Splanchnic and Leg Exchange of Glucose, Amino Acids, and Free Fatty Acids during Exercise in Diabetes Mellitus

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ABSTRACT The influence of exercise on leg and splanchnic exchange of substrates was examined in eight insulin-dependent diabetics 24 h after withdrawal of insulin and in eight healthy controls studied at rest and after 40 min of bicycle ergometer exercise at 55–60% of maximal capacity. In four of the diabetic subjects, basal arterial ketone acid levels were 3–4 mmol/liter (ketotic diabetics) and in the remainder, below 1 mmol/liter (nonketotic diabetics). Free fatty acid (FFA) turnover and regional exchange were evaluated with ^{14}C-labeled oleic acid.

Leg uptake of blood glucose rose 13–18-fold during exercise in both the diabetics and controls and accounted for a similar proportion of the total oxygen uptake by leg muscles (25–28%) in the two groups. In contrast, leg uptake of FFA corresponded to 39% of leg oxygen consumption in the diabetic group but only 27% in controls. Systemic turnover of oleic acid was similar in the two groups.

Splanchnic glucose output increased during exercise 3–4-fold above resting levels in both groups. In the diabetics, splanchnic uptake of lactate, pyruvate, glycerol, and glycogenic amino acids rose more than twofold above resting levels and was fourfold greater than in exercising controls. Total precursor uptake could account for 30% of the splanchnic glucose output in the diabetic group. In contrast, in the controls, total splanchnic uptake of glucose precursors was no greater during exercise than in the resting state and could account for no more than 11% of splanchnic glucose output. The augmented precursor uptake during exercise in the diabetics was a consequence of increased splanchnic fractional extraction as well as increased peripheral production of gluconeogenic substrates. The arterial glucagon concentration was unchanged by exercise in both groups, but was higher in the diabetics.

In the diabetic subjects with ketosis in the resting state, exercise elicited a rise in arterial glucose and FFA, an augmented splanchnic uptake of FFA, and a 2–3-fold increase in splanchnic output of 3-hydroxybutyrate. Uptake of 3-hydroxybutyrate by the exercising leg rose more rapidly than splanchnic production, resulting in a fall in arterial levels of 3-hydroxybutyrate.

It is concluded that (*a*) glucose uptake by exercising muscle in hyperglycemic diabetics is no different from that of controls; (*b*) splanchnic glucose output rises during exercise to a similar extent in diabetics and controls, while uptake of gluconeogenic substrates is markedly higher in diabetics and accounts for a greater proportion of total splanchnic glucose output; (*c*) exercise in diabetic patients with mild ketosis is associated with a rise in blood glucose and FFA levels as well as augmented splanchnic production and peripheral uptake of ketone bodies.

INTRODUCTION

It has long been known that physical exercise improves glucose tolerance and diminishes insulin requirements in patients with diabetes mellitus (1–3). The mechanism(s) underlying the beneficial effects of exercise in diabetes are not well understood. Theoretically, the lowered blood sugar levels after exercise may be ascribed either to suppression of hepatic glucose output and/or to augmented glucose utilization by muscle during and after exercise. Data from exercising healthy individuals indicate that working muscle increases its assimilation of blood glucose in proportion to both duration and severity of the work performed (4–6). In a study of two

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TABLE I

Clinical Data for Diabetic and Control Subjects

Subject	Age	Height	Weight	Maximal oxygen uptake	History of diabetes	Dose of lente insulin*	Arterial concentration of		
							Glucose	3-Hydroxy-butyrate	Aceto-acetate
	yr	*cm*	*kg*	*liter/min*	*yr*	*IU*		*mmol/liter*	
Patients									
H. L.	21	184	71	3.19	6	32+28	21.2	2.09	1.06
K. W.	28	180	84	3.40	5	28+20	14.8	2.89	0.46
G. W.	29	180	68	2.35	11	56	20.9	3.28	1.54
K. M.	42	183	72	4.01	17	44+8	26.9	2.64	0.75
L. B.	30	178	59	2.10	11	44	14.8	0.79	0.29
I. J.	34	168	73	2.93	5	40	15.1	0.13	0.06
T. S.	36	173	73	3.58	9	52	7.1	0.03	0.06
T. B.	30	172	64	3.41	10	60+56	12.4	0.23	0.08
Controls									
C. H.	26	186	77	4.45			4.2		
O. C.	27	188	89	3.26			4.9		
T. J.	26	187	86	4.16			5.1		
B. B.	24	188	76	3.10			4.3		
L. N.	25	185	71	3.45			4.3		
B. C.	23	177	67	3.52			4.1		
M. W.	27	174	61	3.51			4.1		
L. H.	40	178	71	2.05			4.2		

