Exogenous Insulin Replacement in Type 2 Diabetes Reverses Excessive Hepatic Glucose Release, But Not Excessive Renal Glucose Release and Impaired Free Fatty Acid Clearance

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In type 2 diabetes renal and hepatic glucose release are increased and free fatty acids (FFA) clearance is reduced. Restoration of normoglycemia by exogenous insulin replacement normalizes overall glucose release and plasma FFA concentrations. However, it is unclear to what extent normalization of overall glucose release is due to suppression of hepatic (HGR) and renal glucose release (RGR) and whether the abnormal FFA clearance is improved. We therefore determined overall, renal, and hepatic glucose release, as well as systemic FFA release and clearance by tracer techniques in type 2 diabetic subjects with (DM^+) and without (DM^-) physiologic overnight insulin infusion and in nondiabetic volunteers (NV). Insulin infusion normalized plasma glucose (5.3 ± 0.1 *v* 5.2 ± 0.1 mmol/L in NV) and overall glucose release (10.1 ± 0.7 *v* 10.6 ± 0.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV), (both $P > .9$). Values in DM^- were 9.1 ± 0.6 mmol/L and 14.6 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively (both $P < .001$ *v* DM^+ and NV). The correction of overall glucose release in DM^+ was due to suppression of HGR to rates below normal (6.11 ± 0.53 *v* 8.67 ± 0.44 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV, $P < .03$). RGR remained increased (3.91 ± 0.38 *v* 1.90 ± 0.28 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV, $P < .002$) and was similar to DM^- (3.97 ± 0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P > .9$). Insulin infusion also normalized plasma FFA levels (450 ± 45 *v* 476 ± 42 in NV, $P > .9$ and *v* 613 ± 33 μmol/L in DM^-, $P < .04$). This was due to suppression of FFA release to below normal (4.04 ± 0.45 *v* 5.25 ± 0.25 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV, $P < .04$). Plasma FFA clearance remained reduced (7.2 ± 1.0 *v* 11.4 ± 1.2 $mL \cdot kg^{-1} \cdot min^{-1}$ in NV, $P < .04$) and was similar to DM^- (7.3 ± 0.5 $mL \cdot kg^{-1} \cdot min^{-1}$, $P > .9$). We conclude that in contrast to the excessive HGR, excessive RGR and impaired FFA clearance are not corrected by acute exogenous insulin replacement.

IN ADDITION TO increased fasting plasma glucose concentrations, people with type 2 diabetes generally have increased plasma free fatty acid (FFA) concentrations.[1] This seems to be primarily due to reduced plasma FFA clearance,[2-4] because systemic FFA release has generally been found to be normal.[2,3,5-11] Moreover, several studies have found reduced uptake and/or oxidation of FFA in skeletal muscle,[2,12,13] the major site of FFA disposal.[14] Infusion of insulin resulting in physiologic hyperinsulinemia has been shown to increase plasma FFA clearance in nondiabetic volunteers,[15,16] indicating that insulin regulates FFA metabolism by both influencing their release, as well as their uptake. However, the effect of exogenous insulin replacement on FFA clearance has not been determined in type 2 diabetes.

Recent studies suggest that fasting hyperglycemia in type 2 diabetes is due to excessive release of glucose by both the liver and kidney.[4] This is mainly the result of increased gluconeogenesis.[17-19] Although restoration of normoglycemia by exogenous insulin replacement is accompanied by normalization of increased overall glucose release,[20,21] it is presently unclear to what extent this is due to suppression of hepatic glucose release (HGR) and renal glucose release (RGR).

HGR is due to glycogenolysis and gluconeogenesis, whereas RGR is thought to be nearly exclusively due to gluconeogenesis.[22] Because glycogenolysis is more sensitive to suppression by insulin than is gluconeogenesis,[19,23] one may expect that the normalization of overall glucose release during exogenous insulin replacement may be the result of greater suppression of HGR than RGR.

