

Mechanism of Increased Gluconeogenesis in Noninsulin-dependent Diabetes Mellitus

Role of Alterations in Systemic, Hepatic, and Muscle Lactate and Alanine Metabolism

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Abstract

To assess the mechanisms responsible for increased gluconeogenesis in noninsulin-dependent diabetes mellitus (NIDDM), we infused [3-¹⁴C]lactate, [3-¹³C]alanine, and [6-³H]glucose in 10 postabsorptive NIDDM subjects and in 9 age- and weight-matched nondiabetic volunteers and measured systemic appearance of alanine and lactate, their release from forearm tissues, and their conversion into plasma glucose (corrected for Krebs cycle carbon exchange). Systemic appearance of lactate and alanine were both significantly greater in diabetic subjects (18.2 ± 0.9 and 5.8 ± 0.4 $\mu\text{mol/kg/min}$, respectively) than in the nondiabetic volunteers (12.6 ± 0.7 and 4.2 ± 0.3 $\mu\text{mol/kg/min}$, respectively, $P < 0.001$ and $P < 0.01$). Conversions of lactate and alanine to glucose were also both significantly greater in NIDDM subjects (8.6 ± 0.5 and 2.4 ± 0.1 $\mu\text{mole/kg/min}$, respectively) than in nondiabetic volunteers (4.2 ± 0.4 and 1.8 ± 0.1 $\mu\text{mol/kg/min}$, respectively, $P < 0.001$ and $P < 0.025$). The proportion of systemic alanine appearance converted to glucose was not increased in NIDDM subjects (42.7 ± 1.9 vs. $44.2 \pm 2.9\%$ in nondiabetic volunteers), whereas the proportion of systemic lactate appearance converted to glucose was increased in NIDDM subjects (48.3 ± 3.8 vs. $34.2 \pm 3.8\%$ in nondiabetic volunteers, $P < 0.025$); the latter increased hepatic efficiency accounted for $\sim 40\%$ of the increased lactate conversion to glucose. Neither forearm nor total body muscle lactate and alanine release was significantly different in NIDDM and nondiabetic volunteers. Therefore, we conclude that increased substrate delivery to the liver and increased efficiency of intrahepatic substrate conversion to glucose are both important factors for the increased gluconeogenesis of NIDDM and that tissues other than muscle are responsible for the increased delivery of gluconeogenic precursors to the liver. (*J. Clin. Invest.* 1990. 86:2038–2045.) Key words: noninsulin-dependent diabetes mellitus • gluconeogenesis • alanine • lactate • liver

Introduction

Fasting hyperglycemia in noninsulin-dependent diabetes mellitus (NIDDM)¹ is mainly attributable to excess hepatic glucose output (1–3). Recent studies indicate that gluconeogenesis is increased in NIDDM (4, 5) and that it may account for most of the excess hepatic glucose output (5). The mechanisms responsible for this increased gluconeogenesis have not been established. Gluconeogenesis could be increased in NIDDM because of greater delivery of gluconeogenic substrates to the liver, greater efficiency of hepatic uptake, and conversion of these substrates into glucose, or a combination of these processes.

Lactate and alanine are the major gluconeogenic precursors in man (6, 7). Normally, substantial release of these precursors into plasma is thought to occur as a result of glycolysis in muscle and other peripheral tissues (8). In the postabsorptive state, glucose uptake by muscle and other peripheral tissues is increased in NIDDM (9–11). Since there should be no storage of glucose in the postabsorptive state and since glucose oxidation is reduced under this condition in NIDDM (12, 13), it is reasonable to expect that the increased muscle glucose uptake would result in increased release of glycolytic products into the circulation. Indeed, it has been proposed that increased muscle release of lactate and alanine could be responsible for sustaining increased gluconeogenesis in NIDDM (14). To date, however, muscle release of lactate and alanine in NIDDM has not been evaluated. Furthermore, there are reports suggesting that rates of appearance of lactate and alanine in plasma may not be increased in NIDDM (15, 16).

Regarding the other major mechanism by which gluconeogenesis might be increased in NIDDM (i.e., altered hepatic disposal of gluconeogenic precursors), the hyperglucagonemia, insulin resistance, and increased free fatty acid oxidation usually associated with this disorder (2, 3, 12, 13, 17) could all directly promote greater intrahepatic conversion of lactate and alanine into glucose (18, 19). Nevertheless, perhaps because of the limited number of subjects studied (15, 16) or the failure to correct for Krebs cycle carbon exchange (20), increased conversion of lactate or alanine into glucose has yet to be convincingly demonstrated in NIDDM (15, 16, 21).

Therefore, the present studies were undertaken to assess the mechanisms responsible for increased gluconeogenesis in NIDDM. For this purpose, we infused [3-¹⁴C]lactate, [3-¹³C]-

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1. Abbreviations used in this paper: AV, arteriovenous; NIDDM, noninsulin-dependent diabetes mellitus.

alanine, and [6-³H]glucose in 10 postabsorptive NIDDM subjects and in 9 age- and weight-matched nondiabetic subjects and measured the rates of systemic appearance of alanine and lactate and their incorporation into plasma glucose with correction for Krebs cycle carbon exchange; at the same time, we also used the forearm balance technique in conjunction with measurement of forearm alanine and lactate fractional extraction to test the hypothesis that greater release of these precursors from muscle might be important for sustaining the increased gluconeogenesis found in NIDDM.