* Where two numbers are given, they represent morning and evening doses of insulin, respectively.

insulin-dependent diabetic subjects, Sanders, Levinson, Abelmann, and Freinkel (7) reported unchanged or increased arterial-femoral venous (A-FV)[1] glucose differences during exercise, suggesting that in diabetic individuals glucose uptake by muscle may also rise in response to exertion. In contrast to the limited data available in human subjects, studies in lightly exercised, depancreatized, running dogs (2–3-fold increase in oxygen consumption) have indicated reduced rates of peripheral glucose utilization compared to controls (8, 9).

The interaction of diabetes and exercise is also of interest with respect to hepatic glucose production. In normal man the liver responds to short-term exercise by increasing its glucose output (10), the major part of which derives from hepatic glycogenolysis and only a minor part from gluconeogenic processes (4, 6). In the resting state, splanchnic glucose output of diabetic patients is comparable to that of controls but the rate of gluconeogenesis is augmented (11). Although exercising, depancreatized dogs show normal or augmented hepatic glucose output (9), no information appears to be available on hepatic glucose production during exercise in patients with diabetes mellitus.

[1] *Abbreviations used in this paper:* A-FV, arterial-femoral venous; FFA, free fatty acids.

The present study was designed to examine in man the influence of diabetes mellitus on peripheral glucose and free fatty acid (FFA) utilization, as well as on hepatic glycogenolytic and gluconeogenic processes during physical exercise. This was done by the simultaneous determination of leg and splanchnic exchange of substrates during bicycle exercise in insulin-dependent juvenile diabetics and in healthy controls.

METHODS

Subjects and procedures. Eight male, nonobese patients with insulin-dependent diabetes mellitus of several years duration were studied at the Serafimer Hospital, Stockholm. Clinical data are given in Table I. The patients were in good nutritional balance and ingested a weight-maintaining diet made up of approximately 40% carbohydrate and 25–35% each of protein and fat. All patients were followed at regular intervals of 2–3 mo in the outpatient department. None had had periods of weight loss or episodes of hypoglycemia during the year preceding the study. There was no history or evidence of liver disease. No patient had signs or symptoms of peripheral vascular disease. At the time of the study all patients were actively employed. The control group consisted of eight healthy nonobese male volunteers. Data on age and body dimensions are given in Table I. None of the subjects participated in competitive athletics on a regular basis. The mean (±SE) maximal oxygen uptake in the diabetics was 3.12±0.23 liter/min, not significantly different from that found in controls (3.44± 0.25 liter/min). The nature, purpose, and possible risks involved in the study were carefully explained to all pa-

tients and control subjects before their voluntary consent to participate was obtained.

The studies were performed in the morning after an overnight fast (12–14 h). Insulin was withheld for 24 h before the study. Teflon catheters with an outer diameter of 1.2 mm were inserted percutaneously into a femoral artery, both femoral veins, and an antecubital vein. A Cournand catheter (no. 7 or 8) was introduced percutaneously into another antecubital vein and manipulated under fluoroscopic control to a right-sided main hepatic vein. The tip of the catheter was placed 3–4 cm from the wedge position. Patency of the catheters was maintained by intermittent flushing with saline. Heparin was not employed in the study.

When the catheters were introduced the subjects were studied at rest in the supine position and during upright exercise on a bicycle ergometer (Siemens-Elema, Stockholm, Sweden). They exercised for 40 min on a work load calculated to result in a pulmonary oxygen uptake of approximately 60% of the previously determined maximal value. The loads employed were 500–900 kg-m/min (1 kg-m/min = 0.163 W) in the patient group and 490–1,000 kg-m/min for the controls. The relative workloads achieved were 59±1% and 56±2% of maximal oxygen uptake for patients and controls, respectively. Blood samples were collected simultaneously from the femoral artery and vein and from the hepatic vein repeatedly at timed intervals at rest and during exercise. Expired air was collected at rest and after 10 and 40 min of exercise for determination of pulmonary oxygen uptake.

