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**Research Article**

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# Multiple Disturbances of Free Fatty Acid Metabolism in Noninsulin-dependent Diabetes

## Effect of Oral Hypoglycemic Therapy

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## Abstract

To assess the mechanisms for the elevation of free fatty acids in noninsulin-dependent diabetes, free fatty acid metabolism and lipid and carbohydrate oxidation were compared in 14 obese diabetic Pima Indians and in 13 age-, sex-, and weight-matched nondiabetics. The studies were repeated in 10 of the diabetics after 1 mo of oral hypoglycemic therapy. Fasting plasma glucose concentrations were elevated in diabetics (242±14 vs. 97±3 mg/dl, $P < 0.01$) and decreased to 142±12 ($P < 0.01$) after therapy. Fasting free fatty acid concentrations were elevated in diabetics (477±26 vs. 390±39 μmol/liter, $P < 0.01$) and declined to normal values after therapy (336±32, $P < 0.01$). Although free fatty acid transport rate was correlated with obesity ($r = 0.75$, $P < 0.001$), the transport of free fatty acid was not higher in diabetics than in nondiabetics and did not change after therapy. On the other hand, the fractional catabolic rate for free fatty acid was significantly lower in untreated diabetics (0.55±0.04 vs. 0.71±0.06 min$^{-1}$, $P < 0.05$); it increased after therapy to 0.80±0.09 min$^{-1}$, $P < 0.05$, and was inversely correlated with fasting glucose ($r = -0.52$, $P < 0.01$). In diabetics after therapy, lipid oxidation rates fell significantly (from 1.35±0.06 to 1.05±0.01 mg/min per kg fat-free mass, $P < 0.01$), whereas carbohydrate oxidation increased (from 1.21±0.10 to 1.73±0.13 mg/min per kg fat-free mass, $P < 0.01$); changes in lipid and carbohydrate oxidation were correlated ($r = 0.72$, $P < 0.02$), and in all subjects lipid oxidation accounted for only ~40% of free fatty acid transport. The data suggest that in noninsulin-dependent diabetics, although free fatty acid production may be elevated because of obesity, the elevations in plasma free fatty acid concentrations are also a result of reduced removal, and fractional clearance of free fatty acid appears to be closely related to diabetic control. Furthermore, the increase in fractional clearance rate, despite a marked decrease in lipid oxidation, suggests that the clearance defect in the diabetics is due to an impairment in reesterification, which is restored after therapy.

## Introduction

Plasma FFA concentration may be regulated by rates of both appearance and disappearance. Inflow of FFA depends on the rate of lipolysis in adipose tissues and also on re-

lease of FFA during the hydrolysis of circulating triglyceride-rich particles, particularly in the postabsorptive state (1, 2). The rate of FFA removal is determined by both esterification (or reesterification) and lipid oxidation (1, 2). It has been generally accepted that plasma FFA concentration is controlled mainly by FFA production (i.e., by the rate of lipolysis), whereas the efflux rate of FFA is secondary to change in plasma FFA concentration (3–6). This concept implies that the removal of FFA from plasma is not controlled independently (3–6). Initially, the interaction of FFA and glucose metabolism was suggested by Randle and co-workers 20 yr ago, when they proposed a glucose-fatty acid cycle (7, 8). Recently, interest in the interrelations between FFA and glucose metabolism has been rekindled, and several reports have emphasized the close interaction between FFA and glucose metabolism. It has been shown using the euglycemic clamp and indirect calorimetry that in nondiabetic subjects an elevation of plasma FFA is accompanied by an increase in lipid oxidation and a concomitant decrease in glucose oxidation (9, 10), and that lipid oxidation and carbohydrate oxidation in the basal state are also inversely related (10). In diabetics, fasting FFA correlates positively with endogenous glucose production (11), and Ferranini et al. (12) have shown that during a hyperglycemic hypoinsulinemic clamp, glucose production is enhanced in the presence of increased FFA.

In uncontrolled diabetes, the concentration of FFA in plasma is commonly elevated (13–15), but the mechanisms leading to the rise of plasma FFA in diabetes have not been thoroughly studied. It has been assumed that the elevation of plasma FFA is primarily caused by enhanced FFA mobilization as a consequence of decreased insulin. This hypothesis is consistent with the insulin deficiency in untreated type I diabetic patients, but it can be questioned in type II diabetes, where circulating insulin remains available. Recent studies both in vivo and in vitro suggest that the antilipolytic action of insulin in type II diabetics remains very sensitive (16–18), and available kinetic data on FFA metabolism in type II diabetics have indicated that the turnover rate of FFA is increased in some (19), but not in others (20).

