Glucose and Free Fatty Acid Metabolism in Non-insulin-dependent Diabetes Mellitus

Evidence for Multiple Sites of Insulin Resistance

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Abstract

The effect of graded, physiologic hyperinsulinemia (+5, +15, +30, +70, +200 μU/ml) on oxidative and nonoxidative pathways of glucose and FFA metabolism was examined in nine lean non-insulin dependent diabetic patients (NIDDM) and in eight age- and weight-matched control subjects. Glucose and FFA metabolism were assessed using stepwise insulin clamp in combination with indirect calorimetry and infusion of [^{3}H]3-glucose/[^{14}C]palmitate. The basal rate of hepatic glucose production (HGP) was higher in NIDDM than in control subjects, and suppression of HGP by insulin was impaired at all but the highest insulin concentration. Glucose disposal was reduced in the NIDD patients at the three highest plasma insulin concentrations, and this was accounted for by defects in both glucose oxidation and nonoxidative glucose metabolism. In NIDDs, suppression of plasma FFA by insulin was impaired at all five insulin steps. This was associated with impaired suppression by insulin of plasma FFA turnover, FFA oxidation (measured by [^{14}C]palmitate) and nonoxidative FFA disposal (an estimate of reesterification of FFA). FFA oxidation and net lipid oxidation (measured by indirect calorimetry) correlated positively with the rate of HGP in the basal state and during the insulin clamp. In conclusion, our findings demonstrate that insulin resistance is a general characteristic of glucose and FFA metabolism in NIDDM, and involves both oxidative and nonoxidative pathways. The data also demonstrate that FFA/lipid and glucose metabolism are interrelated in NIDDM, and suggest that an increased rate of FFA/lipid oxidation may contribute to the impaired suppression of HGP and diminished stimulation of glucose oxidation by insulin in these patients.

Introduction

Insulin resistance is a characteristic feature of non-insulin dependent diabetes mellitus (NIDDM)[1] (1–4). The impairment in insulin-mediated glucose metabolism affects both hepatic and peripheral tissues, primarily muscle (1–6). On the other hand, the effect of insulin on FFA metabolism in NIDDM is controversial (7–14). A number of studies have demonstrated that basal FFA levels are increased in obese NIDD patients (7, 11, 12–16). However, there is contradictory evidence as to whether lipolysis is resistant to the effect of insulin in these patients (7–10, 14–16). Most in vitro studies have demonstrated normal antilipolytic effect of insulin in fat cells of subjects with NIDDM (8–10). Except for a study in Pima Indians (7), all in vivo studies have shown impaired suppression of plasma FFA by insulin in NIDD patients (12–16). The discrepancy between in vivo and in vitro studies is particularly striking, and could be explained by a number of factors including methodological differences. For example, in vitro lipolysis is measured directly, whereas in vivo changes in lipolysis are inferred from corresponding changes in circulating FFA levels.

More than 20 years ago Randle and co-workers introduced the concept of substrate competition, whereby enhanced availability and oxidation of FFA leads to an impairment in glucose oxidation (17). Thereafter, a number of studies have confirmed that elevated rates of lipid oxidation are associated with impaired oxidative glucose disposal in nondiabetic and NIDD subjects (18–24). However, it remains unproven whether enhanced lipid oxidation also inhibits the nonoxidative pathways of glucose metabolism (21–26). An association between elevated plasma FFA/lipid oxidation and excessive rates of hepatic glucose production (HGP) has also been suggested (4, 12, 19, 27). In normal subjects, elevated plasma FFA levels augment HGP under hyperglycemic and insulinopenic conditions (19). In addition, a positive correlation has been reported between the fasting plasma FFA concentration and the basal rate of HGP (12) and between lipid oxidation and HGP in obese NIDD patients (4).

Despite these potentially important interactions between lipid and glucose metabolism, both at the level of the liver and muscle, very little is known about the effect of physiologic hyperinsulinemia on the pathways of FFA metabolism in NIDDM. The plasma FFA concentration is determined by their rate of appearance from adipose tissue (lipolysis) and their rate of disappearance from plasma. The latter can occur by two pathways, oxidation to CO_2 and water or reesterification to triglycerides. At present, little information is available concerning the effect of insulin on these three pathways of FFA metabolism, nor is it known whether the effect of insulin on FFA metabolism is altered by the diabetic state.

The current study was designed to establish whether the insulin resistance of nonobese NIDD patients also involves FFA metabolism, and whether a deranged FFA metabolism might be linked with the defects in glucose metabolism.

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Received for publication 24 February 1988 and in revised form 25 January 1989.

1. *Abbreviations used in this paper:* CV, coefficients of variation; HGP, hepatic glucose production; NID, non-insulin diabetic; NIDD, non-insulin dependent diabetic; NIDDM, non-insulin-dependent diabetes mellitus; R_a, rate of glucose appearance.

J. Clin. Invest.

0021-9738/89/07/0205/09 $2.00
Volume 84, July 1989, 205–213

Methods

Subjects

Nine patients with onset of NIDDM after the age of 40 were compared with eight age- and weight-matched control subjects (Table I). The fasting plasma glucose concentration in the diabetic group was 172±14 mg/dl (mean±SEM), the duration of diabetes was 6±2 yr. Seven patients were treated with sulfonylureas, and two with diet alone. Sulfonylurea treatment was stopped 1 wk before the studies. None of the subjects had clinical or laboratory evidence of hepatic, renal, or other endocrine disease. The purpose, nature, and potential risks of the study were explained to all subjects, and written consent was obtained from each subject. The study protocol was approved by the Human Investigation Committee of Yale University School of Medicine.

Experimental protocol

A five-step euglycemic insulin clamp was performed on two separate days in random order. In the first study, the subjects received three different infusions of insulin (4, 20, and 100 mU/m^2 · min); during the second study they received insulin infusions of 10 and 40 mU/m^2 · min. All control subjects participated in both studies. Seven of the nine diabetic subjects participated in both studies; two diabetic subjects participated in only one study (10 and 40 mU/m^2 · min). The studies were carried out in combination with indirect calorimetry and infusions of 3-[^{3}H]glucose and [^{14}C]palmitate to allow quantitation of respiratory gas exchange, HGP, and FFA turnover, respectively.