Methods

Subjects. Informed, written consent was obtained from 10 subjects with NIDDM and from 9 nondiabetic healthy subjects matched for age, gender, and body mass index (Table I). None of the diabetic subjects had been treated with insulin. All had been treated with a sulfonylurea which was discontinued ≥ 1 wk before the study. All subjects had normal renal and hepatic function and each consumed a weight-maintaining diet containing at least 200 g carbohydrate for 3 d before the study.

Protocol. Subjects were admitted to the University of Pittsburgh General Clinical Research Center the evening before the experiments between 5:00 and 7:00 p.m., were given a standard meal (10 cal/kg; 50% carbohydrate, 35% fat, and 15% protein) and were studied the next morning after a 12–14-h fast.

At 5:00 a.m., primed continuous infusions of [3-¹⁴C]lactate (custom preparation; Amersham Corp., Arlington Heights, IL) (60 μ Ci, 0.60 μ Ci/min), [3-¹³C]alanine (Merck, Sharp, & Dohme, Dorval, Canada) (174 mg, 1.74 mg/min) and [6-³H]glucose (Amersham Corp.) (25 μ Ci, 0.25 μ Ci/min) were started through an antecubital vein; at the same time, a bolus of 50 μ Ci of [¹⁴C]sodium bicarbonate and of 50 mg of [¹³C]sodium bicarbonate was also administered to prime the endogenous bicarbonate pool. Shortly thereafter the ipsilateral radial artery was cannulated at the wrist and a contralateral deep antecubital vein was cannulated retrogradely to obtain blood draining muscle (22).

After allowing 4 h for isotopic equilibration (Fig. 1), blood samples were taken simultaneously from the radial artery and the deep antecubital

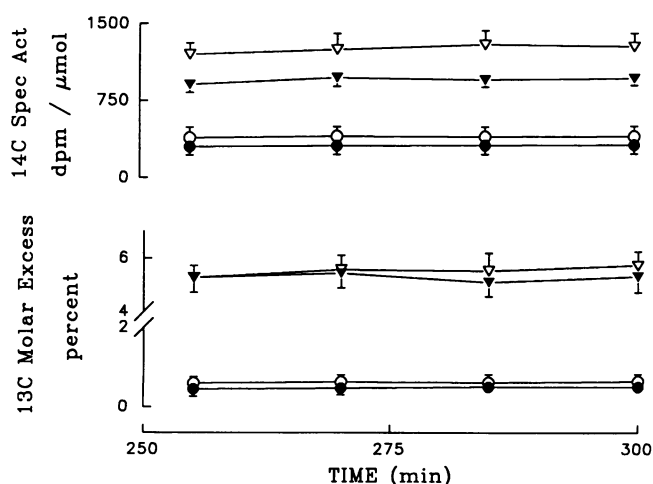


Figure 1. (Top) Specific activities (mean \pm SEM) of [¹⁴C]lactate (triangles) and [¹⁴C]glucose (circles) at the four sampling times for nondiabetic (open symbols) and diabetic (closed symbols) subjects. (Bottom) [¹³C]enrichment (mean \pm SEM) of plasma alanine (triangles) and glucose (circles) at the four sampling times for nondiabetic (open symbols) and diabetic (closed symbols) subjects.

vein at 15-min intervals between 9:00 and 10:00 a.m. After each blood sampling, forearm blood flow was determined using electrocardiogram plethysmography as previously described (22).

Between 9:00 and 10:00 a.m., total CO₂ production was continuously measured using a metabolic measurement cart (Sensor Medic, Anaheim, CA) equipped with an infrared CO₂ analyzer and an in-line turbo transducer for determination of expired gas volume (22). Before and after CO₂ production measurements, breath samples of the subjects were collected through a small plastic tube into 2 ml of a 0.5 M solution of hyamine hydroxide in ethanol, to trap expired CO₂ for later determination of its [¹⁴C]specific activity and [¹³C]enrichment.

Analytical procedures. Plasma glucose was determined with a YSI glucose analyzer (Yellow Springs, OH). Plasma lactate and alanine were determined by standard fluorometric methods (22). The specific activities (dpm/ μ mol) of plasma [³H]glucose, [¹⁴C]glucose and [¹⁴C]lactate were determined after isolating lactate and glucose using ion exchange chromatography (20). The radioactivities of the isolated lactate and glucose were corrected for recovery using external standards. Distribution of the [¹⁴C]radioactivity within the plasma glucose molecule was obtained as previously described (20).

Carbon-13 enrichment in plasma glucose and alanine were measured by chemical ionization, selected ion monitoring gas chromatography mass spectrometry of the pentaacetate (23), and *N*-acetyl-*n*-propyl ester (24) derivatives, respectively. For *N*-acetyl-*n*-propyl alanine, the protonated molecular ion region comprising *m/z* 174 and 175 was monitored. The former ion reflects unlabeled alanine and the latter corresponds to [3-¹³C]alanine when corrected appropriately for natural isotopic abundance.