Therefore, the present investigation was designed to thoroughly study FFA metabolism and its relation to substrate oxidation in type II diabetics. FFA turnover was measured using labeled FFA infusion, and lipid and carbohydrate oxidation were evaluated using indirect calorimetry in 14 obese diabetic Southwest American Indians and in 13 age-, sex-, and weight-matched nondiabetic subjects. To evaluate the influence of diabetic control on these parameters, the studies were repeated in 10 diabetic patients after blood glucose was lowered with 1 mo of oral hypoglycemic therapy.

## Methods

*Subjects.* 14 obese diabetic and 13 obese nondiabetic Pima/Papago Indian volunteers were admitted to the Phoenix Clinical Research

Section for the study (Table I). After written informed consent was obtained, the subjects were placed on a weight-maintaining diet composed of 45% carbohydrate, 40% fat, and 15% protein; patients were weighed daily and calories adjusted to maintain initial weight throughout the study. Known duration of diabetes was <5 yr (8 mo–5 yr), and all patients were untreated at the time of entrance into the study. Five subjects had been treated previously with oral agents (one subject with tolbutamide, four subjects with chlorpropamide, and three subjects had a history of temporary insulin therapy). Previous antidiabetic therapy was stopped at least 1 mo before the study. No subjects were taking any medication at the time of the study, and all had a normal physical examination, electrocardiogram, and blood tests (liver function tests, blood urea nitrogen, creatinine, and thyroid hormones.)

Body composition of the subjects was determined by underwater weighing with correction for simultaneously measured residual lung volume (21). The clinical characteristics of the subjects are summarized in Table I. After at least 4 d on the standard diet, the subjects underwent tests of glucose tolerance, fatty acid metabolism, and indirect calorimetry as described below. After the base-line studies, tolazamide treatment (Tolinase, Upjohn Co., Kalamazoo, MN) was initiated for the diabetics and continued for 6 wk. Glycemic control was monitored by plasma glucose determinations (fasting and 4:00 p.m.) carried out every second day during the first week of therapy and twice per week thereafter. The dose of tolazamide was increased gradually during the first week to obtain optimal glycemic control. The maximal dose of tolazamide was 1,000 mg/d in two doses (500 mg 30 min before breakfast and 500 mg 30 min before supper.) Studies of glucose tolerance, fatty acid metabolism, and indirect calorimetry were repeated after tolinase treatment for 4 wk. Four subjects discontinued the study before the end of the treatment period, and therefore they did not complete the repeat tests.

*Oral glucose tolerance test.* The 75-g oral glucose tolerance test was performed after a 10-h overnight fast. Venous blood samples for plasma glucose were drawn at −15, 0, 30, 60, and 120 min. All control subjects had a normal glucose tolerance (fasting plasma glucose < 115 mg/dl and 2 h < 140 mg/dl) (22).

*Standard breakfast test.* On the morning after the oral glucose tolerance test, an indwelling intravenous catheter was placed in an arm vein at 7:00 a.m. The breakfast contained 30% of the daily weight maintaining caloric requirements with the same caloric distribution of

carbohydrate, fat, and protein as in the standard diet. Samples for plasma glucose, insulin, and C-peptide were drawn 15 min before and just before starting the meal, and thereafter every 30 min for 4 h.

*FFA metabolism.* The measurement of FFA metabolism was performed together with simultaneous indirect calorimetry after a 10-h overnite fast. [1-¹⁴C]Palmitate (New England Nuclear, Boston, MA; 56.3 mCi/mmol) was complexed to human serum albumin (U. S. Pharmacopeia, Albuminar-25; Armour Pharmaceutical Co., Kankakee, IL). The final solution contained 10 mg/ml albumin, 0.5 μCi/ml [1-¹⁴C]palmitate, and 2.3 mg/ml Na palmitate. An indwelling catheter was placed in an antecubital vein at 7:00 a.m. The infusion of labeled palmitate was started at 7:30 a.m. at a rate of 0.5 ml/min and continued for 60 min. Blood samples were drawn from an indwelling catheter in an opposite antecubital vein 30 min before and just before the start of the infusion for measuring the base-line FFA concentration. Blood samples were drawn at 7-min intervals starting 30 min after the commencement of the palmitate infusion for the determination of FFA concentration and its specific activity. The intraindividual variation of FFA specific activity within the four assayed samples ranged from 2.5 to 4.1%, and it has been previously documented that during the experimental conditions employed, a steady state of FFA specific activity was reached during the sampling period (10). Plasma volume was measured after the end of the palmitate infusion using a bolus injection of Evans Blue (23).