Euglycemic insulin clamp. For the two-step insulin clamp (10 and 40 mU/m^2 · min), subjects were admitted to the Clinical Research Center of Yale University School of Medicine on the morning of the study. For the three-step clamp (4, 20 and 100 mU/m^2 · min), subjects were admitted on the evening before the study. All studies were started at 0800 hours after a 12-h overnight fast. After obtaining at least four basal samples for insulin, C-peptide, FFA, and glucose determination, a primed-continuous infusion of regular insulin (Eli Lilly & Co., Indianapolis, IN) was begun to acutely raise and maintain plasma insulin at the desired level. In control subjects, plasma glucose concentration was determined at 5-min intervals, and a variable infusion of 20% glucose was adjusted to maintain glycemia constant at the fasting level (28). Each hyperinsulinemic step lasted 100 min. In the NIDD subjects, no glucose was infused until plasma glucose had declined to the desired level. During the 4- and 10-mU/m^2 · min insulin steps, it was not possible to lower plasma glucose to the desired goal of 100 mg/dl even though the time interval was extended to 130 min. Therefore, from 90 to 130 min plasma glucose was clamped at the hyperglycemic level that was present at the 90-min time point. At 130 min, the insulin space was reprimed for the second step of the insulin clamp (i.e., 20 and 40 mU/m^2 · min) and plasma glucose was allowed to decline to 100 mg/dl, at which level it was clamped. The second (20 and 40 mU/m^2 · min) and third (100 mU/m^2 · min) steps of the insulin clamp each lasted 100 min. Blood samples for determination of plasma insulin, C-peptide, and FFA concentrations were drawn at 15-min intervals throughout the study.

Table I. Clinical Characteristics of the Subjects

	Control subjects	Diabetic subjects
Number (Females/Males)	8 (3/5)	9 (2/7)
Age (*yr*)	56±5	61±2
Body mass index (*kg/m^2*)	22.7±0.8	23.8±0.8
Fasting plasma glucose (*mg/dl*)	86±2	172±14*
Glycohemoglobin (HbA_1, %)	5.6±0.1	9.6±0.7*
Fasting plasma insulin (*μU/ml*)	6±1	7±1

Values represent the mean±SEM.
* $P < 0.01$ vs. control subjects.

Tritiated glucose. 120 min before the start of insulin, a primed (25 μCi)-continuous (0.25 μCi/min) infusion of 3-[^{3}H]glucose (New England Nuclear, Boston, MA) was begun in the control subjects. In diabetic subjects, the priming dose of 3-[^{3}H]glucose was increased in proportion to a degree of hyperglycemia, and the continuous infusion was extended to 180 min to ensure achievement of isotope equilibrium. Baseline samples for determination of 3-[^{3}H]glucose specific activity were drawn at 5-min intervals during the last 30 min of the equilibration period. During the clamp, 3-[^{3}H]glucose samples were drawn at 15-min intervals until the last 20 min of each insulin clamp step, during which time they were obtained at 5-min intervals.

FFA turnover. 1-[^{14}C]palmitate (New England Nuclear, Boston, MA) was used to trace plasma FFA turnover (29). The labeled FFA was supplied in toluene, and after drying under nitrogen it was resuspended in 25% human serum albumin. Before use, albumin-bound [^{14}C]-palmitate was filtered through a Millipore filter and checked for sterility and absence of pyrogens. The final solution contained ~ 0.5 μCi/ml of [^{14}C]palmitate. During the two-step clamp (10 and 40 mU/m^2 · min) a priming dose of 2.5 μCi of 1[^{14}C]palmitate was rapidly injected 150 min before starting the insulin infusion at 0800 hours, and followed by a constant infusion at the rate of 0.1 μCi/min throughout the study. Simultaneously with the administration of palmitate, a bolus injection of 3.5 μCi of 1-[^{14}C]$NaHCO_3$ (New England Nuclear, Boston, MA) was given to prime the bicarbonate pool.

At 2200 hours on the evening before the day of the three-step clamp, a continuous infusion of [^{14}C]palmitate (0.1 μCi/min) was begun and continued throughout the night. The insulin clamp study was then begun at 0800 hours. Baseline samples for determination of ^{14}C-FFA plasma specific activity were drawn at 5-min intervals during the last 30 min of the equilibration periods in both studies. All subjects achieved a steady state level of plasma ^{14}C-FFA specific activity at the end of the equilibration period (Fig. 1). In control subjects, and during the second and third insulin clamp steps in diabetic subjects, ^{14}C-FFA samples were drawn at 30, 60, 75, 90, 95, and 100 min. During the initial insulin clamp step in the diabetic subjects ^{14}C-FFA samples were obtained at 30, 60, 75, 90, 105, 120, 125, and 130 min. A new steady state of ^{14}C-FFA plasma specific activity was achieved during the last 40 min of each insulin clamp step (Fig. 1). To test the effect of time on FFA turnover, three subjects participated in a prolonged 4-mU/m^2 · min insulin clamp (240 min). The basal rate of FFA turnover was 433±78 μmol/min. During the clamp, there was no significant difference between the rate of FFA turnover from 60 to 120 min (234±99 μmol/min) and that from 180 to 240 min (228±110 μmol/min).

FFA oxidation. Oxidation of plasma FFA during [^{14}C]palmitate infusion was calculated from the specific activity of expired CO_2 in air samples obtained at 5-min intervals during the last 30 min of the equilibration period (30, 31). During the initial insulin clamp step in the diabetics, expired air samples were obtained at 30, 60, 75, 90, 105, 120, and 130 min; during all subsequent insulin steps (and during all steps in the control subjects) air samples were taken at 30, 60, 75, 90, and 100 min. Expired air was bubbled through a CO_2 trapping solution (1 M hyamine hydroxide/absolute ethanol:0.1% phenolphthalein;3:5:1). The solution was titrated with 1 N HCl to trap 1 mmol of CO_2 per 3 ml of solution. ^{14}C radioactivity was subsequently measured using a Tricarb scintillation counter (model 320; Packard Instruments Co., Inc., Downers Grove, IL) and expired $^{14}CO_2$ specific activity calculated. Total $^{14}CO_2$ expired per min was determined by multiplying $^{14}CO_2$ specific activity by the total CO_2 production rate measured by indirect calorimetry (see below).