The present studies were therefore undertaken to test the hypotheses that in type 2 diabetes (1) exogenous insulin replacement normalizes overall glucose release by suppressing HGR more than RGR and that (2) it improves plasma FFA clearance. For these purposes, we compared overall, renal, and hepatic glucose release, as well as systemic FFA turnover and clearance in type 2 diabetic subjects with and without restoration of fasting normoglycemia by overnight insulin infusion and in nondiabetic control subjects.

MATERIALS AND METHODS

Subjects

Informed written consent was obtained from 52 subjects, 28 with type 2 diabetes and 24 nondiabetic volunteers (NV), after the protocol had been approved by The University of Rochester Institutional Review Board. Type 2 diabetic subjects who received overnight insulin infusion (DM^+, 8 men, 2 women) were 47 ± 2 years of age and had a body mass index (BMI) of 31.4 ± 1.4 kg/m^2. Type 2 diabetic subjects who did not receive overnight insulin infusion (DM^-, 13 men, 5 women) were 50 ± 2 years of age and had a BMI of 28.9 ± 0.8 kg/m^2. NV (11 men, 13 women) were 44 ± 2 years of age and had a BMI of 29.9 ± 1.0 kg/m^2. Type 2 diabetic subjects had comparable antecedent glycemic control (glycosylated hemoglobin [HbA_{1c}], 7.2 ± 0.3 in DM^+ *v* 7.8 ± 0.3% in DM^-). The mean diabetes duration in DM^+ and DM^- was 3.7 ± 1.2 and 4.2 ± 1.3 years, respectively. All diabetic subjects had been treated with oral hypoglycemic agents, which had been withdrawn 4 days before the experiment, or diet and exercise. In addition to oral hypoglycemic agents, 1 subject was treated with bedtime insulin, which had been omitted the day before the experiment. None of the diabetic subjects had nephropathy (ie, no microalbuminuria), coronary artery disease, proliferative retinopathy, or autonomic neuropathy. NV had normal glucose tolerance tests according to World

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Health Organization (WHO) criteria[24] and no family history of diabetes.

Protocol

All subjects were admitted to the University of Rochester General Clinical Research Center between 6 and 7 PM the evening before experiments, consumed a standard meal between 6:30 and 8 PM and fasted thereafter until experiments were completed.

Ten of the 28 diabetic subjects received an overnight intravenous insulin infusion (DM^+), which was started at 12:00 midnight according to the algorithm by Mokan et al[25] to restore normoglycemia. The insulin infusion, which had rendered them normoglycemic, was maintained during the blood sampling period.

At approximately 5:30 AM, an antecubital vein was cannulated and a primed-continuous infusion of [6-^{3}H] glucose (Amersham International, UK) was started at a rate of 0.15 to 0.5 μCi/minute. The normal prime of [6-^{3}H] glucose (100 times the rate of the continuous infusion in μCi/minute), which was given in diabetic subjects infused with insulin overnight and in control subjects, was adjusted for the prevailing plasma glucose concentration in the diabetic subjects not infused with insulin overnight (normal prime $\times$ millimolar plasma glucose/5 mmol/L). At approximately 8 AM, an infusion of p-aminohippuric acid (PAH; 12 mg/minute) was started for determination of renal blood flow (RBF). Between 8 and 9 AM, a renal vein was catheterized through the right femoral vein under fluoroscopy. The position of the catheter tip was initially ascertained by injecting a small amount of iodinated contrast material and during the blood sampling period by measurement of PAH. The catheter was continuously perfused with a saline infusion (heparinized at 5.6 U/minute). At approximately 9 AM, a continuous infusion of [9,10-^{3}H] palmitate ($\approx$ 0.2 μCi/minute, Amersham) was started. For preparation of the infusate, [9,10-^{3}H] palmitate as supplied by the manufacturer was taken to dryness under nitrogen, resuspended in 25% human free fatty acid free albumin (Sigma Chemical, St Louis, MO) and then diluted with 5 mmol/L Na_2Po_4 buffer, pH 7.8, to a final albumin concentration of 1% as previously described.[26] Shortly thereafter, a dorsal hand vein was cannulated and kept in a thermoregulated plexiglas box at 65°C for sampling arterialized venous blood. Starting at approximately 10 AM, 3 blood samples were collected simultaneously from the dorsal hand vein and the renal vein at 30-minute intervals. Urine was collected during the blood sampling period for determination of glucosuria. Some data of the diabetic subjects without overnight insulin infusion (15 of 18) and of the nondiabetic control subjects (13 of 24) have been included in previous publications.[4,27-32]

