 0.01$), 51±8%, in NIDDM. To assess whether reduced FFA uptake by leg tissue could have limited postabsorptive lipid oxidation, the percentage of leg oxygen consumption which would have been accounted for by immediate oxidation of all of the FFA taken up was calculated. During fasting conditions, immediate oxidation of plasma FFA taken up by leg tissues would have accounted for 137±13% of leg VO_2 in NIDDM subjects and 146±12% in nondiabetic subjects. This suggests that increased basal RQ across the leg in NIDDM subjects is not accounted for solely by the concomitant reduction of FFA uptake.

Systemically, the postabsorptive rate of appearance of oleate was not significantly different in NIDDM and nondiabetic subjects, respectively (1.74±0.20 vs. 1.56±0.26 μmol/min-kg FFM, NS), but systemic clearance of oleate was lower among NIDDM subjects (8.3±0.8 vs. 10.2±1.2 ml/kg-FFM, $P < 0.05$). Postabsorptive systemic RQ (0.78±0.01 vs. 0.79±0.01, NIDDM vs. nondiabetic, NS), systemic rates of lipid oxidation (4.89±0.34 vs. 4.23±0.25 μmol FFA/min-kg FFM, NS), and systemic rates of postabsorptive glucose oxidation (4.54±0.70 vs. 5.44±0.96 μmol/min-kg FFM, NS) were also similar in NIDDM and nondiabetic subjects.

Postprandial FFA and glucose metabolism by leg tissue (Figs. 2 and 3 and Table II). During the 6 h after ingestion of a mixed meal containing ~ 50 g of both fat and glucose, fractional extraction of oleate across the leg continued to be significantly reduced in NIDDM compared with nondiabetic subjects (0.34±0.04 vs. 0.38±0.03, $P < 0.05$). In both NIDDM and nondiabetic subjects, oleate uptake across the leg initially decreased after meal ingestion. The nadir of oleate uptake occurred at 90 min and was lower in nondiabetic subjects (28±6 vs. 42±6 nmol/min, $P < 0.05$). Uptake of oleate across the leg

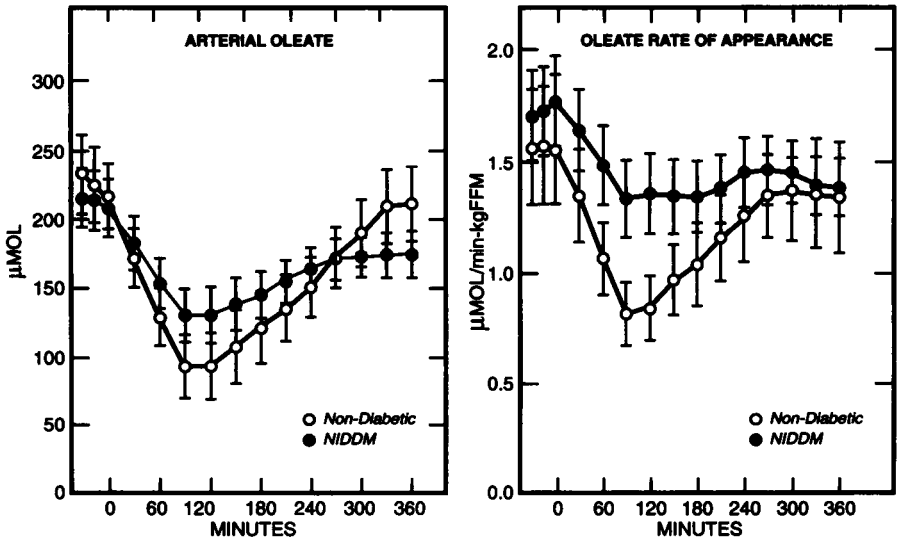

Figure 3. The left panel displays arterial oleate concentrations, and the right panel displays systemic rates of appearance of oleate in NIDDM and nondiabetic subjects during postabsorptive conditions and for 6 h after ingestion of a meal.