During continuous or primed-continuous infusion of [^{14}C]-palmitate, the plasma ^{14}C-FFA specific activity achieves a steady state within 60 min (11, 29). However, using previously published infusion rates of [^{14}C]palmitate and [^{14}C]$NaHCO_3$, at least 8–10 h were required for $^{14}CO_2$ specific activity in expired air to reach steady state conditions. In the present study, we employed two approaches to obtain stable $^{14}CO_2$ specific activity (Fig. 1). In one study, the standard continuous [^{14}C]palmitate infusion method was employed, while for the second study the bicarbonate and palmitate pools were brought to equilibrium with the use of larger priming doses of [^{14}C]palmitate and

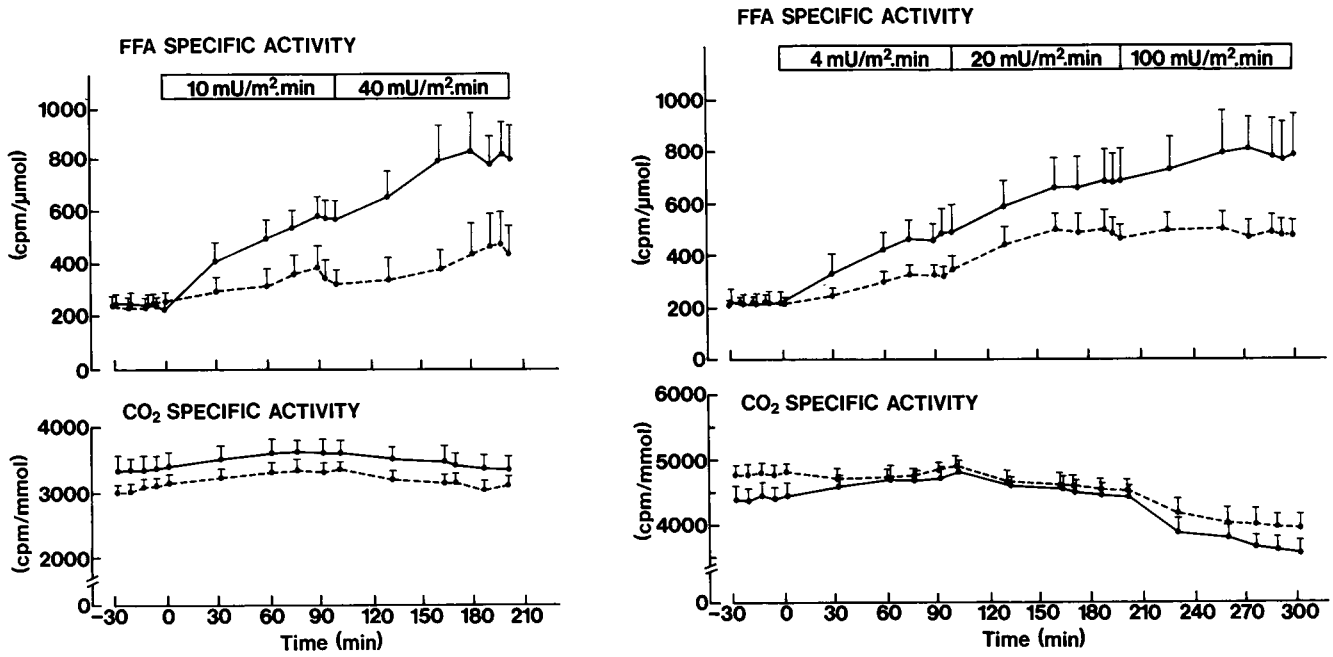

Figure 1. The specific activity of FFA in plasma (*top*) and of CO_2 in expired air (*bottom*) using the short (150 min) and long (720 min) equilibration period in control (*open circles*) and NIDD (*closed circles*) subjects. For the short equilibration period (*left*) a priming dose of 2.5 μCi [^{14}C]-palmitate was rapidly injected 150 min before starting the insulin infusion and followed by a constant infusion at the rate of 0.1 μCi/min. Simultaneously with the administration of [^{14}C]palmitate, a bolus injection of 3.5 μCi of 1-[^{14}C]$NaHCO_3$ was given to prime the bicarbonate pool. For the long equilibration period (*right*) a continuous infusion of [^{14}C]palmitate (0.1 μCi/min) was begun at 2200 hours the evening before the study and continued throughout the night. Values are mean±SEM.

[^{14}C]$NaHCO_3$. Both methods resulted in steady state concentrations of $^{14}CO_2$ in expired air during the last 30 min of the equilibration periods (Fig. 1). The basal rates of FFA oxidation as measured with the two methods were highly correlated with one another ($r = 0.91$; $P < 0.001$). The rate of FFA oxidation in the three subjects who participated in a prolonged 4 mU/m^2·min insulin clamp was 112±20 μmol/min basally and did not change significantly from 60 to 120 min (88±32 μmol/min) or from 180 to 240 min (92±35 μmol/min).

Respiratory exchange measurements. In all studies continuous indirect calorimetry was employed to estimate total net rates of carbohydrate and lipid oxidation (32). Starting 60 min before and throughout the clamp, continuous gaseous exchange measurements were performed with a ventilated hood system (Vista; Vacumed, Ventura, CA). Oxygen was measured by electrochemical analysis, CO_2 by an infrared analyzer using Applied Electrochemistry Instruments (Sunnyvale, CA). Protein oxidation was calculated from the nonprotein urinary nitrogen excretion measured before and during the insulin clamp (32).

Analytical procedures. Plasma glucose concentration was determined in duplicate by the glucose oxidase method on a Beckman glucose analyzer II (Beckman Instruments, Inc., Fullerton, CA). Methods for determination of plasma [3-^{3}H]glucose specific activity have been published previously (33). Plasma insulin and C-peptide concentrations were measured by specific radioimmunoassays (34, 35), urinary nitrogen excretion by the method of Kjeldahl (36), and plasma FFA concentrations by the microfluorometric method of Miles et al. (37). For the determination of ^{14}C-FFA specific activity 1.5 ml of plasma was extracted with 10 ml of Dole's solution. FFA were isolated from the lipid phase using 0.02 N NaOH and reextracted after acidification with heptane (11, 28). The heptane extraction was repeated three times and > 90% of the radioactivity was consistently recovered in the heptane phase. The extracts were dissolved in scintillation liquid (Scinti-Verse; Fisher Scientific, Springfield, NJ) and counted in a Tricarb scintillation counter.