For glucose pentaacetate, the *m/z* ratios 332/331 and 170/169 were monitored simultaneously. The former is the protonated molecular ion region and the latter is a fragment ion that also contains all six glucose carbons. In each instance, the higher mass reflects glucose singly labeled with [¹³C], when appropriate natural isotopic abundance corrections are applied, and the lower mass reflects unlabeled glucose. Monitoring two sets of ions provided an internal check of analytical accuracy and these ion current ratios were nearly identical in all instances. The average of the two ion current ratios at each time point was used in subsequent calculations. In addition, the ion current ratios *m/z* 333/331 and 334/331 were monitored to assess the presence of glucose molecules doubly or triply labeled with carbon-13. In each case, no multiple labeled glucose molecules were observed at these ion current ratios (limit of detection ~ 0.05 – 0.1 atom percent excess). ¹³CO₂ enrichment in expired CO₂ was determined by dual-inlet, dual-collector isotope ratio mass spectrometry (25).

Calculations. At steady-state, the rate of appearance (*R_a*) of a substrate in plasma equals its rate of disappearance (*R_d*) from plasma and these are collectively referred to as the plasma turnover rate (TR) of the substrate (26). Plasma glucose TR was obtained by dividing the [6-³H]glucose infusion rate (dpm/kg body wt/min) by the steady-state arterial [6-³H]glucose specific activity (SA) (dpm/ μ mol) (26). Plasma lactate TR was obtained by dividing the [3-¹⁴C]lactate infusion rate (dpm/kg body wt/min) by the steady-state arterial [¹⁴C]lactate SA (dpm/ μ mol) (22). Plasma alanine TR was obtained according to the following equation (26):

$$\text{Alanine TR} = i [Ei/Ep - 1],$$

where *i* is the [¹³C]alanine infusion rate (μ mol/kg body wt/min), *Ei* is the enrichment of the infusate (mole percent excess) and *Ep* is the arterial isotopic enrichment of plasma alanine (mole percent excess).

Assuming that pyruvate dehydrogenase activity would be negligible in the liver in the postabsorptive state, the correction factor (CF) to account for exchange of label within the Krebs cycle was calculated with the following equation derived from the Katz model of the Krebs cycle (27):

$$\text{CF} = [2 \cdot (1 + 2y) \cdot (1 + y)] / [(5 + 4y) \cdot y],$$

where y is obtained from the ratio of the specific activities of plasma glucose carbons 1, 2, 5, 6 and that of carbons 3, 4 (R), according to the formula (27):

$$y = (R - 2)/2.$$

The percent of plasma glucose rate of appearance derived from lactate was calculated according to the equation:

Percent plasma glucose from lactate

$$= (\text{plasma } [^{14}\text{C}]\text{glucose SA}/2 \times \text{plasma } [^{14}\text{C}]\text{lactate SA}) \cdot \text{CF}.$$

The incorporation of lactate into plasma glucose ($\mu\text{mol/kg body wt/min}$) was calculated by multiplying overall plasma glucose rate of appearance by the percent of the rate of appearance derived from lactate.

The percent of plasma lactate rate of appearance incorporated into plasma glucose was obtained dividing the rate of incorporation of lactate into plasma glucose by the lactate rate of appearance.

Lactate oxidation ($\mu\text{mol/kg body wt/min}$) was calculated according to the following equation (26):

$$\text{Lactate oxidation} = (\text{VCO}_2 \cdot ^{14}\text{CO}_2 \text{ SA})/0.81/\text{plasma } [^{14}\text{C}]\text{lactate SA}.$$

The percent of plasma glucose rate of appearance derived from alanine was calculated according to the equation:

Percent glucose from alanine

$$= (Ep[^{13}\text{C}]\text{glucose}/2 \cdot Ep[^{13}\text{C}]\text{alanine}) \cdot \text{CF}.$$

The incorporation of alanine into plasma glucose was calculated multiplying overall plasma glucose rate of appearance by the percent of the rate of appearance derived from alanine.

The percent of alanine rate of appearance converted to glucose was obtained dividing the rate of alanine incorporation into plasma glucose by alanine rate of appearance.

Alanine oxidation ($\mu\text{mol/kg body wt/min}$) was calculated according to the following equation (28):

Alanine oxidation

$$= (\text{VCO}_2 \cdot Ep \text{ } ^{13}\text{CO}_2/100)/0.81/\left(\frac{(1 - 1)}{([^{13}\text{C}]\text{Ala } EpEi)}\right) \cdot 100,$$

where 0.81 corrects for recovery of label in expired CO_2 . Net forearm substrate balance was calculated by multiplying arteriovenous (AV) differences in substrate concentrations by forearm blood flow (29). Forearm fractional extraction of $[^{14}\text{C}]\text{lactate}$ was calculated as the AV difference in lactate radioactivity divided by the arterial lactate radioactivity (30). Forearm alanine fractional extraction was calculated as the AV difference in $[^{13}\text{C}]\text{alanine}$ concentration divided by the arterial $[^{13}\text{C}]\text{alanine}$ concentration (31). Forearm uptake of lactate and alanine were calculated as arterial concentration times blood flow times fractional extraction (32). Forearm release of lactate and alanine were calculated according to the equation (32):

Release = uptake - net balance.