Hepatic blood flow was estimated at rest and during exercise by the continuous infusion technique (12) with indocyanine-green dye (13). The procedure employed has been described in detail elsewhere (6). Leg blood flow was determined at rest and during exercise by the indicator dilution procedure described by Jorfeldt and Wahren (14). Plasma volume was determined after intravenous injection of Evans blue (25 mg).

Albumin-bound [1-^{14}C]oleic acid was infused intravenously at a constant rate (0.4–0.6 μCi/min) for 30 min at rest and then during the entire exercise period. Constant levels of oleic acid specific activity were reached in each individual at rest and at 20–40 min of exercise (Fig. 1). The mean specific activities from three measurements at rest (obtained between 20 and 30 min after the start of the infusion) and three measurements during exercise (between 30 and 40 min) were used in the calculation of oleic acid turnover. [1-^{14}C]Oleic acid (sp act 53.7 mCi/mmol) was obtained from the New England Nuclear Corp. (Dreieichenhain, Germany) and bound to human serum albumin as described elsewhere (15).

Analytical methods. Glucose was analyzed in whole blood by the glucose oxidase reaction (16). Lactate (17), pyruvate (18), glycerol (19), 3-hydroxybutyrate, and acetoacetate (20) were determined enzymatically in whole blood. Individual acidic and neutral amino acids were measured in plasma by the automated ion-exchange chromatographic technique (21). Plasma glucagon was analyzed by radioimmunoassay (22). Total and individual plasma FFA were determined by a gas chromatographic method (23). Radioactivity in the FFA fraction was measured as described previously (15). Indocyanine green dye was determined spectrophotometrically at 805 nm in serum samples. Oxygen saturation was measured spectrophotometrically (24), and hemoglobin concentration was determined by the cyanmethemoglobin technique (25). Hematocrit was measured with a microcapillary hematocrit centrifuge and corrected for trapped plasma. Expired air was analyzed by the Scholander microtechnique.

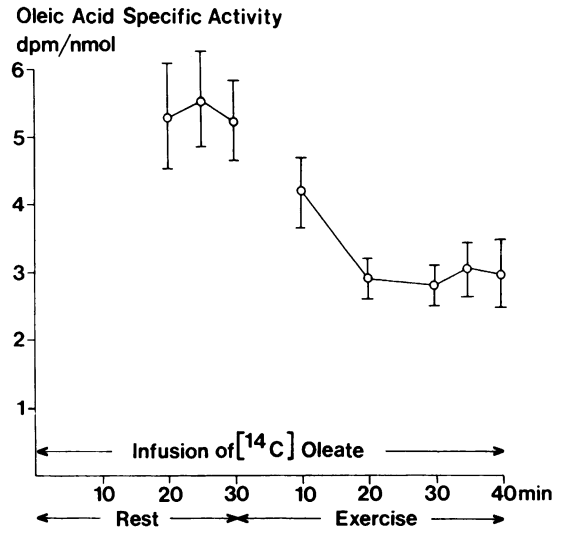

FIGURE 1 Oleic acid specific activity at rest and during exercise in the diabetic group. The mean specific activities from the three measurements in the resting state and from the observations obtained between 30 and 40 min were used in the calculations of oleic acid turnover and regional exchange.

Calculations. The fractional uptake (f) of oleic acid across the leg and splanchnic vascular beds was calculated on the basis of its arterial (A) and venous (V) radioactivity: $f = {}^{14}\text{C-18}:1_{A-V}/{}^{14}\text{C-18}:1_A$. The uptake of oleic acid (U, in micromoles per minute) by the leg or the splanchnic area was calculated as the product of f, the arterial plasma concentration of free oleic acid, and the plasma flow (P). Release of oleic acid (R, in micromoles per minute) was estimated as the difference between U and the net exchange of unlabeled oleic acid: $R = U - (18:1_{A-V}) \cdot P$. The turnover rate of oleic acid was calculated as the amount of radioactivity infused per unit time divided by the oleic acid specific activity. The fractional turnover of oleic acid was calculated as its turnover divided by the product of the arterial concentration times the plasma volume. An average oxygen consumption of 24.7 mol/mol fatty acid was assumed. These calculations have been reported previously (6), and the validity of oleic acid as a tracer for the entire FFA fraction has been established (15, 26).