Calculations. Rates of glucose appearance (R_a) and disposal in non-steady-state were calculated from 3-[^{3}H]glucose data according to the model described by Radziuk et al. (38). The infusion rate of cold glucose was integrated over 20-min intervals and subtracted from the total R_a to obtain HGP. Negative numbers for HGP were observed only at the 40- and 100-mU/m^2·min clamp steps. As recently demonstrated on theoretical as well as experimental grounds (39), such underestimation of glucose turnover by the tracer method is largely accounted for by a model error emerging at high rates of glucose metabolism. Because a simple and satisfactory correction for changes in the glucose system during the non-steady state is not yet available, we took the negative numbers to indicate a nil HGP. The basal rates of HGP obtained during the two studies differed from each other by < 10% in all subjects; the basal values shown in the figures represent the mean of the two studies. The plasma insulin concentration for half-maximal insulin effects were determined using log-logit plots (8).

Total body glucose metabolism was calculated by adding the mean rate of HGP (if a positive number) during the last 40 min of each insulin step to the mean glucose infusion rate during the same period. Nonoxidative glucose metabolism was calculated as the difference between total body glucose uptake and glucose oxidation, as determined by indirect calorimetry.

Net glucose and lipid oxidation rates were estimated from indirect calorimetric measurements. The constants to calculate glucose, lipid, and protein oxidation from gas exchange data and nonprotein urinary nitrogen excretion were those given in reference 32. To allow direct comparison with ^{14}C-FFA oxidation, whole body net lipid oxidation was expressed in molar units by using the molecular weight of palmitate (mol wt = 256).

To examine whether the primed-continuous and the continuous infusion techniques would give the same rates of FFA turnover and oxidation, five healthy young subjects participated in each two experiments utilizing the short (150 min) and the long (720 min) equilibration periods. Plasma FFA concentrations were similar in both experiments, 570±90 and 540±60 μmol/liter. In addition, plasma FFA turnover (409±25 vs. 394±41 μmol/min) and oxidation (132±30 vs. 124±21 μmol/min) rates were approximately similar during the two

protocols. The basal rates presented therefore represent the mean from the two experiments. Palmitic acid accounts for about 30% of plasma FFA regardless of the plasma FFA concentration (40, 41). Since the fractional turnover of palmitate is very similar to that of total FFA, labeled palmitate can be taken to trace total FFA (42). FFA turnover was calculated as the ratio of labeled palmitate infusion rate to the steady state plasma FFA specific activity and is expressed as micromoles per square meter per min. Plasma FFA concentration and specific activity were constant during the last 30 min of the equilibration period and the last 40 min of each insulin clamp step (Fig. 1). Therefore, all calculated rates of FFA turnover pertain to steady state conditions.

Plasma FFA oxidation in the basal state and during the clamp was calculated from the ^{14}C radioactivity in expired CO_2 divided by the product of plasma FFA specific activity and a factor k, which takes into account the incomplete recovery of labeled $^{14}CO_2$ from the bicarbonate pool (29).

FFA oxidation rate (μmol/m^2 · min) = (specific activity $^{14}CO_2$) × VCO_2/ k × (specific activity ^{14}C-FFA), where VCO_2 = total CO_2 production (in μmol/min), and k = 0.81.

Nonoxidative FFA metabolism was calculated as the difference between FFA turnover and oxidation (31). Portal insulin concentrations were estimated as follows: $(I_{pv})_{ss} = (I_p)_{ss} + (I_{pv})_o [(CP)_o - (CP)_{ss}/(CP)_o]$, where I_{pv} and I_p are the portal and peripheral plasma insulin concentrations, respectively, CP is the arterialized plasma C-peptide concentration, and the subscripts ss and o indicate the steady state and the baseline, respectively (43). The portosystemic insulin concentration gradient was assumed to be 3 (44).

Statistical analysis. All data are presented as the mean±SEM. Basal values differed by < 10% between the short and long equilibration period; therefore, basal values presented represent the mean from the two experiments. Differences in time-course of any variable between diabetic and control subjects was tested by two-way analysis of variance for repeated measures (ANOVA) using a Biomedical Data Processing (Los Angeles, CA) computer program (4V). The significance of difference between single time points was tested with Student's unpaired t test. Correlation coefficients were calculated by standard formulae.

Results

During the 4- and 10-mU/m^2 · min insulin clamp steps, the diabetic patients were clamped at higher plasma glucose concentrations (152±17 and 119±11 mg/dl, respectively) than during the 20, 40, and 100 mU/m^2 · min insulin steps during which the plasma glucose concentrations were 116±5, 104±2, and 105±2 mg/dl, respectively (Fig. 2). The coefficients of variation (CV) in glycemia during the five steps were 4.9±0.4% in controls, and 3.5±0.4% in diabetic patients, respectively.

In control subjects, basal plasma insulin concentration was 6±1 μU/ml and rose to 10±1, 22±2, 37±4, 75±5, and 208±16 μU/ml during the five insulin steps (Fig. 2). The CV of plasma insulin concentrations during all five steps was 7.9±0.9%. In diabetic patients, basal plasma insulin level was 7±1 μU/ml and rose to 11±2, 24±4, 48±6, 82±5, and 236±8 μU/ml, with a CV of 7.8±0.6%.

Basal C-peptide concentrations in control and diabetic subjects were 0.22±0.04 and 0.27±0.06 nmol/liter, respectively (Fig. 2). At an insulin infusion rate of 10 mU/m^2 · min, there was a significant suppression of basal C-peptide concentration in both the control and diabetic subjects.

Glucose metabolism

Hepatic glucose production (Fig. 3). Basal HGP was significantly higher in diabetics than in controls (83±4 vs. 71±2 mg/m^2 · min; $P < 0.01$). In the diabetic patients, fasting plasma glucose showed a strong positive correlation with basal HGP (r = 0.82, $P < 0.01$). In the control group, an increment in estimated mean portal insulin level of only 5 μU/ml (from 19 to 24 μU/ml) was associated with a 50% reduction in HGP; HGP was suppressed by > 90% at a portal insulinemia of 45 μU/ml, and half-maximal suppression of HGP was achieved with a mean portal insulinemia of 17±2 μU/ml (peripheral insulin concentration of 14±1 μU/ml). Suppression of HGP by insulin was impaired in diabetic compared to control subjects at all but the highest insulin concentration ($P < 0.05$–0.01). Complete suppression of HGP was observed at portal insulin concentrations > 100 μU/ml in the diabetic group, with half-maximal suppression at 26±4 μU/ml ($P < 0.05$ vs. controls).