Table II. Leg Indirect Calorimetry in NIDDM and Nondiabetic Subjects during Postabsorptive Conditions and for 6 h after Meal Ingestion

		Postprandial					
	Postabsorptive	1 h	2 h	3 h	4 h	5 h	6 h
Leg RQ							
Nondiabetic	0.77±0.02	0.81±0.05	0.79±0.04	0.75±0.05	0.79±0.04	0.87±0.04	0.82±0.05
NIDDM	0.85±0.03*	0.84±0.08	0.89±0.06*	0.87±0.05*	0.91±0.03*	0.86±0.03	0.85±0.04
Glucose oxidation							
Nondiabetic	155±46	191±107	203±60	137±44	272±85	387±99	266±78
NIDDM	339±64*	341±91*	464±78	285±63	452±78	362±90	298±98
Lipid oxidation							
Nondiabetic	143±16	92±21	98±21	121±24	114±19	83±26	95±27
NIDDM	81±13*	58±15*	64±18	95±20	58±16	80±20	95±20

Mean±units for glucose and lipid oxidation by leg tissue are nmol/min-100 ml leg tissue. * $P \leq 0.05$ nondiabetic vs. NIDDM.

thereafter gradually increased during the next 4.5 h, toward resumption of postabsorptive rates. The pattern of oleate uptake across the leg corresponded to the postprandial pattern for arterial concentrations of FFA and for arterial oleate in particular. Thus, despite lower fractional extraction among NIDDM subjects, there was increased mean postprandial oleate uptake across the leg in NIDDM (54±7 vs. 45±8 nmol/min, $P < 0.01$). There was a 41±8% decrease in postprandial oleate uptake across the leg compared with basal rates in nondiabetic subjects, but a lesser suppression ($P < 0.05$) of 10±12% among NIDDM subjects. Based on the fractional extraction across the leg determined for oleate, mean postprandial uptake of FFA across the leg was 223±49 nmol/min in subjects with NIDDM, which was increased compared with the value among nondiabetic subjects (157±23 nmol/min, $P < 0.01$). Among subjects with NIDDM, postprandial and fasting FFA uptake across the leg were not significantly different, while among nondiabetic subjects postprandial FFA uptake across the leg was significantly suppressed ($P < 0.01$) compared with basal values. Mean postprandial uptake of plasma triglyceride across the leg was not significantly different in NIDDM and nondiabetic subjects (75±80 vs. 63±46 nmol/min, NS). The mean rate of glucose uptake across the leg during the 6 h postprandially was lower among NIDDM subjects (207±172 vs. 288±104 nmol/min, $P < 0.05$). Among both groups of subjects, there was increased glucose uptake across the leg (relative to basal) during the initial 3 h after meal ingestion followed by lower rates during the next 3 h, such that the mean postprandial values for leg glucose uptake were not significantly different from basal values. There was net release of lactate and alanine across the leg during postprandial metabolism, with similar mean values in NIDDM and nondiabetic subjects (185±44 vs. 173±57 nmol/min), and neither value different from postabsorptive rates.

The RQ across the leg remained higher in NIDDM subjects compared with nondiabetics during postprandial metabolism ($P < 0.01$), with mean values of 0.87±0.04 vs. 0.81±0.03. The mean value for postprandial oxidation of lipid by leg tissue was lower in NIDDM subjects (76±18 vs. 107±23 nmol/min, $P < 0.01$), while postprandial oxidation of glucose by leg tissues was greater in NIDDM (363±85 vs. 227±81 nmol/min, $P < 0.01$). Among nondiabetics immediate oxidation of FFA taken up postprandially by leg tissues could have accounted for 110±14% of postprandial oxygen consumption, and among NIDDM subjects oxidation of postprandial leg FFA uptake could have accounted for 156±27% of leg oxygen consumption. This indicates that decreased postprandial lipid oxidation by leg tissue in NIDDM subjects is not attributable to a deficit of FFA uptake. However, mean systemic RQ was slightly lower in NIDDM subjects during the 6 h after meal ingestion (0.77±0.03 vs. 0.79±0.03, $P < 0.05$). The mean respective rates in NIDDM and nondiabetic subjects for postprandial systemic glucose oxidation (4.86±1.0 vs. 5.37±1.23 μmol/min-kg fat-free mass [FFM]) and lipid oxidation (5.37±1.09 vs. 5.07±1.06 μmol/min-kg FFM) were not significantly different.

Postprandial release of oleate from leg tissue decreased steadily for the first 3 h after meal ingestion and then progressively increased toward postabsorptive rates by the sixth hour after meal ingestion, as shown in Fig. 2. The mean rate of postprandial release of oleate across the leg was greater in NIDDM subjects compared with nondiabetics (131±31 vs. 90±22 nmol/min, $P < 0.01$), and mean postprandial release of FFA across the leg (as estimated from the pattern measured for oleate) was also increased in subjects with NIDDM (409±78 vs. 282±48 nmol/min, $P < 0.01$). Among both groups of subjects, these postprandial values represented significant decreases compared with postabsorptive rates of oleate and FFA release from leg tissues ($P < 0.01$). The systemic appearance of oleate was greater in NIDDM subjects during postprandial metabolism (1.43±0.24 vs. 1.16±0.18 μmol/min-kg FFM, $P < 0.01$), as shown in Fig. 3. Postprandial oleate rate of appearance was reduced compared with postabsorptive rates in both NIDDM and nondiabetic subjects ($P < 0.01$), while the nadir observed after meal ingestion was lower in nondiabetics ($P < 0.01$). Systemic clearance of oleate was not significantly different in NIDDM compared with nondiabetic subjects during postprandial conditions (9.2±0.9 vs. 10.2±1.4 ml/kg-FFM, $P = 0.07$).