Data in the text, tables, and figures are given as means ±SE. Standard statistical methods (27) have been employed, with the paired t test when applicable.

RESULTS

Heart rate, oxygen uptake, and regional blood flow. Data on oxygen uptake and circulatory adaptation at rest and during exercise are given in Table II. Oxygen uptake for the patients at rest as well as during exercise was no different from that of the controls. However, during exercise the patients attained a higher heart rate by about 20 beats/min, the mean maximal heart rate being 167±3 for the patients and 145±8 for the controls. The respiratory exchange ratio was higher in the

TABLE II

*Heart Rate, Oxygen Uptake, and Regional Blood Flow in Diabetic Patients (P) and Controls (C) at Rest and during Exercise**

			Exercise			
		Rest	10 min	20 min	30 min	40 min
Heart rate, *beats/min*	P	73±4	148±6‡	156±5	163±3‡	167±3§
	C	66±4	129±7	141±8	145±9	145±8
Pulmonary oxygen uptake, *ml/min*	P	302±15	1,834±118			1,924±214
	C	294±21	1,950±149			1,928±142
Respiratory exchange ratio	P	0.86±0.06‡	0.86±0.01			0.80±0.01‡
	C	0.73±0.02	0.86±0.02			0.85±0.02
Leg blood flow,‖ *liter/min*	P	0.90±0.12	6.90±0.36			6.82±0.48
	C	0.86±0.10	7.20±0.62			7.24±0.72
A-FV oxygen difference, *ml/liter*	P	48.3±6.5‡	159.8±6.2			174.0±9.6
	C	68.7±7.1	165.7±3.7			169.0±5.2
Leg oxygen uptake,‖ *ml/min*	P	40±5	1,105±76			1,128±82
	C	58±9	1,190±100			1,207±102
Estimated splanchnic blood flow, *liter/min*	P	1.28±0.06	0.74±0.05	0.71±0.06	0.71±0.09	0.69±0.09
	C	1.14±0.11	0.58±0.09	0.60±0.09	0.64±0.08	0.66±0.07
Arterial-hepatic venous oxygen difference, *ml/liter*	P	57.7±3.9	116.1±11.5			165.9±13.8‡
	C	48.6±3.3	98.3±5.3			133.9±10.4
Splanchnic oxygen uptake, *ml/min*	P	74.4±7.8‡	87.1±11.2§			106.5±13.0‡
	C	54.7±5.1	55.0±6.4			85.6±7.7
Hematocrit, %	P	42.3±1.0	46.3±1.1			45.7±0.8
	C	42.9±0.8	45.9±1.0			46.2±1.0

* Data are given as means ±SE.
‡ Significantly different from the corresponding control value, $P < 0.05$.
§ Significantly different from the corresponding control value, $P < 0.025$.
‖ Data are presented for both legs.

patient group in the resting state (0.86±0.06) than in controls (0.73±0.02, $P < 0.05$). Mechanical efficiency during exercise was similar in the two groups. No differences of statistical significance were discernible in leg blood flow or leg oxygen uptake. A-FV oxygen difference was lower in the patient group at rest only. Estimated splanchnic blood flow tended to be slightly higher (11–27%) in the patient group both at rest and during exercise. The arterial-hepatic venous oxygen difference was also higher in the patient group both at rest and during exercise ($P < 0.05$). The calculated splanchnic oxygen uptake for the diabetic patients exceeded that of controls by 35% in the resting state and by 25–60% during exercise.