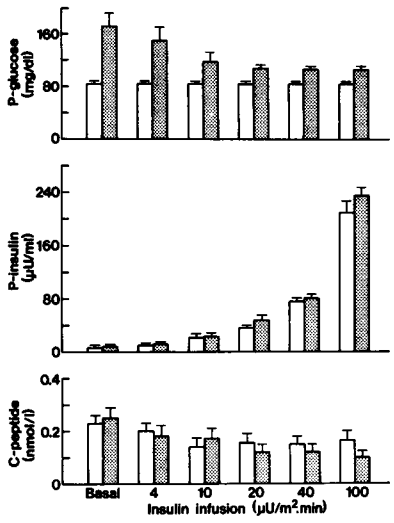

Figure 2. Basal and steady-state concentrations (mean±SEM) of plasma glucose, insulin and C-peptide during 4, 10, 20, 40, and 100 mU/m^2 · min insulin clamp steps in lean NIDD subjects (shaded bars) and in controls subjects (*open bars*). Values are mean±SEM.

Total body glucose metabolism (Fig. 4). In the postabsorptive state the rate of glucose disappearance equals the rate of glucose appearance (HGP). Therefore, in the basal state the diabetic patients metabolized more glucose than the controls (83±4 vs. 71±2 mg/m^2 · min; $P < 0.01$). Total body glucose metabolism did not increase significantly at the two lowest insulin infusion rates, and the rate of glucose uptake was not different between the diabetic and control subjects. During the 20 mU/m^2 · min insulin infusion, total glucose metabolism rose significantly in both control and diabetic groups with a

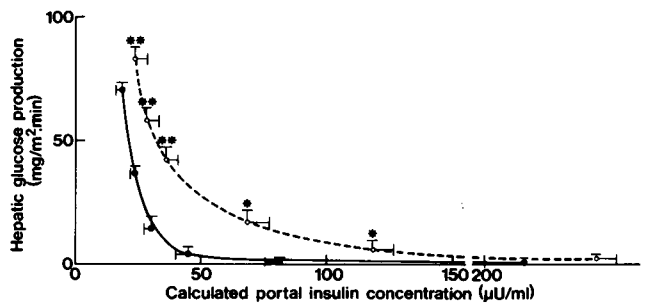

Figure 3. Rate of hepatic glucose production in the basal state and during graded hyperinsulinemia in nonobese NIDD (*broken line*) and in matched control (*solid line*) subjects. The x-axis shows the estimated portal insulin concentrations. Values are mean±SEM. *$P < 0.05$; **$P < 0.01$ versus control subjects.

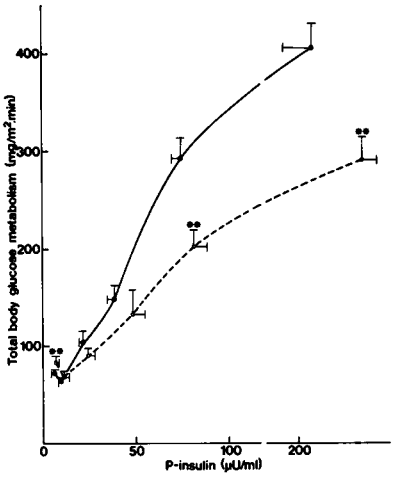

Figure 4. Rate of total body glucose disposal in the basal state and during graded hyperinsulinemia in nonobese NIDD (*broken line*) and in matched control (*solid line*) subjects. Values are mean±SEM. $^{**}P < 0.01$ versus control subjects.

slightly greater rise in the former. At the two highest insulin infusion steps, total glucose uptake was decreased by ~ 30% in the diabetic group compared to controls (both $P < 0.01$).

Glucose oxidation (Fig. 5). Basal glucose oxidation was slightly although not significantly lower in the diabetic versus control subjects (33±5 vs. 48±11 mg/m^2 · min; $P < 0.1$). Glucose oxidation was significantly enhanced by insulin only at insulin infusion rates > 20 mU/m^2 · min in both controls and diabetics. Compared to the controls, the patients showed impaired stimulation of glucose oxidation by insulin at the two highest insulin infusion rates (77±9 vs. 117±12, $P < 0.02$, and 91±4 vs. 132±11 mg/m^2 · min, $P < 0.01$, during the 40- and 100-mU/m^2 · min insulin clamp steps, respectively).

Nonoxidative glucose disposal (Fig. 5). In the basal state, nonoxidative glucose metabolism was greater in diabetic compared to control subjects (50±6 vs. 24±11 mg/m^2 · min, $P < 0.05$). A rise in plasma insulin concentrations to > 30 μU/ml was necessary before a significant enhancement of nonoxidative glucose disposal was observed in both the control and diabetic group. In the latter, nonoxidative glucose disposal was significantly reduced at the two highest plasma insulin concentrations.

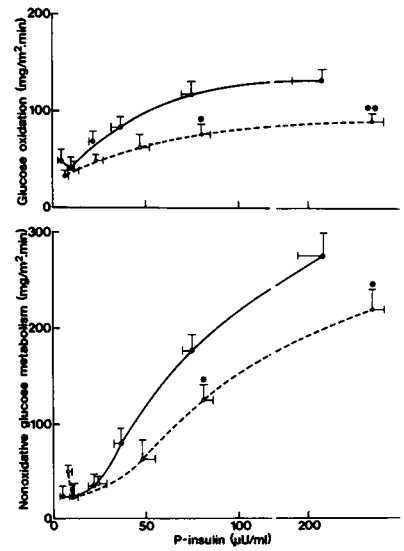

Figure 5. Rates of glucose oxidation (*top*) and nonoxidative glucose metabolism (*bottom*) in the basal state and during graded hyperinsulinemia in nonobese NIDD (*broken line*) and matched control (*solid line*) subjects. Values are mean±SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ versus control subjects.

FFA metabolism

Plasma FFA concentration (Fig. 6). The variation in fasting plasma FFA concentration between the two experiments was 8±2%. Basal plasma FFA concentration was slightly greater in the diabetics compared to controls (867±63 vs. 758±80 μmol/liter; P = NS). In the control group, an elevation of plasma insulin of only 4–5 μU/ml caused a 60% decrease in plasma FFA levels, and maximal suppression of these occurred at insulin concentrations less than 40 μU/ml; half-maximal suppression of plasma FFA could be estimated to occur at plasma insulin levels of 9±1 μU/ml. In the diabetic group, suppression of plasma FFA by insulin was significantly impaired at each insulin step ($P < 0.05$–0.01), and the ED_{50} was slightly higher, 15±3 μU/ml (P = NS).