Muscle metabolic profile and fiber type characteristics (Table III). The activity levels of seven metabolic enzyme markers, fiber type proportion, and capillary number of vastus lateralis muscle are shown in Table III. HK, PFK, and GAPDH activity reflects the glycolytic capacity of muscle, CK activity is an indicator of capacity for ATP resynthesis from creatine phosphate, CS activity is a marker of Krebs cycle capacity, while COX activity is an index of oxidative phosphorylation, and HADH activity marks mitochondrial capacity for FFA β-oxida-

Table III. Fiber Type Proportion and Areas, Capillary Density, and Metabolic Profile of Skeletal Muscle (Vastus Laterlis) in Nondiabetic and NIDDM Subjects

Variables	Nondiabetic	NIDDM
Proportion (%)		
Type I	35±3	37±2
Type IIA	38±6	37±2
Type IIB	27±8	26±5
Area (μm^2)		
Type I	4810±373	5523±367
Type IIA	4593±413	4924±427
Type IIB	3992±570	3676±622
Capillaries per fiber		
Type I	3.93±0.32	4.33±0.24
Type IIA	3.55±0.38	3.66±0.20
Type IIB	2.96±0.30	2.59±0.37
CK	381±15	365±20
Glycolytic potential		
HK	1.9±0.2	1.9±0.1
PFK	53±5	55±5
GAPDH	411±35	436±21
Oxidative potential		
CS	9.0±0.4	8.6±0.3
COX	6.8±0.3	6.0±0.5
HADH	13.6±1.2	13.0±0.5

Data are expressed as mean±SEM. Enzyme activities are expressed as units per gram of wet weight.

tion. The profile of skeletal muscle for these five metabolic capacities was entirely similar between NIDDM and nondiabetic subjects and are somewhat low, consistent with values found in sedentary individuals (23, 27). Also, the proportions of types I, IIa, and IIb muscle fibers, as well as the numbers of capillaries per fiber type and the area of the different fibers, were equivalent in NIDDM and nondiabetic subjects. Type I muscle fibers comprised ~ 40% of vastus lateralis muscle fibers in both groups, which is also consistent with findings in sedentary subjects (28).

Discussion

NIDDM is associated with several abnormalities of FFA metabolism, including increased plasma FFA concentration (3), elevated rates of lipolysis (4), insulin resistance in suppression of lipolysis (5), and decreased systemic clearance of FFA during hyperglycemia (6). In the current study, subjects with NIDDM had each of the abnormalities. In previous studies, we found that the RQ across the leg was increased in untreated NIDDM subjects during postabsorptive conditions (13), but not after overnight euglycemia had been maintained in NIDDM subjects by moderate hyperinsulinemia (29). These findings suggested that hyperglycemia in NIDDM could be associated with impaired FFA oxidation by muscle during postabsorptive conditions and perhaps with reduced uptake of FFA by muscle. Therefore, this study was undertaken to test the hypothesis that fasting rates of FFA uptake by skeletal muscle are reduced in NIDDM. In the only previous study which has measured muscle FFA uptake in NIDDM, Capaldo and colleagues (14) found that fractional extraction of palmitate across the forearm was slightly though not significantly reduced in lean NIDDM subjects compared with nondiabetic volunteers. However, in that study the NIDDM subjects were rendered euglycemic, so these findings may not pertain to hyperglycemic conditions.

It is well established that in healthy (nondiabetic) individuals, lipid is the principal oxidative substrate of skeletal muscle during postabsorptive conditions and that uptake of plasma FFA is considerable (10–12). In a large number of healthy volunteers, Baltzan et al. (10) found that the RQ across the forearm during postabsorptive conditions was 0.76±0.02, indicating that lipid oxidation accounted for ~ 80% of forearm O_2 uptake. In later studies by this group of investigators, fractional extraction of arterial [^{14}C]oleate across the forearm in healthy volunteers was shown to be ~ 40% during postabsorptive conditions (11). In the present study, nearly identical rates for postabsorptive RQ across the leg and for fractional extraction of [^{3}H]oleate were found in nondiabetic subjects, reconfirming the importance of plasma FFA as a substrate for postabsorptive skeletal muscle metabolism. However, postabsorptive utilization of FFA by skeletal muscle was found to be perturbed in subjects with NIDDM. Subjects with NIDDM had reduced fractional extraction and uptake of oleate by leg tissues. Also, NIDDM subjects had an elevated value for leg RQ, with lower rates of lipid oxidation by muscle and increased rates of glucose oxidation. However, the reduced rate of FFA uptake by muscle in NIDDM does not explain per se the impairment in postabsorptive lipid oxidation by muscle. It can be estimated that immediate oxidation of FFA taken up by leg tissue in NIDDM subjects would have been sufficient to account for oxygen consumption, as was also found in nondiabetic subjects and as has been noted previously in healthy individuals (11).