Arterial substrate concentrations. Table III presents the arterial concentrations of substrates for patients and controls in the resting state and during exercise. The arterial glucose concentration was measured on three occasions at 15-min intervals in all subjects in the resting state. The mean values obtained in the diabetic group were 17.3±2.7, 17.4±2.7, and 17.4±2.6 mmol/liter. Similarly, lactate, pyruvate, glycerol, and FFA determinations at 15-min intervals yielded mean values varying less than 4%, indicating a relative steady state at rest with regard to the concentrations of circulating metabolites.

The arterial glucose level was approximately four times higher in the patient group than in the control group, both at rest and during exercise. In neither group did the mean arterial glucose concentration change significantly during exercise, although there were variations among the diabetics, depending upon the presence or absence of ketosis (see below). The arterial levels of lactate and pyruvate were similar at rest but rose more in response to exercise in the patients ($P < 0.01$). The patients' arterial glycerol concentrations exceeded that of controls, both at rest and during exercise, and

were directly correlated to their arterial FFA ($r = 0.79$, $P < 0.05$ at rest; $r = 0.93$, $P < 0.01$ during exercise).

Four of the diabetic subjects showed a mild degree of ketosis at the time of the study (Table I), the mean arterial level of 3-hydroxybutyrate for the entire group being 1.51±0.48 mmol/liter, while that for acetoacetate was 0.54±0.19 mmol/liter. Among the diabetics, the concentration in the resting state of both 3-hydroxybutyrate and acetoacetate correlated directly with the level of FFA ($r = 0.75$ and 0.71, respectively; $P < 0.05$). During exercise the concentration of 3-hydroxybutyrate fell by 25% ($P < 0.05$) while the level of acetoacetate remained largely unchanged.

The arterial concentration of FFA at rest and the initial fall in FFA level induced by exercise was similar in the diabetic and control subjects. The subsequent increase was on the average greater in the diabetics so that their arterial FFA levels at 30 and 40 min of exercise exceeded those of the controls by 30–35%. This difference, however, did not attain statistical significance, due to the considerable variation between the response in the ketotic and nonketotic diabetic groups (see below).

Data on concentration, pool size, and turnover of oleic acid are shown in Table IV. The turnover rate of oleic acid did not differ between the groups at rest or during exercise, but the diabetics showed a higher fractional turnover at rest ($P < 0.05$), which may in part be referable to the fact that the control subjects had greater plasma volumes than did the diabetics ($P < 0.01$). However, since the plasma oleic acid concentration was somewhat higher in the diabetics, no difference in pool size of oleic acid was observed.

The arterial concentrations of plasma amino acids are shown in Table V. As expected (11), the levels of branched chain amino acids were 65–100% higher in the diabetic group. The arterial concentration of alanine rose during exercise to a similar extent (80–100%, $P < 0.01$) in both groups, while all other amino acids showed no significant change.

Arterial glucagon concentrations (Table III) were significantly higher in the diabetic group throughout the exercise period ($P < 0.025$). This difference reflected an increase in the basal concentrations, inasmuch as arterial glucagon did not rise above resting levels with this degree of exercise in either the diabetic or control groups.

Leg exchange of substrates. Glucose uptake by the legs at rest was similar for patients and controls (Table VI). During exercise leg glucose uptake rose 13- to 18-fold in both groups, the mean rise being greater but not significantly so for the diabetics. Lactate and pyruvate output did not differ between the two groups at rest but was greater for the patients during exercise ($P < 0.02$).

A significant uptake of acetoacetate by the leg was observed in the diabetics in the resting state (Table VI). The A-FV difference of acetoacetate at rest was 0.058±

TABLE III

*Arterial Concentrations of Substrates and Glucagon in Diabetic Patients (P) and Control Subjects (C) at Rest and during Exercise**