Forearm data per 100 ml/tissue were converted to kg/forearm muscle by multiplying the data by 13.3, assuming that muscle tissue has a density of 1.0, that it represents about 60% of the forearm volume and that 80% of the blood flow to the forearm is directed to the muscle tissue (29). Forearm volume was determined using the equations for a truncated cone (22, 29). Total body muscle mass (kg) was calculated according to the equations by Heymsfield (33). Total body muscle alanine and lactate uptake and release were calculated by multiplying forearm muscle data by total body muscle mass. Data are given as mean \pm SEM and were evaluated statistically using analysis of variance and, where appropriate, by least squares linear regression analysis.

Results

Plasma glucose, lactate, alanine, insulin, and glucagon concentrations (Table I). Plasma glucose averaged 12.9 ± 0.6 mmol/liter in the NIDDM subjects and 5.4 ± 0.1 mmol/liter in the nondiabetic volunteers. Both plasma insulin and plasma glucagon levels were significantly greater in the NIDDM subjects. However, neither plasma lactate nor plasma alanine were significantly different in the two groups.

Rates of appearance of glucose, lactate, and alanine in plasma (Figs. 2–4). As expected, plasma glucose appearance was greater in the NIDDM subjects (17.4 ± 0.7 $\mu\text{mol/kg/min}$) than in the nondiabetic subjects (11.6 ± 0.1 $\mu\text{mol/kg/min}$, $P < 0.001$) and there was a significant correlation between plasma glucose appearance and fasting plasma glucose concentration in the NIDDM subjects ($r = 0.78$, $P < 0.01$; Fig. 2).

Both plasma lactate and alanine appearances were increased in the NIDDM subjects. Plasma lactate appearance was $\sim 50\%$ greater in the NIDDM subjects than in the nondiabetic volunteers (18.2 ± 0.9 vs. 12.6 ± 0.7 $\mu\text{moles/kg/min}$, $P < 0.001$; Fig. 2). Plasma alanine appearance was $\sim 40\%$ greater in the NIDDM subjects than in the nondiabetic volunteers (5.8 ± 0.4 vs. 4.2 ± 0.3 $\mu\text{mol/kg/min}$, $P < 0.01$, Fig. 4).

Krebs cycle carbon exchange and conversion of lactate and alanine into plasma glucose (Table II, Figs. 3 and 4). The ^{14}C -specific activities of plasma glucose carbons 1, 2, 5, and 6 and carbons 3 and 4 are given in Table II. Y , the ratio between flux from pyruvate and flux from acetyl CoA into the Krebs cycle, was 1.05 ± 0.14 and 0.90 ± 0.08 in the NIDDM and in the nondiabetic subjects, respectively ($P = \text{NS}$, Table II). The correction factor for Krebs cycle carbon exchange, derived from this ratio according to the equation proposed of Katz (27), averaged in the NIDDM subjects 1.42 ± 0.05 and was not significantly different from the value of 1.39 ± 0.06 observed in the nondiabetic subjects.

Using this factor to correct for Krebs cycle carbon exchange, incorporation of lactate into plasma glucose was found to be more than twofold greater in the NIDDM subjects than in the nondiabetic subjects (8.6 ± 0.5 vs. 4.2 ± 0.4 $\mu\text{mol/kg/min}$, $P < 0.0001$, Fig. 3). Incorporation of alanine into glucose was found to be one-third greater in the NIDDM subjects than in the nondiabetic subjects (2.4 ± 0.1 vs. 1.8 ± 0.1 $\mu\text{mol/kg/min}$, $P < 0.025$, Fig. 4).

The percentage of plasma lactate appearance that was incorporated into plasma glucose was greater in the NIDDM

Table I. Clinical and Biochemical Characteristics of the Subjects

	NIDDM subjects	Nondiabetic subjects	P
Gender (F/M)	2/8	2/7	NS
Age (yr)	51.2 ± 1.4	52.4 ± 1.4	NS
Weight (kg)	83.0 ± 3.2	80.9 ± 3.3	NS
Body mass index (kg/M^2)	27.7 ± 0.8	27.4 ± 0.6	NS
Plasma glucose (mmol/liter)	12.9 ± 0.6	5.5 ± 0.1	< 0.001
Plasma insulin (pmol/liter)	102 ± 19	59 ± 3	< 0.05
Plasma glucagon (ng/liter)	387 ± 23	186 ± 15	< 0.01
Plasma lactate (mmol/liter)	1.35 ± 0.19	0.90 ± 0.07	NS
Plasma alanine (mmol/liter)	0.30 ± 0.02	0.33 ± 0.03	NS

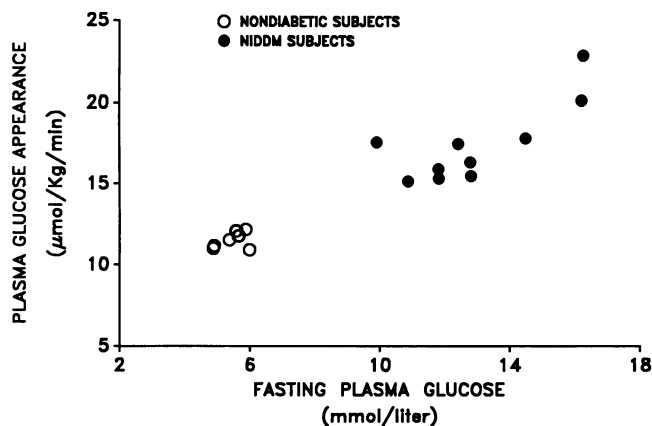


Figure 2. Rates of plasma glucose appearance in NIDDM and nondiabetic subjects in relation to their fasting plasma glucose concentrations.