Analytical Procedures

Blood samples were collected for glucose, lactate, glycerol, alanine, and FFA concentrations and for glucose and palmitate specific activities (SA) in oxalate-fluoride tubes. Whole blood glucose was immediately determined in triplicate with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma [^{3}H] glucose and [9,10 ^{3}H] palmitate SAs and plasma FFA concentrations were determined by high-performance liquid chromatography (HPLC) methods with a coefficient of variation of 0.5%, 3.8%, and 2.3%, respectively.[27] Plasma PAH concentrations were measured by a colorimetric method,[33] and plasma lactate, alanine, and glycerol concentrations were measured by standard microfluorometric assays.[34,35]

Calculations

Assumptions and methodologic limitations of the combined net balance and isotopic approach for determining glucose release by liver and kidney have been previously discussed in detail.[22,27,30] Standard steady-state equations were used to determine systemic turnover (uptake and release) of glucose and palmitate,[36] ie, the infusion rate of the tracer in dpm $\cdot$ kg^{-1} $\cdot$ min^{-1} divided by the arterial tracer SA in dpm/μmol. Because palmitate represents 31% of total plasma FFA,[37] systemic palmitate turnover was divided by 0.31 to extrapolate data to total FFA. Systemic glucose clearance was calculated as systemic glucose uptake divided by arterial glucose concentration. Assuming that systemic palmitate clearance is representative of total plasma FFA clearance, the latter was calculated as systemic palmitate uptake divided by arterial palmitate concentrations. Renal plasma flow (RPF) was determined by the PAH acid clearance technique[33] and RBF was calculated as RPF/(1 - hematocrit). Renal glucose tissue net balance (NB) was calculated as RBF $\times$ (arterial glucose concentration - renal vein glucose concentration) - glucosuria. Renal glucose fractional extraction was calculated (arterial [6-^{3}H] glucose SA $\times$ arterial glucose concentration - renal vein [6-^{3}H] glucose SA $\times$ renal vein glucose concentration)/(arterial glucose SA $\times$ arterial glucose concentration). In 4 of the 24 nondiabetic volunteers and in 2 of the 10 diabetic subjects infused with insulin, physiologically impossible negative values were obtained for renal glucose fractional extraction, most likely representing analytical imprecision. To avoid bias, these values were included without rerunning the samples. Renal glucose uptake (RGU) was calculated as RBF $\times$ arterial glucose concentration $\times$ Fx - glucosuria. RGR was calculated as RGU - NB. HGR was calculated as the difference between overall glucose release and RGR. Renal net balance of lactate, alanine, and glycerol were calculated as (arterial concentration – renal vein concentration) $\times$ RBF or RPF. RBF was used for lactate, whereas RPF was used for alanine and glycerol, because organ exchange of these substrates occurs via plasma.[34,38]

Statistical Analysis

Unless stated otherwise, data are expressed as mean $\pm$ SEM. Differences of various parameters among DM^+, DM^-, and NV were analyzed using analysis of variance (ANOVA) followed by the post hoc Scheffe test when parameters were significantly different by ANOVA. Least-squares linear regression was used to assess correlations. A P value less than .05 was considered statistically significant.