			Exercise			
		Rest‡	10 min	20 min	30 min	40 min
Glucose, *mmol/liter*	P	17.37±2.68	17.54±2.80	17.81±3.13	18.19±3.41	17.46±4.12
	C	4.32±0.17	4.20±0.23	4.22±0.26	4.17±0.21	4.33±0.24
Lactate, *mmol/liter*	P	0.78±0.09	3.51±0.53			3.58±0.88
	C	0.58±0.06	2.23±0.46			1.27±0.21
Pyruvate, *μmol/liter*	P	71±4	189±20			212±28
	C	63±8	129±7			101±14
Glycerol, *μmol/liter*	P	63±9				399±70
	C	37±3				128±31
3-hydroxybutyrate, *mmol/liter*	P	1.51±0.48	1.06±0.36			1.14±0.45
	C	0.10±0.06	—			—
Acetoacetate, *mmol/liter*	P	0.54±0.19	0.53±0.17			0.45±0.17
	C	0.06±0.04	—			—
FFA, *μmol/lier*	P	791±89	588±95	904±137	1,049±165	1,095±271
	C	734±133	644±99	728±95	761±110	802±133
Glucagon, *pg/ml*	P	78±11	79±10	76±10	76±7	78±7
	C	50±9	44±9	45±7	49±8	51±6

* Data are given as means ±SE.

‡ Data in the resting state for glucose, lactate, pyruvate, glycerol, and FFA were calculated from the mean of three observations obtained at 5–10-min intervals in each subject.

TABLE IV

*Systemic and Regional Turnover Data for Oleic Acid in Diabetic Patients and Controls at Rest and during Exercise**

	Rest		Exercise (30–40 min)	
	Diabetic patients	Controls	Diabetic patients	Controls
Arterial concentration, *μmol/liter*	297±33	248±61	395±67	272±48
Plasma pool, *μmol*	825±158	889±212	1,048±299	908±153
Turnover rate, *μmol/min*	299±57	253±54	599±97	445±64
Fractional turnover rate, min^{-1}	0.36±0.01	0.29±0.02	0.60±0.06	0.57±0.04
Leg exchange‡				
Fractional uptake	0.35±0.05	0.49±0.05	0.20±0.04	0.19±0.02
Uptake, *μmol/min*	49±11	72±14	248±50	200±31
Uptake, *% of turnover*	17±2	31±5	43±7	45±4
Release, *μmol/min*	65±13	77±14	127±17	95±20
Splanchnic exchange				
Fractional uptake	0.42±0.07	0.37±0.03	0.58±0.06	0.52±0.05
Uptake, *μmol/min*	76±11	63±20	84±22	43±8
Uptake, *% of turnover*	32±5	25±2	16±4	9±1
Release, *μmol/min*	20±9	14±3	42±11	32±7

* Data are given as means ±SE and represent the mean of three observations obtained in the resting state and between 30 and 40 min of exercise.
‡ Data for both legs are presented.

0.025 mmol/liter and showed a positive regression on its arterial concentration ($r = 0.83$, $P < 0.05$). For 3-hydroxybutyrate, on the other hand, there was no resting uptake (Table VI), the mean A-FV difference being 0.00±0.07 mmol/liter. In contrast, during exercise an uptake of 3-hydroxybutyrate was observed ($P < 0.05$), while the mean exchange of acetoacetate reverted from an uptake at rest to a net release during exercise. The net uptake of total ketone acids by the exercising legs amounted to 430±250 μmol/min.

The fractional uptake and rate of uptake of oleic acid by the legs in the resting state were lower in the diabetics, accounting for 17±2% of the total oleic acid turnover as compared to 31±5% in controls ($P < 0.05$) (Table IV). With exercise, fractional uptake fell to 20% in both groups while the rate of uptake by the legs increased 3–5-fold. The rate of leg uptake and release

TABLE V

*Arterial Concentration of Plasma Amino Acids in the Basal State and during Exercise in Patients with Diabetes and Control Subjects**

	Rest		Exercise, 40 min	
	Diabetes	Controls	Diabetes	Controls
	μmol/min			
Taurine	40±1	33±6	46±4	41±5
Threonine	118±10	96±9	114±8	107±7
Serine	131±12	109±9	117±8	112±9
Proline	184±30	146±20	166±6	168±25
Citrulline	37±2	37±8	30±3	34±5
Glycine	214±26	213±17	218±20	227±13
Alanine	206±16	208±35	408±36	378±44
α-Aminobutyrate	41±4‖	24±3	31±4	24±3
Valine	347±39‡	209±25	336±44§	222±25
Cystine	109±6	135±35	99±4	106±10
Methionine	17±1	18±3	22±2	23±4
Isoleucine	100±23	53±9	99±20	59±9
Leucine	205±36§	110±11	199±33§	119±11
Tyrosine	48±5	44±6	59±6	50±5
Phenylalanine	52±4	48±8	61±5	57±10

* Data are presented as means ±SE.
‡ Significantly different from the corresponding value for controls, $P < 0.02$.
§ Significantly different from the corresponding value for controls, $P < 0.05$.
‖ Significantly different from the corresponding value for controls, $P < 0.01$.