*Indirect calorimetry.* 15 min before the start of the palmitate infusion, a clear plastic ventilated hood was placed over the subject's head. Room air was drawn through the hood and the flow rate was measured using a pneumotachograph (Gould Inc., Cleveland, OH). A constant fraction of expired air was withdrawn and analyzed for oxygen and carbon dioxide content. The oxygen analyzer was a zirconium cell analyzer (Applied Electrochemistry, Sunnyvale, CA), and the carbon dioxide analyzer was infrared analyzer (Applied Electrochemistry). The analyzers and flow meter outputs were connected to a desk top computer (Hewlett-Packard Co., Palo Alto, CA), which recorded continuous integrated calorimetric measurements over 5-min intervals. The recorded values over the last 45 min were averaged and used for calculations. The protein oxidation during the test was estimated from the urinary nitrogen production rate. The nonprotein respiratory quotient was then calculated and the substrate rates determined from the tables of Lusk (24).

*Analytic methods.* Glycosylated hemoglobin was analyzed by using the method of Welch et al. (25). Plasma insulin concentrations were determined by the method of Herbert et al. (26). C-Peptide was measured by the method of Heding (27), and human C-peptide was used as standard.

All the samples for fatty acid determinations were collected in tubes containing diethyl *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO; 1.1 mg/ml) and kept on ice. Concentrations of FFA in plasma were measured using the microfluorometric method of Miles et al. (28). The FFA assay was standardized using reference pools whose values were assayed by titration. For the determinations of FFA specific activity, 2 ml of plasma were extracted with 10 ml of Dole's extraction mixture (29). Fatty acids were then isolated from the lipid extract by using 0.02 N NaOH. After acidification they were reextracted using heptane. A portion of the extract was evaporated to dryness and the radioactivity was measured in Atomlight (New England Nuclear) scintillation liquid in an 1800 counter (Beckman Instruments Inc., Fullerton, CA). Another portion of the heptane extract was used for the determination of FFA concentration by the method of Ho (30).

*Calculations.* FFA transport rate (turnover) was calculated as the rate of infusion divided by steady state plasma FFA specific activity: FFA transport (μmol/min) = [Infusion rate (dpm/min)]/[specific activity (dpm/μmol)].

The values reported are the mean of the four steady state samples. The fractional catabolic rate (FCR)[1] for FFA is the fraction of plasma

*Table I. Clinical Characteristics of the Subjects*

| | | Diabetics | |
| --- | --- | --- | --- |
| | Nondiabetics | Before therapy | After therapy |
| Females/males | 7/6 | 9/5 | 6/4 |
| Age (*yr*) | 27±2 | 31±3 (31±4) | 31±4 |
| Weight (*kg*) | 107±6 | 97±7 (98±7) | 100±9 |
| Height (*cm*) | 165±2 | 163±2 (162±3) | 162±3 |
| IBW (%) | 181±13 | 169±11 (171±12) | 176±12 |
| Percent fat | 37±3 | 37±2 (37±2) | 38±2 |
| FFM (*kg*) | 68±3 | 60±4 (62±5) | 63±5 |
| Plasma volume (*ml*) | 3,172±184 | 3,076±199 (2,952±253) | 2,952±253 |

The data are mean±SEM. Numbers in parentheses are pretreatment values for the 10 subjects who completed the therapy. IBW, ideal body weight.

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1. *Abbreviations used in this paper:* FCR, fractional catabolic rate; FFM, fat-free mass.

pool cleared per minute, and it was calculated as follows: FCR (min⁻¹) = [turnover ($\mu$mol/min)]/[pool size ($\mu$mol)].

Because of the large range of obesity in the subjects, FFA transport rate was expressed as micromole per minute (net transport rate), as well as micromole per minute per kilogram body weight, micromole per minute per kilogram fat-free mass (FFM), and micromole per minute per kilogram fat mass.

Percentage of FFA oxidized = [lipid oxidation (mg/min)]/[FFA transport (mg/min)].

*Statistics.* All the statistical analyses were performed using the Statistical Analysis System, SAS Institute, Inc., Cary, NC. All data were expressed as the mean±SEM and relationships were evaluated using simple Pearson correlation coefficients. To assess possible relationships between FFA metabolism, substrate oxidation, glucose tolerance, and obesity, correlation analyses were performed on diabetics before and after therapy, and also on all subjects before therapy. Significance of differences between nondiabetics and diabetics was evaluated using unpaired $t$ test and, of differences between diabetics before and after therapy, by paired $t$ test procedure.

## Results

The diabetic subjects had marked hyperglycemia before the therapy. After 1 mo of tolazamide therapy, mean fasting and 2-h glucose concentrations decreased significantly, but glucose tolerance was not normalized (Table II). The improvement of glycemic control during the treatment was reflected also in a significant decrease in glycosylated hemoglobin (Table II). The mean weight of the diabetic subjects remained constant during the treatment period.