subjects ($48.3 \pm 3.9\%$) than in the nondiabetic subjects ($34.2 \pm 4.3\%$, $P < 0.025$). On the other hand, incorporation of alanine into plasma glucose represented a comparable percentage of plasma alanine appearance in the NIDDM and in the

Table II. [^{14}C]-Specific Activities of Glucose Carbons 1,2,5,6 and 3,4: Ratio Between Flux from Pyruvate and Flux from Acetyl CoA into Krebs Cycle, and Correction Factor for Krebs Cycle Carbon Exchange in NIDDM and Nondiabetic Subjects

NIDDM subjects	Plasma glucose specific activity*		Y^\dagger	Correction factor for Krebs cycle carbon
	Carbons 1,2,5,6	Carbons 3,4		
Exchange ‡				
1	22.0	7.2	0.53	1.67
2	47.2	10.1	1.35	1.24
3	71.7	17.8	1.02	1.33
4	62.7	15.0	1.09	1.30
5	74.1	19.3	0.95	1.35
6	96.6	30.7	0.58	1.61
7	94.5	24.0	0.97	1.35
8	70.7	18.6	0.90	1.37
9	45.9	11.0	1.09	1.30
10	34.9	11.6	0.50	1.71
Mean	62.0	16.5	0.90	1.42
SEM	7.4	2.1	0.08	0.05
Nondiabetic subjects (n = 9)				
Mean	75.0	18.4	1.05	1.39
SEM	8.7	2.9	0.14	0.06

* dpm/ $\mu\text{mol C}$.

† Ratio between flux from pyruvate and flux from acetyl CoA into Krebs cycle, calculated as $(R - 2)/2$ where R is the ratio of the specific activity of glucose carbons 1,2,5 and 6 to the specific activity of glucose carbons 3 and 4.

‡ Calculated as $[2 * (1 + 2Y) * (1 + Y)] / [(5 + 4Y) * Y]$.

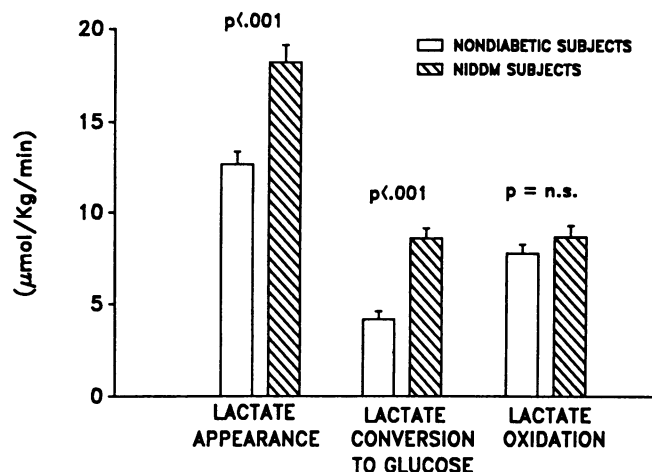


Figure 3. Rates of systemic lactate appearance, conversion to glucose oxidation in NIDDM and nondiabetic volunteers.

nondiabetic subjects (42.7 ± 1.9 and $44.2 \pm 2.9\%$, respectively, $P = \text{NS}$).

Plasma lactate and alanine oxidation (Figs. 3 and 4). Plasma lactate oxidation averaged $8.7 \pm 0.6 \mu\text{mol/kg/min}$ in the NIDDM subjects; this was not significantly different from the value of $7.8 \pm 0.5 \mu\text{mol/kg/min}$ observed in the nondiabetic subjects (Fig. 3). Plasma alanine oxidation was also not significantly different in NIDDM and nondiabetic subjects (1.5 ± 0.1 vs. $1.3 \pm 0.1 \mu\text{mol/kg/min}$, respectively; Fig. 4). Nevertheless, the percentage of plasma lactate disappearance accounted for by oxidation was reduced in the NIDDM subjects (47.5 ± 2.4 vs. $61.9 \pm 3.8\%$ in the nondiabetic groups, $P < 0.01$). On the other hand the percent of plasma alanine appearance accounted for by oxidation was not significantly different in the NIDDM and nondiabetic subject (27.3 ± 2.2 vs. 31.4 ± 3.5 percent, respectively, $P = \text{NS}$).