RESULTS

Arterial Glucose and Hormone Concentrations

In diabetic subjects infused with insulin overnight, plasma glucose concentrations were 9.2 $\pm$ 1.0 mmol/L before the insulin infusion. During the insulin infusion, plasma glucose concentrations at 2, 4, 6, and 8 AM were 6.8 $\pm$ 0.5, 5.6 $\pm$ 0.2, 5.6 $\pm$ 0.1, and 5.4 $\pm$ 0.2 mmol/L; corresponding rates of insulin infusion averaged 2.5 $\pm$ 0.7, 1.4 $\pm$ 0.4, 1.1 $\pm$ 0.3, and 1.8 $\pm$ 0.4 U/h. None of the DM^+ had a plasma glucose concentration below 5.0 mmol/L during the entire study. During the 1-hour blood sampling period, plasma glucose concentrations in DM^+ were similar to those in NV (5.3 $\pm$ 0.1 *v* 5.2 $\pm$ 0.1 mmol/L NV, $P > .9$). Values in the diabetic subjects not infused with insulin were 9.1 $\pm$ 0.6 mmol/L ($P < .001$ *v* DM^+ and NV; Table 1). Plasma insulin levels were approximately 2-fold greater in DM^+ (137 $\pm$ 23 pmol/L) than in NV (57 $\pm$ 4 pmol/L) and DM^- (65 $\pm$ 6 pmol/L), both $P < .001$. Plasma glucagon concentrations were similar among the groups. Plasma epinephrine concentrations in DM^+ were about 3-fold greater than in NV ($P < .02$) and about 2-fold greater than in DM^- ($P = .075$), but plasma cortisol and growth hormone were comparable in all groups ($P > .3$) (Table 2).

Table 1. RBF, RPF, Arterial Substrate Concentrations, and Renal Substrate Net Balances in Type 2 Diabetic Subjects With (DM^+) and Without (DM^-) Overnight Insulin Infusion and in Nondiabetic Volunteers

	DM^+ (n = 10)	NV (n = 24)	DM^- (n = 18)
RBF (mL/min)	1,803 ± 153	1,408 ± 84	1,554 ± 132
RPF (mL/min)	1,030 ± 77	872 ± 55	944 ± 78
Glucose concentration (mmol/L)	5.3 ± 0.1*	5.2 ± 0.1*	9.1 ± 0.6
Renal tissue NB (μmol/min)	−214 ± 61*†	−45 ± 15	−28 ± 35
Lactate concentration (μmol/L)	1,006 ± 61†	666 ± 27	1,056 ± 89†
Renal NB (μmol/min)	423 ± 59†	185 ± 12	328 ± 51‡
Alanine concentration (μmol/L)	275 ± 30	249 ± 19	295 ± 20
Renal NB (μmol/min)	−23 ± 15	−3 ± 3	−5 ± 4
Glycerol concentration (μmol/L)	66 ± 12	81 ± 7	86 ± 7
Renal NB (μmol/min)	29 ± 6	35 ± 4	39 ± 5

NOTE. Data are mean ± SEM.
*$P < .01$ *v* DM^-.
†$P < .01$ *v* NV.
‡$P < .02$ *v* NV.

Glucose-Specific Activities Systemic, Renal, and Hepatic Glucose Kinetics

Glucose-specific activities (dpm/μmol) after 240, 270, and 300 minutes of [6-^{3}H] glucose infusion were not significantly different from one another (338 ± 107, 338 ± 107, and 340 ± 108 in DM^+; 925 ± 130, 906 ± 125, and 912 ± 126 in DM^-; 1,155 ± 203, 1,122 ± 203, and 1,161 ± 203 in NV) indicating that isotopic steady state had been achieved.

Overnight insulin infusion normalized overall glucose release in DM^+ (10.1 ± 0.7 *v* 10.6 ± 0.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV, $P > .9$). Values in the diabetic subjects not infused with insulin were 14.6 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < .001$ versus DM^+ and NV (Fig 1). Systemic glucose clearance was similar in DM^+ and NV (1.93 ± 0.16 *v* 2.05 ± 0.06 $mL \cdot kg^{-1} \cdot min^{-1}$ in NV, $P > .7$), but was reduced in DM^- (1.63 ± 0.08 $mL \cdot kg^{-1} \cdot min^{-1}$, $P < .004$ *v* NV).