*Standard breakfast test.* Compared with nondiabetics, the untreated diabetic patients had marked hyperglycemia after breakfast. The postbreakfast glucose profile improved markedly after therapy, but it did not increase to the nondiabetic level (Fig. 1 *A*). Fasting plasma insulin and C-peptide concentrations were the same in nondiabetics and in diabetics before and after therapy (Fig. 1, *B* and *C*). In untreated diabetics, the early postprandial insulin response was deficient, and plasma insulin values were significantly lower than in nondiabetics at 30, 60, and 90 min (Fig. 1 *B*). The mean insulin response, as measured by the area under the curve, was threefold less in
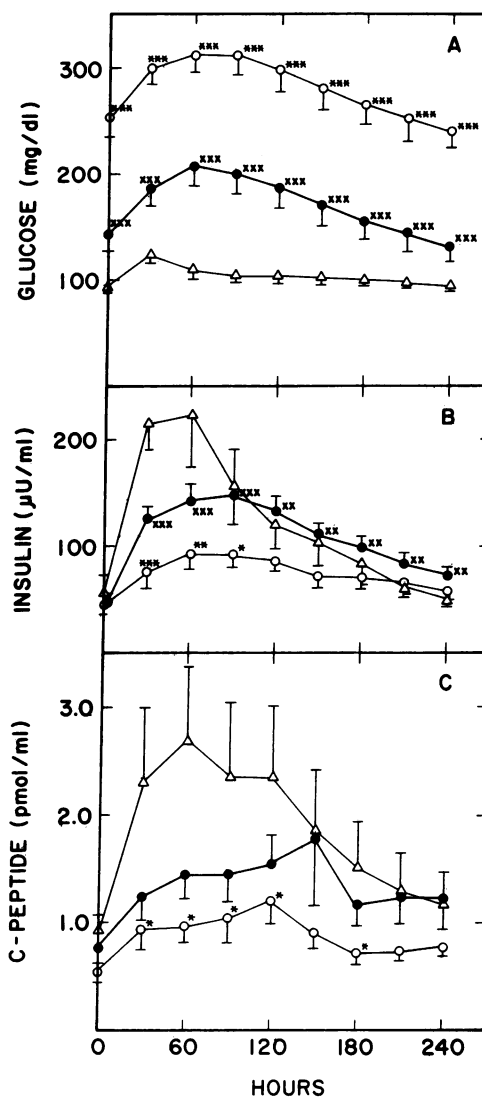
### Table II. Glycemic Control Before and After Therapy

| | | Diabetics | |
| --- | --- | --- | --- |
| | Nondiabetics | Before therapy | After therapy |
| Glucose (*mg/dl*) | | | |
| Fasting | 97±3 | 247±14* (256±17) | 147±12‡ |
| 2 h after OGTT | 135±9 | 382±17* (385±22) | 285±20‡ |
| HBA₁ (%) | 5.8±0.2 | 12.4±0.5* (12.8±0.06) | 9.4±0.6‡ |

The results are mean±SEM. Numbers in parentheses are pretreatment values for the 10 subjects who completed the therapy. HBA₁, hemoglobin A₁; OGTT, oral glucose tolerance test.
* $P < 0.01$ for difference from values in nondiabetics.
‡ $P < 0.01$ for difference from values before therapy.



*Figure 1.* Responses of plasma glucose (*A*), plasma insulin (*B*), and C-peptide (*C*) concentrations to a standard breakfast consumed after time zero values were measured in obese nondiabetic subjects (△) and in obese diabetic subjects before therapy (○) and after therapy (●). Values are the mean±SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ in comparison between values for diabetics before and after therapy.

diabetics before therapy than in controls (mean±SEM; 6,678±2,246 vs. 20,107±3,515 $\mu$U·min/ml, $P < 0.01$). After therapy, the mean insulin response (area under the curve) was not significantly different than in controls (mean±SEM, 16,481±2,306 $\mu$U·min/ml). In agreement with the deficient insulin response, postbreakfast C-peptide concentrations were significantly reduced in untreated diabetics (Fig. 1 *C*). The mean C-peptide response (area under the curve) averaged 40% of that observed in nondiabetics (91±20 vs. 226±51 pmol·min/ml, $P < 0.01$). After therapy, the mean response improved to 139±27 pmol·min/ml, but it was still significantly lower than in nondiabetics ($P < 0.01$).