To examine one potential mechanism for abnormal patterns of FFA utilization by skeletal muscle in NIDDM subjects, muscle fiber type and oxidative profile of marker enzymes were examined. Muscle fiber type and related biochemical characteristics of skeletal muscle have often been considered to be important determinants of substrate utilization. A high proportion of muscle fibers poorly equipped with an aerobic-oxidative capacity, such as type IIb fibers which generally possess reduced mitochondrial content and high glycolytic capacity (15, 30), could contribute to a reduced capacity to use FFA. Increased proportion of type IIb muscle fibers and a lower capillary density have been associated with insulin resistance (31). Capillary density as a determinant of diffusion radius could potentially affect FFA uptake in muscle due to the hydrophobicity of this substrate. In this study, NIDDM subjects exhibited similar proportions of type I, IIa, and IIb fibers, along with similar capillary density (whether expressed per fiber or relative to fiber area, data not shown), and fiber areas to those found among NIDDM subjects in a recent report by Marin et al. (32). However, these factors per se seem unlikely to account for abnormalities in the utilization of FFA, since the nondiabetic subjects of the current study had equivalent fiber type distributions.

More appropriate than fiber type distribution in delineating metabolic capacity of skeletal muscle is the determination of activity of key glycolytic and aerobic-oxidative enzymes, chosen as markers of the capacity of these pathways (15, 28). Of the seven enzymes assayed in the current study, none was significantly different in NIDDM in comparison with nondiabetic subjects. Both groups had values for enzyme activity,

and for fiber type distribution and capillary density, which are consistent with a physically untrained status (23, 27, and Simoneau, J.-A., unpublished observations). Although there were no differences in these factors, it remains to be determined whether enzymes more specific to FFA catabolism or proteins involved in FFA transport, such as fatty acid binding proteins (33), are affected by NIDDM, and are potentially important in the mechanism of decreased fasting uptake and oxidation of FFA as found in the current study.

Hyperglycemia can increase muscle glucose uptake and glucose oxidation independent of insulin (34). Therefore, hyperglycemia could be an important factor which suppresses muscle FFA utilization. In recent studies in lean nondiabetic volunteers, Mandarino et al. (35) found that hyperglycemia (maintained during somatostatin infusion and basal insulin replacement) was associated with increased leg RQ and activation of muscle pyruvate dehydrogenase (PDH). The latter is particularly important since PDH is considered to be the rate-limiting enzyme for glucose oxidation and its activity is inversely related to that of carnitine palmitoyl transferase (36). Among untreated NIDDM subjects, increased muscle PDH activity was found in association with elevated leg RQ (13). Furthermore, overnight euglycemia in NIDDM patients is associated with normal postabsorptive RQ across the leg and for muscle PDH activity (29). Also, Calpaldo et al. (14) found that FFA uptake across the forearm is normal in NIDDM subjects who have been rendered euglycemic. These studies suggest that hyperglycemia may be a key factor for increased postabsorptive glucose oxidation and reduced lipid oxidation by skeletal muscle in NIDDM.

The finding of an increased postabsorptive leg RQ in subjects with NIDDM was not apparent from the postabsorptive values for systemic RQ, which were equivalent in NIDDM and nondiabetic individuals, as has been found previously (5, 9). With respect to the potential impact of gas exchange by muscle upon systemic RQ, muscle oxygen consumption during resting conditions constitutes a relatively small fraction of systemic oxygen consumption, ~ 30% (37), and postabsorptive muscle glucose uptake is an even smaller fraction of systemic glucose utilization (approximately one-tenth). Thus, it is difficult to interpolate muscle RQ and glucose oxidation from systemic values. In addition, it is well recognized that indirect calorimetry is a net measurement and, as such, is affected by lipogenesis and gluconeogenesis. Gluconeogenesis has an estimated RQ value of ~ 0.13 (26). Though gluconeogenesis does not influence limb indirect calorimetry per se, its effects on systemic RQ might counterbalance those of increased muscle RQ in patients with NIDDM, particularly as gluconeogenesis is increased in this disorder (9). It is also possible that impaired muscle FFA oxidation in NIDDM could be offset by increased FFA oxidation in other tissues, perhaps the liver, as has been proposed in the pathophysiology of visceral obesity (38). As reviewed recently by Frayn et al. (39), the technique of limb indirect calorimetry can be a useful measurement particularly within a context of overall substrate utilization. In the current study, both leg RQ and leg uptake of oleate indicated that there is impaired postabsorptive utilization of FFA by skeletal muscle in patients with NIDDM.