RBF was not significantly different between the groups ($P > .1$). Glucosuria ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) averaged 0.012 ± 0.004 in DM^+, 0.51 ± 0.26 in DM^-, and 0.017 ± 0.004 in NV. Renal tissue glucose net balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) was negative in DM^+ (-2.08 ± 0.58) and in NV (-0.48 ± 0.16), but was not significantly different from zero in DM^- (-0.26 ± 0.38). Thus, DM^+ had markedly greater net RGR than NV ($P < .008$) and DM^- ($P < .004$) (Fig 2).

In contrast, renal glucose uptake was greater in DM^- (3.63 ± 0.39 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) than in DM^+ (1.85 ± 0.68 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < .05$) and NV (1.41 ± 0.30 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < .001$), which were not significantly different from one another ($P > .8$). Because renal glucose fractional extraction in DM^+ (2.1% ± 0.8%), DM^- (2.6% ± 0.3%), and in NV (1.9% ± 0.5%) were not significantly different ($P > .5$), differences in RGU were primarily due to mass action effects of hyperglycemia. RGR was increased in DM^+ (3.91 ± 0.38 *v* 1.90 ± 0.28 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV, $P < .002$) and was similar to values in DM^- (3.97 ± 0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P > .9$). In contrast, overnight insulin infusion suppressed HGR in DM^+ to levels significantly below those of normal volunteers (6.11 ± 0.53 *v* 8.67 ± 0.44 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NV, $P < .03$). Values in the diabetic subjects not infused with insulin were 10.6 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < .001$ *v* DM^+ and $P < .03$ *v* NV) (Fig 1).

Table 2. Arterial Plasma Hormone Concentrations in Type 2 Diabetic Subjects With (DM^+) and Without (DM^-) Overnight Insulin Infusion and in Nondiabetic Volunteers

	DM^+ (n = 10)	NV (n = 24)	DM^- (n = 18)
Insulin (pmol/L)	137 ± 23*†	57 ± 4	65 ± 6
C-peptide (pmol/L)	344 ± 76‡†	590 ± 34	704 ± 61
Glucagon (ng/L)	94 ± 6	85 ± 6	76 ± 5
Epinephrine (pmol/L)	295 ± 76‡	99 ± 11	146 ± 19
HGH (μg/L)	1.7 ± 0.3	1.4 ± 0.4	1.0 ± 0.3
Cortisol (μg/dL)	8.4 ± 0.9	8.4 ± 0.8	8.0 ± 1.0

NOTE. Data are mean ± SEM.
*$P < .001$ *v* NV.
‡$P < .02$ *v* NV.
†$P < .001$ *v* DM^-.

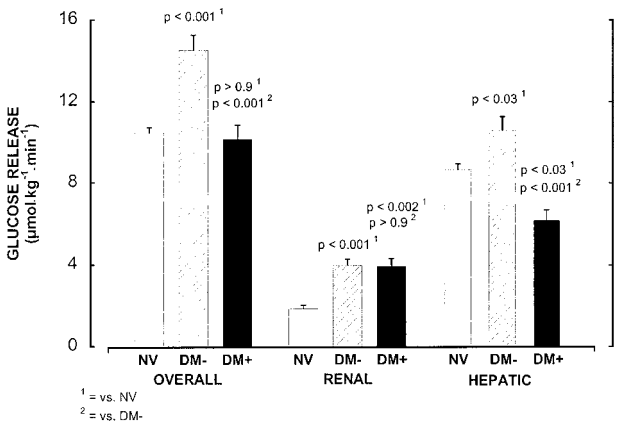

Fig 1. Overall, renal, and HGR in NV (n = 24) and in type 2 diabetic subjects with (DM^+, n = 10) and without (DM^-, n = 18) overnight insulin infusion to restore normoglycemia (mean ± SEM).