*Plasma FFA.* Plasma FFA concentrations were higher in untreated diabetics than in controls (Table III), although the difference did not reach statistical significance ($P = 0.07$). FFA

Table III. Parameters of FFA Metabolism

| | Nondiabetics (n = 13) | Diabetics Before therapy (n = 14) | Diabetics After therapy (n = 10) |
|---|---|---|---|
| Plasma FFA (μmol/liter) | 390±39 | 477±26 (508±31) | 336±32* |
| Total FFA transport (μmol/min) | 813±76 | 776±60 (811±74) | 784±152 |
| FFA transport per kilogram FFM (μmol/min per kg FFM) | 12.3±0.9 | 12.8±0.6 (13.0±0.8) | 11.9±1.3 |
| FFA transport per kilogram body weight (μmol/min per kg body wt) | 7.6±0.5 | 7.9±0.4 (8.1±0.5) | 7.5±0.8 |
| FFA transport per kilogram fat mass (μmol/min per kg fat) | 22.0±2.1 | 22.3±2.1 (22.3±2.2) | 21.3±2.8 |
| FCR (min⁻¹) | 0.71±0.06 | 0.55±0.04‡ (0.56±0.05) | 0.80±0.09§ |
| Percentage of FFA oxidized | 44±5 | 42±2 (42±2) | 39±5 |

Values are mean±SEM. Numbers in parentheses are pretreatment values for the 10 diabetics who completed the therapy. Calculation of percent of FFA oxidized is a maximum value that assumes all fat oxidation is derived from plasma FFA.
* $P < 0.01$ for differences from values before therapy.
‡ $P < 0.05$ for difference from values in nondiabetics.
§ $P < 0.05$.

concentration was related to the degree of obesity, being positively correlated with percentage fat (Table IV A) in the group of nondiabetics and diabetics before therapy. FFA concentration in diabetics fell significantly after therapy (Table III). After therapy, FFA concentration in diabetics was similar to the nondiabetic group, even though glucose tolerance was not normalized. In diabetics, FFA concentration showed a significant positive correlation with plasma glucose ($r = +0.73$, $P < 0.001$, Table IV B).

*FFA transport.* The total transport rate (turnover) of FFA, as well as transport rates expressed per kilogram FFM, per kilogram body weight, and per kilogram fat mass, were not different between nondiabetics and diabetics before and after therapy (Table III). FFA concentration did not correlate with the net FFA transport rate (Table IV, A and B) or with FFA transport expressed per kilogram body weight or per kilogram fat mass (data not shown). In this group of subjects, FFA transport rates were not related to either lipid or carbohydrate oxidation, and the turnover of FFA was not related to fasting plasma glucose (Table IV, A and B). FFA transport rate was related to body composition, being positively correlated with both fat mass and FFM (Fig. 2). After therapy, the mean for

FFA transport was not significantly different from that before therapy (Fig. 3, A and B).

*Clearance of FFA.* The major difference in FFA metabolism between the nondiabetics and diabetics before and after therapy was observed in the clearance of FFA. The mean for FFA FCR was significantly lower in untreated diabetics than in the controls (Fig. 3), and, in 13 of the 14 diabetics, FCR was less than the mean for nondiabetics. Plasma FFA correlated negatively with FCR (Table IV, A and B). The FCR for FFA was not significantly related to either fat mass or FFM (data not shown). FCR correlated inversely with fasting blood glucose (Table IV, Fig. 4) as well as with the glucose response after breakfast ($r = -0.53$, $P < 0.01$ for nondiabetics and untreated diabetics, and $r = -0.58$, $P < 0.01$ for diabetics before and after therapy). After therapy, the clearance of FFA increased in seven of the nine patients, and the mean value for FCR was significantly higher after therapy than before (Fig. 3 B).

*Lipid and carbohydrate oxidation rates.* Nonprotein RQs in the diabetics before therapy were not different from the nondiabetics (0.79±0.01 vs. 0.80±0.01). After therapy, the RQ increased to 0.83±0.01 ($P < 0.01$).

The mean rates of carbohydrate and lipid oxidation (Fig. 5, A and B) were not significantly different in untreated diabetics compared with nondiabetics. Lipid oxidation correlated negatively with carbohydrate oxidation (Table IV). Both lipid and carbohydrate oxidation were related to body composition; lipid oxidation was positively and carbohydrate oxidation negatively related to percent fat (Table IV). In the group of diabetics before and after therapy, lipid oxidation correlated positively with fasting plasma glucose (Table IV B), and carbohydrate oxidation showed an inverse but not significant relation with fasting plasma glucose ($r = -0.23$, NS). After treatment for 1 mo, the lipid and carbohydrate oxidation rates in diabetics showed reciprocal changes; the lipid oxidation rates fell significantly, whereas the carbohydrate oxidation rates increased (Fig. 5, A and B). When the changes of lipid oxidation during the therapy were compared with those of carbohydrate oxidation, a highly significant correlation was observed (Fig. 6). Thus, the patients with the greatest fall of lipid oxidation showed the highest rise in carbohydrate oxidation and vice versa. The changes of neither lipid nor carbohydrate oxidation were significantly related to changes in plasma glucose.

*Fraction of FFA oxidized.* The lipid oxidation compared as fraction of FFA turnover was similar in nondiabetics and diabetics before and after therapy (Table III). The fraction of FFA oxidized was not related to plasma FFA concentration, fasting blood glucose, or body composition (data not shown). The percentage of FFA oxidized correlated negatively with RQ ($r = -0.66$, $P < 0.001$), with the net FFA transport ($r = -0.49$, $P < 0.01$), and with the FCR ($r = -0.52$, $P < 0.001$).