Taken together, plasma lactate oxidation and incorporation into plasma glucose accounted for 95.8 ± 2.8 and $96.1 \pm 4.5\%$ of plasma lactate turnover in the NIDDM and nondiabetic subjects, respectively ($P = \text{NS}$). The sum of

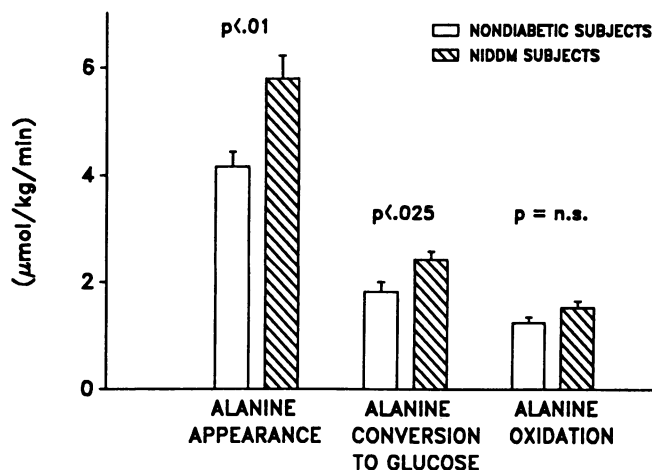


Figure 4. Rates of systemic alanine appearance, conversion to glucose, and oxidation in NIDDM and nondiabetic volunteers.

plasma alanine oxidation and incorporation into plasma glucose accounted for 70.0 ± 3.3 and $75.7 \pm 5.1\%$ of overall plasma alanine turnover in the NIDDM and nondiabetic subjects, respectively, ($P = \text{NS}$).

Muscle glucose, lactate, and alanine metabolism (Table III, Fig. 5). Forearm blood flow was not significantly different in the two groups (2.97 ± 0.10 and 2.76 ± 0.07 ml/100 ml tissue/min in the NIDDM and nondiabetic subjects, respectively). Forearm glucose uptake in the diabetic subjects was nearly twofold greater than in the nondiabetic volunteers (0.82 ± 0.10 vs. 0.48 ± 0.03 $\mu\text{mol}/100$ ml tissue/min, $P < 0.01$). Forearm lactate and alanine fractional extraction, net balance, uptake and release are given in Table III. There was no statistically significant difference between the NIDDM and the nondiabetic subjects for any of these parameters.

Total body skeletal muscle mass was comparable in the NIDDM subjects (32.1 ± 1.9 kg) and nondiabetic volunteers (30.2 ± 2.0 kg, $P = \text{NS}$). Extrapolation of the values for forearm tissue to total body skeletal muscle yielded rates of glucose of 4.24 ± 0.49 and 2.36 ± 0.18 $\mu\text{mol}/\text{kg}$ body weight/min in the diabetic and nondiabetic subjects, respectively ($P < 0.01$). Although muscle glucose uptake accounted for a somewhat greater percentage of overall glucose disposal in the diabetic subjects (24.6 ± 2.3 vs. 20.3 ± 1.5 percent, $P = \text{NS}$), muscle glucose clearance was reduced by 25% in the diabetic subjects (0.87 ± 0.09 vs. 1.16 ± 0.09 ml/kg muscle/min, $P < 0.05$).

Muscle uptake of lactate and alanine not significantly different in the NIDDM and in the nondiabetic subjects (4.2 ± 0.5 vs. 3.9 ± 0.3 $\mu\text{mol}/\text{kg}$ body weight/min, respectively, for lactate and 1.8 ± 0.3 vs. 1.6 ± 0.2 $\mu\text{mol}/\text{kg}$ body weight/min, respectively, for alanine, Fig. 5). These represented 24.0 ± 2.4 and $26.6 \pm 2.3\%$ of overall lactate disposal and 31.7 ± 5.4 and 39.3 ± 3.0 of overall alanine disposal in the NIDDM and in the nondiabetic subjects, respectively. Muscle release of lactate and alanine were also not significantly different in the NIDDM and in the nondiabetic subjects (6.9 ± 0.7 vs. 5.7 ± 0.4 $\mu\text{mol}/\text{kg}$ body weight/min, respectively, for lactate and 3.4 ± 0.3 vs. 3.0 ± 0.3 $\mu\text{mol}/\text{kg}$ body weight/min, respectively,