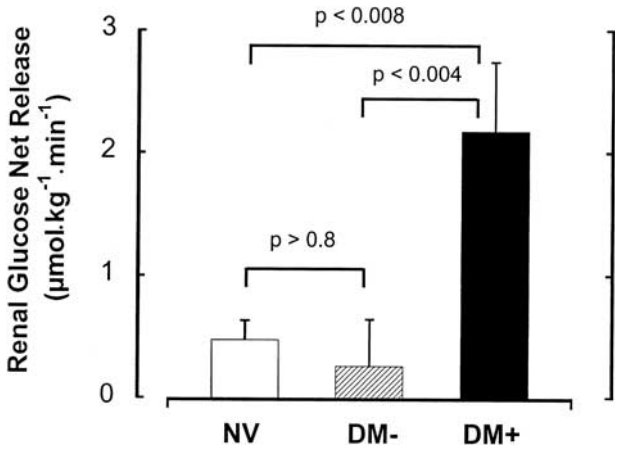

Fig 2. Renal glucose net release in NV (n = 24) and in type 2 diabetic subjects with (DM^+, n = 10) and without (DM^-, n = 18) overnight insulin infusion to restore normoglycemia (mean ± SEM).

Arterial Substrate Concentrations and Renal Substrate Balances

Arterial lactate concentrations and renal lactate net uptake in DM^+ and DM^- were comparable ($P > .3$) and were greater than in NV (all $P < .02$) (Table 2). Arterial concentrations and renal net balances of alanine and glycerol were similar in all groups. When all subjects were analyzed together, RGR correlated significantly with the sum of renal net balances of lactate, alanine, and glycerol ($r = .55$, $P < .001$).

Arterial FFA Concentrations and Systemic FFA Kinetics

Palmitate-specific activities (dpm/μmol) after 60, 90, and 120 minutes of [9,10-^{3}H] palmitate infusion were not significantly different from one another (4,738 ± 1,498, 4,582 ± 1,449 and 5,101 ± 1,613 in DM^+; 4,888 ± 831, 4,813 ± 784, and 4,983 ± 897 in DM^-; 4,732 ± 690, 4,681 ± 643, and 5,059 ± 714 in NV) indicating that isotopic steady state had been achieved. After overnight insulin infusion, plasma FFA concentrations were comparable in DM^+ and in NV (450 ± 45 *v* 476 ± 42 μmol/L, $P > .9$) and were significantly lower than in DM^- (613 ± 33 μmol/L, both $P < .04$). In contrast, plasma FFA clearance in DM^+ remained reduced compared with that in NV (7.2 ± 1.0 *v* 11.4 ± 1.2 mL · kg^{-1} · min^{-1}, $P < .04$) and was not significantly different from that in DM^- (7.3 ± 0.5 mL · kg^{-1} · min^{-1}, $P > .9$). The reduction in plasma FFA concentrations in DM^+ was due to the suppression of FFA release to rates below those in NV (4.04 ± 0.45 *v* 5.25 ± 0.25, $P < .04$). Rates in the diabetic subjects not infused with insulin were 5.40 ± 0.27 μmol · kg^{-1} · min^{-1} ($P < .04$ *v* DM^+, $P > .9$ *v* NV) (Fig 3).

DISCUSSION

The present studies were undertaken to test the hypotheses that restoration of normoglycemia in type 2 diabetes by exogenous insulin replacement corrects excessive endogenous glucose release by preferentially suppressing HGR and that this would be accompanied by an improvement in the reduced FFA clearance. For these purposes, we compared overall, renal, and hepatic glucose release, as well as FFA turnover and clearance in subjects with type 2 diabetes, with and without overnight insulin infusion designed to restore normoglycemia, and in matched nondiabetic controls.