Plasma FFA turnover (Fig. 6). Plasma FFA turnover closely paralleled the behavior of plasma FFA concentrations in both the control and the diabetic group ($r = 0.73$; $n = 16$; $P < 0.01$). In the NIDD subjects, basal FFA turnover was slightly, although not significantly, higher than in controls (302±32 vs. 249±27 μmol/m^2 · min). At the lowest insulin dose, plasma FFA turnover was nearly maximally suppressed in the controls. The NIDD patients showed a marked impairment in the ability of insulin to inhibit FFA turnover during each insulin step ($P < 0.05$), with a clear defect in maximal suppression (168±33 vs. 101±15 μmol/m^2 · min; $P < 0.05$).

Plasma FFA oxidation (Fig. 7). Approximately 30% of plasma FFA turnover was accounted for by FFA oxidation in the basal state in both study groups. In the postabsorptive state, plasma FFA oxidation was slightly increased in diabetics compared to controls (101±14 vs. 76±10 μmol/m^2 · min). During the lowest insulin infusion, plasma FFA oxidation was suppressed by 50% in controls. Increasing the rate of insulin infusion caused no further reduction in plasma FFA oxidation. At all plasma insulin concentrations within the physiologic and pharmacologic range, FFA oxidation was at least twice as great in the diabetic compared to control subjects ($P < 0.05$–0.01). Plasma FFA concentrations correlated positively with FFA oxidation in controls ($r = 0.68$; $P < 0.05$) and diabetics ($r = 0.85$; $P < 0.85$) during each insulin clamp step. The ED_{50} for suppression of FFA oxidation by insulin was not

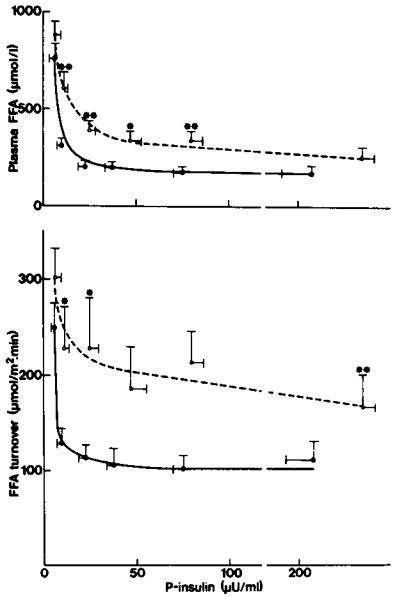

Figure 6. Plasma FFA concentrations and rate of plasma FFA turnover in the basal state and during graded hyperinsulinemia in nonobese NIDD (*broken line*) and in matched control (*solid line*) subjects. Values are mean±SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ versus control subjects.

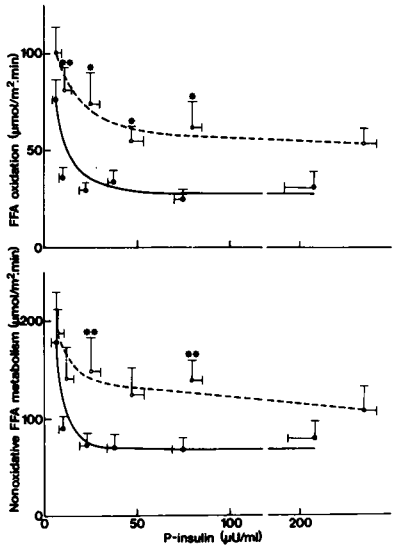

Figure 7. Rates of plasma FFA oxidation and nonoxidative FFA metabolism (an estimate of reesterification of FFA) in the basal state and during graded hyperinsulinemia in nonobese NIDD (*broken line*) and in matched control (*solid line*) subjects. Values are mean±SEM. *$P < 0.05$; **$P < 0.01$ versus control subjects.

significantly different between NIDD and control subjects (16±2 vs. 11±1 μU/ml).

Nonoxidative FFA metabolism (Fig. 7). In the basal state, ~ 70% of total FFA disposal was channeled through nonoxidative metabolism, i.e., reesterification to triglycerides. The basal rate of nonoxidative FFA metabolism did not differ between control and NIDD subjects (177±18 vs. 186±24 μmol/m² · min). In control subjects, nonoxidative FFA metabolism was suppressed by 50% at a plasma insulin concentration of 20 μU/ml, and did not decrease further at higher plasma insulin levels. Nonoxidative FFA metabolism remained 40% higher in the NIDDM patients compared to the controls during each insulin clamp step, and showed a strong positive correlation with the plasma FFA concentration both in controls ($r = 0.86$; $P < 0.01$) and in NIDDs ($r = 0.72$; $P < 0.01$).

Total net lipid oxidation (Fig. 8). The basal rate of total net lipid oxidation was similar in control and diabetic subjects (133±21 vs. 132±10 μmol/m² · min); of this plasma FFA oxidation accounted for 60% in controls and 75% in diabetics, respectively (P = NS). Inhibition of lipid oxidation was less sensitive to the effect of small increments in plasma insulin concentration than was plasma FFA oxidation; a significant reduction in the rate of lipid oxidation was not observed until plasma insulin was raised to > 20 μU/ml, while maximal suppression of lipid oxidation was observed at plasma insulin concentrations above 200 μU/ml. At the highest insulin infusion rate, plasma FFA oxidation exceeded total net lipid oxidation, suggesting the occurrence of net lipid synthesis (−10±6 vs. −6±4 μmol/m² · min in controls vs. diabetics). These values, however, were not significantly different from zero.

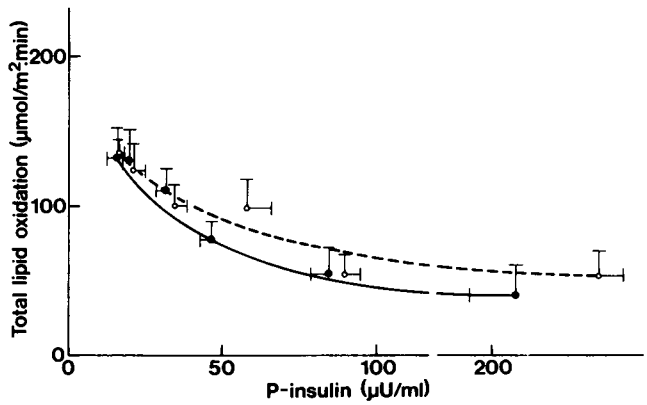

Figure 8. Rate of net lipid oxidation in the basal state and during graded hyperinsulinemia in nonobese NIDD (*broken line*) and in matched control (*solid line*) subjects. Values are mean±SEM.