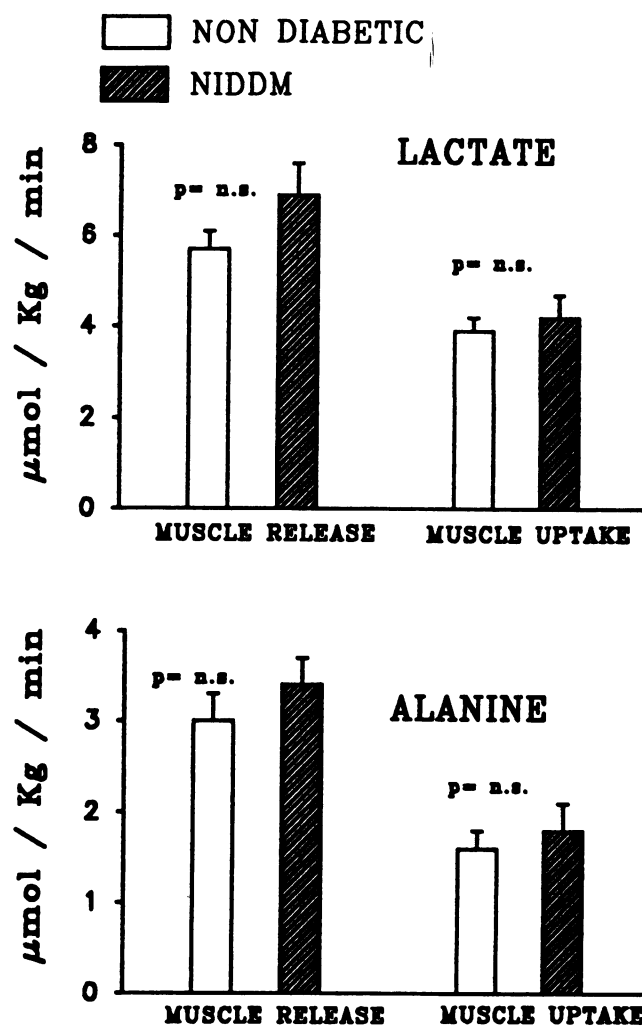


Figure 5. Rates of whole body muscle lactate and alanine release and uptake in NIDDM and nondiabetic volunteers.

Table III. Lactate and Alanine Forearm Net Balance, Fractional Extraction, Uptake, and Release in NIDDM and Nondiabetic Patients

Subjects	Fractional extraction	Forearm net balance	Forearm uptake	Forearm release
$\mu\text{mol}/100$ ml tissue/min				
Lactate NIDDM				
Mean	0.23	0.53	0.80	1.33
SEM	0.01	0.07	0.09	0.14
Nondiabetic				
Mean	0.27	0.47	0.68	1.15
SEM	0.02	0.05	0.07	0.08
Alanine NIDDM				
Mean	0.38	0.31	0.35	0.66
SEM	0.04	0.03	0.06	0.07
Nondiabetic				
Mean	0.38	0.28	0.28	0.61
SEM	0.03	0.03	0.03	0.04

for alanine; Fig. 5). Muscle lactate release accounted for $39.6 \pm 3.8\%$ of overall lactate appearance in plasma in the NIDDM subjects compared to $45.2 \pm 2.7\%$ in the nondiabetic volunteers ($P = \text{NS}$). Muscle alanine release accounted for $61.6 \pm 6.1\%$ of overall alanine appearance in plasma in the NIDDM compared with $72.1 \pm 3.7\%$ in the nondiabetic subjects, respectively ($P = \text{NS}$). Thus, neither the absolute rates of muscle lactate and alanine release nor the percentage of the systemic appearance rates of these gluconeogenic precursors accounted for by muscle release were increased in the NIDDM subjects.

Discussion

Normally, after an overnight fast, gluconeogenesis accounts for $\sim 30\%$ of overall hepatic glucose output (20). In individuals with NIDDM who have fasting hyperglycemia, these rates of hepatic glucose output are increased in the postabsorptive state (1–3, 5, 9–11), and a substantial proportion has been attributed to increased gluconeogenesis (5). The present studies were undertaken to assess the mechanisms responsible for this increased gluconeogenesis; specifically, we sought to de-

termine whether the primary factor was an increased hepatic gluconeogenic efficiency or an increased delivery of gluconeogenic precursors to the liver. Moreover, since it had been proposed (14) that increased glycolysis in muscle might provide the additional lactate and alanine to sustain the increased gluconeogenesis in NIDDM, we compared muscle release of lactate and alanine in our NIDDM and nondiabetic subjects.

Postabsorptive rates of hepatic glucose output were increased by $\sim 6 \mu\text{mol/kg/min}$ in our NIDDM subjects, while the amount of plasma glucose derived from lactate and alanine in these subjects was increased by 2.2 and $0.3 \mu\text{mol/kg/min}$, respectively. Thus, the measured increase in gluconeogenesis from these precursors accounted for $\sim 40\%$ of the observed increase in overall hepatic glucose output. It should be pointed out, however, that gluconeogenesis from these precursors probably represents only $\sim 80\%$ of all gluconeogenesis (7). Moreover, use of arterial specific activities/isotope enrichments rather than intrahepatic values could cause underestimation of their incorporation in glucose by as much as 30% (34). Taking these facts into consideration, our data would indicate that gluconeogenesis could account for nearly 70% of our NIDDM subjects' increase in overall hepatic glucose output. The results of the present study thus support the concept that increased gluconeogenesis is the major process responsible for the increased overall hepatic glucose output found in NIDDM in the postabsorptive state. Indeed two recent studies (35, 36) indicate that gluconeogenesis from glycerol is increased threefold in NIDDM.

Regarding the mechanism(s) responsible for the increased gluconeogenesis in NIDDM, both plasma lactate and alanine rates of appearance in plasma were increased in our NIDDM subjects. To our knowledge this is the first study in which rates of plasma lactate appearance have been compared in NIDDM and nondiabetic subjects. Yki-Jarvinen et al. (21) examined rates of appearance of lactate in plasma before and after insulin therapy in NIDDM but did not present data for nondiabetic volunteers; the values reported for the NIDDM subjects before insulin therapy in that study ($\sim 16 \mu\text{mol/kg/min}$) were greater than those observed in our nondiabetic volunteers ($\sim 12 \mu\text{mol/kg/min}$). As far as the rates of plasma alanine appearance are concerned, our results are in agreement with the preliminary data of Comstock et al. (37), who found a 39% increase in plasma alanine appearance in NIDDM subjects as compared with nondiabetic subjects. In an earlier study, Chochinov et al. (16) failed to demonstrate an increase in plasma alanine appearance in NIDDM subjects; however, the subjects in that study were quite heterogeneous in age and degree of obesity, and some did not have fasting hyperglycemia.