Impaired systemic clearance of FFA has been reported previously in hyperglycemic patients with NIDDM during postabsorptive conditions (6). The current study confirms this finding and expands upon it by indicating that skeletal muscle is one of the tissues responsible for impaired clearance of FFA. A relative impairment of muscle FFA utilization during postabsorptive conditions could contribute to several of the metabolic abnormalities associated with NIDDM. Plasma FFA not utilized by muscle could provide additional substrate for hepatic FFA oxidation and esterification. Respectively, these could contribute to gluconeogenesis and synthesis of very low density lipoproteins, processes which are considered to be increased in NIDDM (1, 2, 9). Also, impaired oxidation of FFA by muscle could contribute to fat deposition within muscle. Skeletal muscle triglyceride content is increased in obese patients with NIDDM (40), and fat content within muscle is a marker of insulin resistance.

Among nondiabetic subjects, ingestion of a meal reduced utilization of FFA across the leg compared with rates during postabsorptive conditions. This occurred in conjunction with postprandial suppression of arterial FFA and stimulation of leg glucose utilization. In NIDDM subjects, the transition between postabsorptive and postprandial patterns of leg FFA uptake did not emerge as distinctly. The decrement of leg FFA uptake in NIDDM subjects after meal ingestion was smaller and there was less suppression of arterial FFA in NIDDM subjects. As a consequence, mean postprandial FFA uptake across the leg in NIDDM subjects was essentially unchanged from fasting rates and consequently greater than that of nondiabetic subjects. This study indicates that because of the insulin resistance of lipolysis in NIDDM there may be less suppression of FFA uptake by muscle in NIDDM during insulin-stimulated conditions. Inability to suppress muscle FFA uptake to the same extent as present in nondiabetics may be an additional characteristic of impaired insulin action in skeletal muscle of patients with NIDDM and could contribute to insulin resistance of skeletal muscle in this disorder. However, the current findings of impaired lipid oxidation by muscle in patients with NIDDM do not support the classic tenets of glucose–FFA substrate competition as a basis for insulin resistance (41). Nevertheless, several recent studies indicate that FFA impair insulin stimulation of muscle glucose storage and activation of the enzyme glycogen synthase (42, 43) and have possible adverse effects on glucose transport (44). Therefore, it remains an open question as to whether and by which mechanisms FFA could affect insulin action on skeletal muscle in patients with NIDDM.

In summary, the normal predominance of skeletal muscle lipid oxidation during postabsorptive conditions does not prevail in NIDDM, instead there is reduced utilization of FFA by muscle. After meal ingestion there is less suppression of FFA uptake by muscle, yet the defect of muscle lipid oxidation persists in NIDDM. Characteristics of muscle fiber type, capillary density, and of enzyme markers for glycolytic and aerobic-oxidative metabolism were equivalent in NIDDM and nondiabetic subjects, making it unlikely that these factors account for the observed differences in FFA utilization. Further studies are needed to determine the responsible mechanisms, particularly the roles of hyperglycemia, skeletal muscle proteins involved in FFA transport, and of enzymes regulating lipid oxidation. The findings of the current study indicate that, in addition to the well described impairment of insulin-stimulated glucose metabolism in skeletal muscle of patients with NIDDM, there is also an impaired pattern of FFA utilization by muscle.

Acknowledgments

The cooperation of our research volunteers is gratefully acknowledged, along with the patient care rendered by the nursing and nutritional staff of the General Clinical Research Center, and in particular the expert

clinical skills of Janice Beattie. Sue Andreko, Alycia Crawley, Christina Thompson, and Yves Gélinas provided expert technical and analytic support.

This project was supported by funding from the Department of Veterans Affairs Merit Award (D. Kelley), by a Career Development Award from the American Diabetes Association (D. Kelley), by the Fonds de la Recherche en Santé du Québec (J.-A. Simoneau), the National Sciences and Engineering Research Council of Canada (J.-A. Simoneau), and by a National Institutes of Health General Clinical Research Center grant (5MO1RR00056).

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