## Discussion

The present study demonstrated that in obese, untreated diabetics, the mean plasma FFA concentration was elevated; FFA transport was not higher than in obese nondiabetics, but there was a markedly reduced FCR for FFA. After therapy, there was a significant fall of FFA concentration, together with an increase of FFA fractional clearance rate, whereas FFA transport was not changed. Furthermore, enhancement of FFA

## Table IV. Correlation Coefficients

| | Fasting FFA | FFA turnover | FCR | Lipid oxidation | Carbohydrate oxidation | Fasting glucose | Percent fat |
|---|---|---|---|---|---|---|---|
| **A Simple (Pearson) correlation coefficients in nondiabetics and untreated diabetics ($n = 27$)** | | | | | | | |
| Fasting FFA ($\mu mol/liter$) | 1.00 | NS | −0.59§ | NS | −0.39* | 0.53‡ | 0.51§ |
| FFA turnover ($\mu mol/min$) | | 1.00 | +0.42* | NS | NS | NS | NS |
| FCR ($min^{-1}$) | | | 1.00 | NS | NS | −0.52‡ | −0.39* |
| Lipid oxidation ($mg/min$ per $kg$ FFM) | | | | 1.00 | −0.56‡ | NS | +0.70§ |
| Carbohydrate oxidation ($mg/min$ per $kg$ FFM) | | | | | 1.00 | NS | −0.58‡ |
| Fasting glucose ($mg/dl$) | | | | | | 1.00 | NS |
| Percent fat | | | | | | | 1.00 |
| **B Simple (Pearson) correlation coefficients in diabetics before and after therapy ($n = 24$)** | | | | | | | |
| Fasting FFA ($\mu mol/liter$) | 1.00 | NS | −0.59§ | NS | −0.40* | 0.73§ | NS |
| FFA turnover ($\mu mol/min$) | | 1.00 | 0.60§ | NS | NS | NS | NS |
| FCR ($min^{-1}$) | | | 1.00 | NS | NS | −0.51‡ | NS |
| Lipid oxidation ($mg/min$ per $kg$ FFM) | | | | 1.00 | −0.75‡ | 0.38* | 0.54‡ |
| Carbohydrate oxidation ($mg/min$ per $kg$ FFM) | | | | | 1.00 | NS | −0.45* |
| Fasting glucose ($mg/dl$) | | | | | | 1.00 | NS |
| Percent fat | | | | | | | 1.00 |

Correlations for nondiabetics alone were: FFA vs. FCR, $r = −64$, $P < 0.05$; FFA vs. carbohydrate oxidation, $r = −0.56$, $P = 0.05$; FFA vs. fasting glucose, $r = 0.47$, $P = 0.10$; FFA vs. percent fat, $r = 0.70$, $P < 0.01$; FFA turnover vs. FCR, $r = 0.43$, $P = 0.14$; FCR vs. fasting glucose, $r = 0.54$, $P = 0.05$; FCR vs. fat, $r = 0.46$, $P = 0.11$; lipid oxidation vs. carbohydrate oxidation, $r = −0.55$, $P = 0.06$; lipid oxidation vs. percent fat, $r = 0.49$, $P = 0.04$; carbohydrate oxidations vs. percent fat, $r = −60$, $P < 0.05$.
Correlations for diabetics before therapy: FFA vs. FCR, $r = −0.33$, $P = 0.28$; FFA vs. carbohydrate oxidation, $r = −0.21$, $P = 0.48$; FFA vs. fasting glucose, $r = 0.64$, $P < 0.01$; FFA turnover vs. FCR, $r = 0.37$, $P = 0.21$; FCR vs. fasting glucose, $r = −0.30$, $P = 0.32$; lipid oxidation vs. carbohydrate oxidation, $r = 0.66$, $P < 0.01$; lipid oxidation vs. fasting glucose, $r = 0.27$, $P = 0.35$; lipid oxidation vs. percent fat, $r = 0.87$, $P < 0.001$; carbohydrate oxidation vs. percent fat, $r = −0.55$, $P < 0.05$.
* $P < 0.05$.  ‡ $P < 0.01$.  § $P < 0.001$.