Protein oxidation

Protein oxidation, as estimated from urinary nitrogen excretion, was slightly greater in NIDD compared to control subjects (31±3 vs. 24±2 mg/m² · min; $P < 0.10$).

Relationship between glucose and lipid metabolism

In control subjects, basal lipid oxidation showed an inverse correlation with basal and insulin-stimulated glucose oxidation (r = −0.72–0.80; P < 0.05–0.01). Likewise, basal FFA oxidation showed an inverse relationship with basal glucose oxidation ($r = -0.65$; $P < 0.05$). Total net lipid oxidation was positively correlated with HGP during the 4-mU/m² · min insulin clamp ($r = 0.73$; $P < 0.05$).

In the NIDD subjects, basal lipid oxidation was inversely correlated with basal and insulin-stimulated glucose oxidation (r = −0.80–0.93; P < 0.01–0.001). Furthermore, basal FFA oxidation was inversely correlated with the rate of basal glucose oxidation ($r = -0.66$; $P < 0.05$). Basal lipid oxidation was positively correlated with basal HGP ($r = 0.72$; $P < 0.05$) and with HGP during the 4 mU/m² · min insulin clamp ($r = 0.76$; $P < 0.05$).

To examine the relationship between HGP and FFA/total lipid oxidation over a wider range of oxidation rates, results obtained in the basal state and during the 4, 10, and 20 mU/m² · min insulin clamp steps were pooled. There was a positive correlation between the rate of FFA oxidation and the rate of HGP ($r = 0.53$; $P < 0.001$) and between the rate of lipid oxidation and the rate of HGP ($r = 0.45$; $P < 0.01$).

Discussion

The present results demonstrate that insulin resistance is a ubiquitous feature of normal weight NIDDs as it involves both FFA and glucose metabolism. They further suggest that the disturbances in FFA metabolism may, at least in part, be linked with the abnormalities in glucose utilization. Several previous studies have demonstrated that, in the basal state the diabetic liver overproduces glucose despite normal or elevated plasma insulin levels (2–6, 43, 45). However, the effect of small physiologic increments (< 50 μU/ml) in plasma insulin on the suppression of HGP in NIDD individuals has not been examined. The dose-response curve relating plasma insulin to HGP, documents the exquisite sensitivity of the liver to very small increases in portal vein insulin concentration. Thus, an increment of only 5 μU/ml (i.e., from 19 to 24 μU/ml) caused a 50% inhibition of HGP while a further increase to 30 μU/ml decreased it by 80%. In contrast, during the two lowest insulin infusions neither glucose oxidation nor nonoxidative glucose disposal increased significantly above baseline. These findings clearly demonstrate that with low, physiologic increments in plasma insulin, the liver is the primary determinant of whole body glucose homeostasis.

In the diabetics, severe hepatic resistance to insulin was observed at all plasma insulin concentrations throughout the

physiologic range. Thus, during the three lowest insulin infusion steps, achieving plasma insulin plateaus of less than 50 μU/ml, the dose-response curve was markedly shifted to the right. Furthermore, if one compares the dose-response curve for insulin-stimulated tissue glucose uptake (Fig. 4) with that for insulin-mediated suppression of HGP (Fig. 3), it is apparent that at plasma insulin < 50 μU/ml impaired suppression of HGP contributes quantitatively more than defective tissue glucose uptake to the disturbance in glucose homeostasis. Given the fact that hyperglycemia has been shown to inhibit HGP independently of insulin (43), and that the diabetics were studied at slightly hyperglycemic levels during the 4- and 10-mU/m^2 · min insulin steps, the liver may be even more resistant to insulin than concluded from this study.

Our results also provide some insight into the factor(s) that might be responsible for the elevated rate of basal HGP and its impaired suppression in response to insulin. In the diabetic patients, total net lipid oxidation was positively correlated with HGP in the postabsorptive state and during the 4 mU/m^2 · min insulin clamp. Additionally, during the low insulin infusions the rates of FFA/total lipid oxidation were positively correlated with the rates of residual HGP. These results suggest that the rate of lipid/FFA oxidation (both basal and insulin mediated) may be an important determinant of glucose production by the liver. The interpretation is consistent with many in vivo and in vitro observations. In vivo, when plasma FFA is acutely elevated, HGP is enhanced and the ability of hyperglycemia to inhibit basal HGP is impaired (19). A positive correlation between fasting plasma FFA levels and basal HGP has been reported in obese NIDD subjects (4, 12). In vitro, the addition of FFA to cultured hepatocytes has been shown to augment gluconeogenesis (46). As for the mechanisms, FFA oxidation consumes NAD, thereby resulting in accumulation of acetyl CoA, a powerful allosteric inhibitor of pyruvate dehydrogenase (47, 48). The depletion of NAD conspires with the increase in acetyl CoA to inhibit pyruvate dehydrogenase, thus making more pyruvate available for gluconeogenesis (49). Furthermore, the enhanced rate of FFA oxidation provides an abundance of high energy phosphate bonds to drive gluconeogenesis, which is an energy requiring process (27). Finally, the accumulation of acetyl CoA activates pyruvate carboxylase, the first enzymatic step in the gluconeogenetic pathway (47). Obviously, further studies are needed to define the precise contribution of enhanced lipid/FFA oxidation to the excessive rate of HGP in NIDDM.

NIDD subjects also displayed a number of disturbances in FFA and total lipid metabolism. At physiologic insulin concentrations there was an impaired suppression of both plasma FFA concentration and turnover in the diabetic compared with control subjects. In the latter group, suppression of plasma FFA concentrations and total FFA flux were extremely sensitive to small changes in plasma insulin. Thus, an increment of only 4–5 μU/ml caused a > 50% decline in both plasma FFA concentration and turnover (Fig. 6).