Overnight insulin infusion, which normalized overall glucose release in our type 2 diabetic subjects, was accompanied by suppression of HGR to levels below normal. In contrast, RGR remained excessive and was comparable to that of diabetic subjects not infused with insulin. These findings suggest that the kidney may be more resistant to insulin than the liver in type 2 diabetes. Alternatively, this may simply reflect the fact that glycogenolysis is more sensitive to suppression by insulin than is gluconeogenesis,[19] because HGR is the result of both glycogenolysis and gluconeogenesis, whereas RGR is probably all due to gluconeogenesis.[22]

Using the deuterated water technique to assess the effects of insulin on gluconeogenesis and glycogenolysis in type 2 diabetic subjects, Gastaldelli et al[19] found that infusion of insulin (40 mU · m^{-2} · min^{-1}) during a 2.5-hour euglycmic clamp completely suppressed glycogenolysis, whereas gluconeogenesis decreased only approximately 16%. Assuming that in our diabetic subjects not infused with insulin glycogenolysis accounted for about 40% of overall glucose release, as has been previously reported,[18,19,39] hepatic glycogenolysis would have been approximately 5.8 μmol · kg^{-1} · min^{-1}, ie, 14.5 μmol · kg^{-1} · min^{-1} × 0.4. Because the difference in overall glucose release between diabetic subjects who were and were not infused with insulin was 4.5 μmol · kg^{-1} · min^{-1}, suppression of HGR could have been wholly accounted for by near total suppression of glycogenolysis.

Regarding the failure of exogenous insulin replacement to suppress the increased renal gluconeogenesis in our diabetic subjects, the increased substrate availability might have been an important factor. Arterial lactate concentrations and renal lactate net balance remained increased in diabetic subjects infused with insulin and were comparable to those not infused with insulin. If wholly used for gluconeogenesis by the kidney, the increased renal lactate net uptake could have accounted for approximately 50% of the excessive RGR in both diabetic groups. Moreover, RGR correlated significantly with the sum of renal net balances of lactate, alanine, and glycerol ($r = .55$,

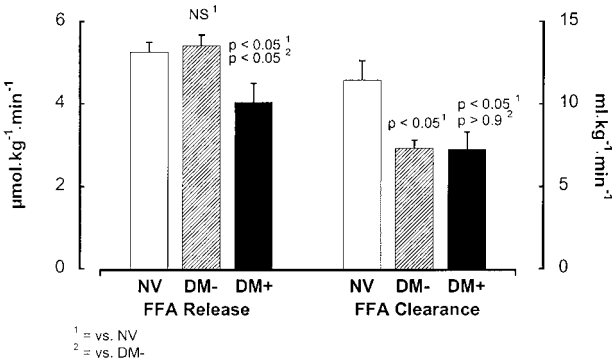

Fig 3. Systemic FFA release and clearance in NV (n = 24) and in type 2 diabetic subjects with (DM^+, n = 10) and without (DM^-, n = 18) overnight insulin infusion to restore normoglycemia (mean ± SEM).

$P < .001$). It should be pointed out, however, that net uptake underestimates true uptake and that uptake of glutamine and other gluconeogenic amino acids were not measured.

Another potential factor contributing to the persistent increased RGR after overnight insulin infusion may have been the associated increase in plasma catecholamine concentrations. Plasma epinephrine concentrations were almost 3-fold greater in diabetic subjects infused with insulin than in normal volunteers and approximately 2-fold greater than in diabetic subjects not infused with insulin. This hyperepinephrinemia would be expected to cause a persistent increase in RGR, but only a transient increase in HGR[31] and is consistent with the finding of increased catecholamine release at normoglycemia in similar subjects with type 2 diabetes infused with insulin.[40]

The second aim of the present studies was to examine the effects of exogenous overnight insulin replacement on plasma FFA metabolism in type 2 diabetes. Acute restoration of normoglycemia by the insulin infusion normalized the elevated plasma FFA concentrations in our diabetic subjects in addition to normalizing their glucose turnover and clearance. The normalization of plasma FFA concentrations was wholly due to suppression of FFA release to below normal since plasma FFA clearance remained unaffected. These findings suggest that FFA clearance may be more insulin resistant than FFA release, glucose release, and clearance in type 2 diabetes.