fractional clearance rate occurred despite a marked decrease in lipid oxidation. The data are consistent with the hypothesis that in uncontrolled diabetes, esterification of FFA is impaired; consequently FFA removal is reduced and plasma FFA concentration is elevated. Furthermore, the results suggest that improvement of diabetic control restores the esterification capacity, resulting in enhanced fractional clearance of FFA and, consequently, in a fall of FFA concentration.
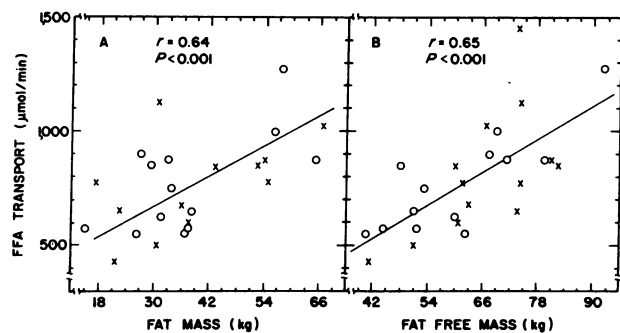


Figure 2. Relationships between fat mass (A) or FFM (B) and FFA transport in nondiabetics (x) and diabetic before therapy (o). FFA turnover data were not available for one diabetic subject, and body composition data were not available for one nondiabetic. For nondiabetics FFA transport vs. fat mass, $r = 0.70$, $P < 0.001$, and FFA transport vs. FFM, $r = 0.45$, $P = 0.14$; for diabetics FFA transport vs. fat mass, $r = 0.62$, $P < 0.05$, and FFA transport vs. FFM, $r = 0.81$, $P < 0.001$.
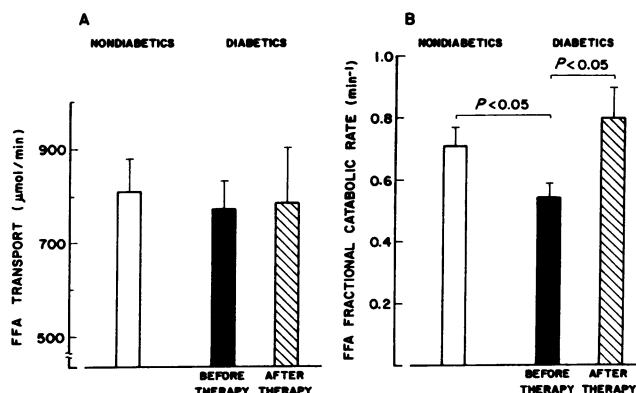


Figure 3. FFA transport (turnover rate) (A) and FFA FCR (B) in nondiabetics and in diabetics before and after therapy. The results are mean±SEM.
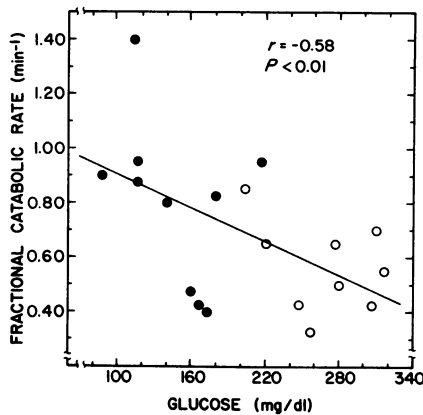
Figure 4. Relationship between FCR of FFA and fasting plasma glucose in diabetics before (o) and after (●) therapy. FFA turnover data were not available for one diabetic subject before therapy. For diabetics before therapy, $r = -0.30$, $P = 0.32$.

The proposed hypothesis would appear to be in contradiction to the concept that FFA turnover and concentration are determined solely by the rate of FFA influx (i.e., by lipolysis). This concept is based primarily on experiments in animals in which large pertubations of plasma FFA were induced (3–5). Under these conditions, FFA concentration shows a highly significant correlation with plasma turnover (3, 4). A significant relationship between FFA turnover and plasma FFA concentration has been demonstrated also in studies of nonobese and nondiabetic humans (6), but data on obese human subjects have been inconsistent. Some studies have confirmed a positive relation in obese subjects (31, 32), and others have not been able to demonstrate a correlation between turnover and plasma FFA (5, 33, 34). Furthermore, several previous observations suggest that changes of FFA input and output can occur separately. One example is the changes in FFA that occur at the initiation of exercise, when the fractional clearance rate of FFA is increased without change in FFA turnover (35). The dissociation of FFA production and removal has also been demonstrated after prolonged glucose consumption, which appears to increase the removal of FFA (36). In further studies, Nestel et al. (37) have shown that in obese subjects, the fractional clearance rate of FFA is diminished and is not stimulated by glucose to the same extent as in lean subjects. It is possible that the removal of FFA may be dependent on esterification capacity as well as lipid oxidation; the former
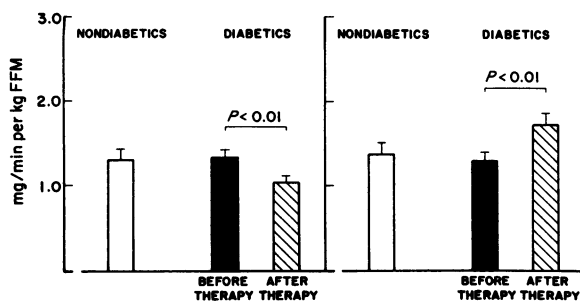