Hyperglycemia has been shown to inhibit lipolysis independently of insulin in dogs (50). If this was the case also in humans, we would have underestimated the severity of insulin resistance at the 4 and 10 mU/m^2 · min infusion steps, i.e., when the diabetics were studied at slightly higher plasma glucose levels than the controls. However, recent data in humans indicate that hyperglycemia per se has no effect by itself on FFA metabolism in NIDD (51–52) or IDD (53) subjects.

The inhibitory effect of insulin on FFA oxidation requires further elaboration. Before oxidation, FFA is activated to its acyl-CoA derivative, and then transported across the outer mitochondrial membrane as a carnitine ester in a reaction catalyzed by carnitine palmitoyl transferase I (CPT I) (54, 55). Since CPT I is inhibited by both insulin and malonyl-CoA, which is generated by condensation of acetyl-CoA (56), the insulin-mediated decline in FFA oxidation could have been due to a direct effect of the hormone on CPT I. However, FFA oxidation also is regulated by substrate availability (57, 58). The positive correlation between plasma FFA concentration and FFA oxidation rate indicates that the fall in FFA oxidation is, at least in part, secondary to the fall in plasma FFA concentration. In diabetic subjects the ability of insulin to inhibit FFA oxidation was impaired at all plasma insulin concentrations (Fig. 7). This defect most likely is secondary to the higher circulating plasma FFA levels that occur as a result of insulin's impaired antilipolytic action. These findings could be compatible with a V_{max} defect in the suppression of lipolysis by insulin in NIDDM. Whether the ability of insulin to inhibit CPT I also is impaired in NIDD individuals remains to be established.

In the postabsorptive state FFA oxidation accounted for approximately two-thirds of total body lipid oxidation in both controls and NIDDs (Figs. 7 and 8). Since [^{14}C]palmitate traces only the circulating FFA pool, whereas indirect calorimetry measures total (intracellular plus extracellular) lipid oxidation (32, 59), the significant difference between total lipid oxidation and FFA oxidation indicates direct oxidation of intracellular lipids (59–62). Consistent with this observation, Dagenais et al. (61) have shown that intracellular oxidation of triglycerides and, possibly phospholipids, make a substantial contribution to total lipid oxidation. The different ED_{50} values for suppression of plasma FFA oxidation and total lipid oxidation by insulin in control (11±1 vs. 21±3 μU/ml; $P < 0.01$) as well as in NIDD subjects (16±2 vs. 32±6 μU/ml; $P < 0.01$) suggest that oxidation of intracellular lipids is differently regulated than plasma FFA oxidation.

In NIDDs, in contrast to the marked impairment in insulin-mediated suppression of plasma FFA oxidation, suppression of whole-body net lipid oxidation was approximately normal. From the present data, explanation for this dissociation can only be descriptive. Indirect calorimetry measures the net result of lipid oxidation and de novo synthesis (32). Therefore, normal rates of net lipid oxidation in the face of raised FFA oxidation, as observed in the NIDD group, suggest that either tissue lipid oxidation is proportionately reduced, or de novo lipid synthesis is proportionately increased.

Taskinen et al. (11) have recently proposed that impaired reesterification of FFA may contribute to elevated plasma FFA levels in obese NIDD patients. Our findings do not support this hypothesis. In contrast, the contribution of oxidation and reesterification to FFA disposal was approximately similar in controls (28/72%) and NIDDs (32/68%). Although FFA reesterification (i.e., nonoxidative FFA disposal) was acutely inhibited by insulin in both control and NIDDM subjects, the absolute rate of reesterification was higher in diabetics compared to controls (Fig. 6), possibly as a consequence of their higher plasma FFA concentrations. Such an explanation is supported by the strong positive correlation between the rate of reesterification and plasma FFA concentrations both in the basal and insulin-stimulated state.

Our data support the negative interaction between lipid

oxidation and glucose metabolism that forms a branch of Randle's cycle (17). Thus, an increased rate of lipid oxidation, both during the basal and insulin-stimulated states, was associated with a decrease in glucose oxidation in NIDDs as well as in controls. It should be pointed out, that we do not know whether in NIDDM the enhanced rate of FFA oxidation precedes the impairment in glucose metabolism, or vice versa. In the latter case, reduced glucose uptake by adipocytes could result in enhanced rate of lipolysis as a consequence of impaired generation of alpha-glycerophosphate. This, in turn, could alter FFA reesterification, and allow more FFA to reach the bloodstream. The elevated plasma concentration of FFA could then trigger a vicious cycle in which glucose and lipid metabolism act negatively on each other.

One last finding is worthy of comment. Under hyperinsulinemic conditions nonoxidative glucose disposal accounts for the majority (approximately two-thirds) of glucose metabolism. During the 20, 40, and 100 $mU/m^2 \cdot min$ insulin clamp steps, nonoxidative glucose metabolism (22, 29, and 26%, respectively) and glucose oxidation (25, 34, and 31%, respectively) both were impaired by the same percentage in the diabetics and in controls. This suggests that, under the present experimental conditions, a defect in some early step in glucose metabolism, uphill to the branching point of oxidative and nonoxidative pathways, may be responsible for the insulin resistance in NIDDM.

In summary, the present findings demonstrate that the insulin resistance in NIDDM involves multiple pathways in the metabolism of two major fuels, glucose and lipid. Furthermore, our results provide evidence that the defects in glucose and lipid metabolism may be interrelated. This observation may have important clinical implications. In NIDD subjects the plasma FFA concentration has been shown to rise excessively with and between meals (13). This, in turn, could lead to an elevated rate of FFA oxidation as demonstrated in the present study. Given the association between FFA oxidation and hepatic glucose production, and between lipid/FFA oxidation and glucose oxidation, one would anticipate that an increased rate of postprandial FFA oxidation could lead to impaired suppression of hepatic glucose output and defective stimulation of glucose oxidation. Both would contribute to the fasting and postprandial hyperglycemia of NIDDM.

Acknowledgments

We thank all the subjects who participated in the study for their cooperation, the staff of the Clinical Research Center for their excellent care of patients, Syed Hasan for his expert technical assistance and Dr. Rosa Hendler for kindly performing the insulin determinations. Dr. Leif Groop was the recipient of a Fogarty International Fellowship (FO5 TWO 3451) and Dr. Stefano DelPrato was the recipient of a Nato Fellowship (215-14-216.-1020). Dr. Riccardo Bonadonna was the recipient of a Fellowship from the Italian Ministry of Public Education.

This research was supported in part by National Institutes of Health grant AM-24092 and CRC grant RR125.

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