There are several possible explanations for the failure of exogenous insulin replacement to correct plasma FFA clearance in type 2 diabetic subjects. Skeletal muscle is normally one of the major tissues utilizing plasma FFA in the postabsorptive state.[14] Considerable evidence indicates that in type 2 diabetes FFA uptake and oxidation are reduced in skeletal muscle.[2,12] Recently, Blaak et al[12] found a decreased content of cytoplasmatic fatty acid-binding protein in skeletal muscle, which is involved in skeletal muscle FFA uptake, and decreased activities of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase, which are involved in muscle FFA oxidation. Decreased FFA oxidation could lead to increased intracellular FFA concentrations and consequently to impairment in FFA transport. Because these defects involve at least in part a deficiency in cell protein content, acute restoration of normoglycemia by insulin replacement may not have been long enough for their reversal. Another possible explanation is that the increase in plasma epinephrine may have had an adverse effect on FFA clearance in our diabetic subjects infused with insulin. However, in normal postabsorptive subjects, infusion of epinephrine at rates from 0.1 to 5.0 μg/minute, which resulted in plasma epinephrine concentrations ranging from 12 to 870 pg/mL, had no effect on plasma FFA clearance.[41]

Alternatively, it is possible that impaired plasma FFA clearance may represent a fundamental defect in type 2 diabetes, which is not due to insulin deficiency or insulin resistance. This concept is supported by studies of Blaak et al[42] who examined the effect of weight loss in obese diabetic subjects on plasma FFA kinetics and on fatty acid-binding protein content and oxidative enzymes in skeletal muscle. After subjects had lost approximately 14% of their body weight, which would be expected to markedly improve insulin sensitivity, plasma FFA concentrations were not significantly different from baseline. However, plasma FFA disposal was reduced, clearly indicating that plasma FFA clearance did not improve. Moreover, fatty acid-binding protein content and the activity of oxidative enzymes in skeletal muscle were unchanged.[42]

If impaired FFA clearance represented a fundamental defect not due to insulin resistance and not be corrected by insulin, it would be expected to contribute to the development of type 2 diabetes rather than to be the result of the diabetic state. The reduced plasma FFA clearance would predispose to elevated plasma FFA concentrations, which are associated with various adverse metabolic effects: diminished postprandial suppression of endogenous glucose release,[43,44] decreased insulin-stimulated glucose uptake,[45,46] impaired β-cell function,[47] and increased plasma triglyceride-rich lipoprotein concentrations.[48]

It is of interest to note that in addition to the increased isotopically determined RGR, the diabetic subjects infused with insulin had increased net RGR, which was more than 4-fold greater than that in normal volunteers (2.1 v 0.5 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}). In diabetic subjects not infused with insulin, renal glucose net release was not significantly different from that in normal volunteers, and in fact, not significantly different from zero, because their increased RGR was compensated for by their increased RGU. These observations thus suggest that increased RGR may contribute to the initial development of hyperglycemia in type 2 diabetes until mass action effects of hyperglycemia increase RGU. It should be pointed out that the same argument may also hold for the liver. All studies to date have found net splanchnic glucose release not to be significantly increased in hyperglycemic diabetic patients.[49-61] This failure to detect increased net HGR could be due to simultaneously increased splanchnic glucose uptake and release. However, in a previous study[4] using the combined isotopic renal net balance approach, in which subtraction of increased RGR from increased overall glucose release was used to calculate HGR, it was demonstrated that HGR was also increased in hyperglycemic type 2 diabetic patients.

In conclusion, the present studies provide evidence that in type 2 diabetes exogenous insulin replacement suppresses RGR less than HGR and, therefore, one cannot equate normalization of overall glucose release with normalization of glucose release by both liver and kidney. Moreover, our studies suggest that the impaired FFA clearance found in type 2 diabetes may represent a fundamental defect independent of insulin deficiency or insulin resistance.

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