Figure 5. Lipid oxidation (left) and carbohydrate oxidation (right) rates in obese nondiabetic subjects and in diabetic patients before and after therapy.
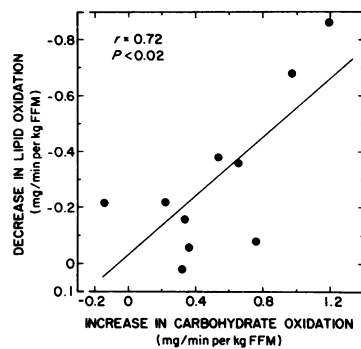


Figure 6. Relationship between the increase in carbohydrate oxidation and the decrease in lipid oxidation observed in diabetic subjects after 4 wk oral hypoglycemic therapy.

may be independently regulated, whereas the latter may be closely related to plasma FFA concentration, as emphasized in previous studies (38).

There was no relationship between FFA concentration and turnover in any of the groups in the present study. This suggests that in the presence of hyperglycemia, FFA concentration may be regulated by fractional removal of FFA as well as by production. This possibility is consistent with several of our observations. First, in untreated diabetics, the fractional clearance rate of FFA was markedly lower than in nondiabetics, without any elevation of FFA production. Secondly, the improvement of diabetic (glycemic) control during the therapy was associated with a significant rise of FFA removal, with no change in FFA production. Thirdly, both fractional clearance rate and FFA concentration were inversely correlated with fasting plasma glucose, whereas the transport rate of FFA was not related to plasma glucose. Finally, the indirect calorimetry measurements indicate that less than half of FFA turnover can be accounted for by oxidation, which implies that a significant amount may be reesterified. Thus, as proposed above, the data suggest that esterification may be diminished in diabetes, and that the correction of hyperglycemia may improve the esterification capacity.

There is in vitro evidence that there may be decreased esterification in diabetics (39). In adipocytes from type II diabetics, there is less incorporation of glucose (the only source in adipocytes of $\alpha$-glycerophosphate used in the esterification process) into triglycerides, and glucose conversion to triglyceride improves after the correction of hyperglycemia (17). Thus, even though plasma glucose is higher in diabetics, the relative insulin deficiency and/or insulin resistance may make it less available for glycerol formation. Another possibility is that the changes in FCR in diabetics are due to changes in the rate of incorporation of FFA into very low density lipoprotein (VLDL). The bulk of FFA removal could not be accounted for by this mechanism, however, since VLDL production rates in this population are only ~15% of the total fatty acid turnover (40).

Our findings are in agreement with both Csorba et al. (6) and Bolzano et al. (20), who also did not find any effect of diabetes on turnover of FFA. Lewis et al. (19) has reported increased fatty acid turnover for untreated type II diabetics compared with normal subjects; however, the majority of their diabetics were more obese than their controls. It has been shown by several workers that FFA turnover is higher in obese than in lean subjects (5, 31, 32), and FFA was correlated with obesity in the present group of subjects. Thus, increased FFA production appears to be closely related to obesity, and obesity

may contribute to the elevation of FFA concentration observed in type II diabetes.

Glycemic control also appeared to have a marked effect on substrate metabolism. First, lipid and carbohydrate oxidation showed reciprocal changes upon correction of hyperglycemia, and these changes occurred concomitantly with a fall of plasma FFA concentration. Secondly, lipid oxidation correlated positively with fasting glucose in diabetics before and after therapy. In a larger group of Pima Indians it has been shown that lipid oxidation is positively and carbohydrate oxidation is negatively related to fasting plasma glucose (11).

Elevation of plasma FFA concentration has also been observed to be associated with increased lipid oxidation and concomitant suppression of carbohydrate oxidation (9–11, 41–42). We have previously reported a positive correlation between lipid oxidation and plasma FFA (10), and a similar trend was observed also in this study, even though the correlation did not reach statistical significance. The present study design does not allow us to distinguish between the effects of glucose and FFA on substrate metabolism. However, it is plausible that, if esterification is impaired in untreated diabetes, more FFA is available for oxidation at a given level of plasma FFA. This could modulate the relation between lipid oxidation and plasma FFA.

In summary, in obese type II diabetes, elevation of plasma FFA may be caused by dual mechanisms: increased FFA production and decreased FFA removal. Increased FFA production rate may be associated with the degree of obesity and not influenced by severity of diabetes. Instead, reduced FFA removal capacity appears to be closely related to diabetic control, and is proposed to be due to impaired esterification due to deficient availability of $\alpha$-glycerophosphate. The improvement of diabetic control with oral sulphonylureas thus may enhance esterification capacity in peripheral tissues, improve FFA removal, and, consequently, result in a fall of plasma FFA.

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