Although the systemic appearance of alanine was increased in our NIDDM subjects, the proportion of the appearance of alanine that was incorporated into plasma glucose was not increased. Therefore, the increased gluconeogenesis from alanine in these subjects could be accounted for solely by the increase in plasma alanine appearance. In contrast both the rate of lactate appearance in plasma and the proportion of lactate appearance that was converted to glucose were increased in our NIDDM subjects. Had the proportion of lactate appearance that was converted to glucose not been increased, gluconeogenesis from lactate would have been increased by 32%. On the other hand, had the appearance of lactate not been increased, gluconeogenesis from lactate would have been

increased by 28%. Thus, as far as lactate is concerned, the increase in its availability and the increase in the hepatic efficiency of its conversion to glucose were about equally important in explaining the increased lactate gluconeogenesis.

Regarding the possible mechanism for the increased hepatic efficiency in converting lactate to glucose, it is noteworthy that plasma glucagon levels were increased in our diabetic subjects. This could certainly be one factor (18). Another factor could be hepatic insulin resistance (3). Recently increased lipolysis and efficiency for hepatic conversion of glycerol to glucose has been found in NIDDM (35). Conceivably increased hepatic fatty acid oxidation might also enhance the hepatic gluconeogenic pathway in NIDDM (19).

The reason for the discrepancy between the roles of substrate availability and efficiency of intrahepatic conversion (as reflected by the proportion of systemic appearance rates that were incorporated into glucose) for lactate and alanine is unclear. Since lactate and alanine are thought to equilibrate fairly rapidly with pyruvate (38), one would have expected similar results for each precursor. It is possible, however, that intrahepatic alanine enrichment might have been diluted to a greater extent than intrahepatic lactate specific activity. This would lead to a relative underestimation of alanine incorporation into glucose relative to that of lactate and could provide an explanation for the failure to detect an increased proportion of alanine appearance that was incorporated into glucose.

The present results, nevertheless, indicate that increased provision of both lactate and alanine is of considerable importance for the increased gluconeogenesis found in NIDDM. The tissues providing the increase in lactate and alanine availability are therefore of interest. It has been proposed (14) that there might be increased release of gluconeogenic precursors from muscle in NIDDM because of greater muscle glucose uptake. In the present study, we therefore compared lactate and alanine release from muscle in NIDDM and nondiabetic subjects. To do this, it was necessary to combine isotopic and balance techniques so that individual rates of uptake and release could be determined. Mere measurement of net balance of these substrates, which represents the difference between uptake and release, would markedly underestimate their release (30), because muscle simultaneously produces and consumes these substrates (30, 39).

In both groups of subjects muscle release contributed appreciably to the overall appearance of lactate and alanine into the systemic circulation. In the nondiabetic subjects, muscle release accounted for 45% of plasma lactate appearance and 72% of plasma alanine appearance. In the diabetic subjects, muscle accounted for 40% of lactate appearance and 61% of alanine appearance.

Nevertheless, although we confirmed that muscle glucose uptake is substantially increased in NIDDM individuals with fasting hyperglycemia (9–11), absolute rates of muscle lactate and alanine release were not significantly different in the two groups. Approximately 45% of plasma lactate is due to glycolysis of plasma glucose (40); the remainder is presumably due to glycolysis of glycogen carbons. Comparable data for alanine are not available. It is possible that the hyperglycemia and hyperinsulinemia of our diabetic subjects had reduced muscle glycogenolysis, therefore offsetting an increase in muscle production of lactate and alanine from plasma glucose. Regardless, although muscle contributes substantially to the amount

of lactate and alanine released into the systemic circulation, our data indicate that muscle cannot be regarded as a major source for their increased systemic delivery in NIDDM.

The present studies, therefore, suggest that tissues other than muscle are responsible for increased release of lactate and alanine into the systemic circulation in NIDDM. Erythrocytes, skin and gut are currently considered to be important sources of lactate in nondiabetic individuals (3). Moreover, several recent studies (41–43) indicate that subcutaneous adipose tissue could also be an important source of systemic lactate. Indeed Newby et al. (42) have recently reported that in adipocytes of obese diabetic rats there is an increased in both absolute and relative amounts of glucose conversion to lactate. Thus, adipose tissue may also be an important source for increased lactate release in NIDDM.

Regarding the possible source of increased systemic delivery of alanine in NIDDM, the gut is a major site of production of alanine (44). This occurs to a considerable extent from glutamine that has been released from muscle (45). Although muscle release of glutamine has not as yet been assessed in NIDDM, it is possible that an increased release of glutamine from muscle could increase production of alanine by the gut, which could explain at least in part the increased systemic appearance of alanine in NIDDM. Further studies are needed to test this possibility.

In conclusion, the present studies demonstrate that gluconeogenesis from alanine and lactate is increased in NIDDM and that this is due to both increased delivery of substrates to the liver and increased hepatic efficiency in converting substrates to glucose. Regarding the specific tissues responsible for the increased delivery of substrates to the liver, muscle release of lactate and alanine was not significantly increased in our NIDDM subjects; therefore, tissues other than muscle are the probably source.

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