TABLE VI

*Leg Exchange of Substrates in Diabetic Patients (P) and Control Subjects (C) at Rest and during Exercise**

		Rest	Exercise 10 min	Exercise 40 min
Glucose uptake, *mmol/min*	P	0.20±0.04	2.10±0.82	3.66±0.78
	C	0.16±0.02	2.02±0.50	2.56±0.64
Lactate production, *mmol/min*	P	0.14±0.06	3.48±0.58‡	2.62±0.70§
	C	0.06±0.02	1.94±0.56	0.52±0.20
Pyruvate production, *μmol/min*	P	0±2	54±107	30±29
	C	2±2	−12±57	7±56
3-Hydroxybutyrate, uptake, *μmol/min*	P	−3±60		520±210
Acetoacetate uptake, *μmol/min*	P	60±30		−90±110

* Data are given as means ±SE for both legs.
‡ Significantly different from the corresponding control value, $P < 0.05$.
§ Significantly different from the corresponding control value, $P < 0.02$.

TABLE VII

*Leg Exchange of Amino Acids at Rest and during Exercise in Diabetic Patients and Controls**

	Rest		Exercise (40 min)	
	Diabetes	Controls	Diabetes	Controls
		μmol/min		
Taurine	0.3±1.2	−1.5±1.1	−21.1±21.7	−7.4±2.6
Threonine	−9.4±2.6	−10.1±3.3	−0.2±4.8‡	−14.7±5.7
Serine	−4.3±3.7	−1.6±3.1	26.9±19.1§	−28.1±13.3
Proline	−3.5±9.1	−12.5±1.1	38.6±23.7	−20.6±50.6
Citrulline	1.8±1.7	0.8±1.9	0.1±13.8	1.0±8.9
Glycine	−6.6±37	−12.5±3.9	0.3±12.6	−10.7±21.3
Alanine	−32.9±8.2	−36.4±2.3	−58.7±14.1	−92.2±22.4
α-Aminobutyrate	1.0±1.8	−2.1±1.5	0.8±3.6	−5.7±7.1
Valine	0.3±3.1‡	−9.7±5.7	32.9±12.0§	−16.0±3.3
Cystine	4.3±2.3	4.0±2.0	−15.5±16.0	−7.5±6.3
Methionine	−1.4±0.4	−1.5±0.4	−5.1±3.1	−7.1±2.7
Isoleucine	−1.7±0.6	−3.6±1.2	10.8±5.5§	−12.7±5.5
Leucine	−3.2±1.3	−3.8±3.4	15.3±5.5§	−24.9±7.2
Tyrosine	−2.9±0.6	−3.9±1.9	9.7±5.4	−9.4±16.5
Phenylalanine	−2.5±1.4	−3.0±1.9	5.6±5.6§	−40.3±13.7

Data are presented as the means ±SE for both legs.
‡ Significantly different from the corresponding control value, $P < 0.1$.
§ Significantly different from the corresponding control value, $P < 0.02$.

of oleic acid during exercise did not differ significantly between the groups.

Table VII presents the leg exchange of amino acids. The output of most amino acids from the leg tissues was not significantly different in the two groups in the resting state or during exercise. In both groups a rise in alanine release of 130–140% was observed during exercise. It is noteworthy that while a release of branched-chain amino acids was seen in controls during exercise, there was a significant uptake of these amino acids by the exercising leg in the diabetics.

Splanchnic exchange of substrataes (Table VIII). Splanchnic glucose production was similar in the two groups in the resting state. After onset of exercise there was a gradual increase in glucose output, and after 40 min of exercise a 3–4-fold rise was seen in both groups. On the average, glucose production was slightly but not significantly higher in the diabetic group. Lactate uptake by the splanchnic region was 80% greater for the diabetics in the resting state ($P < 0.05$). This difference became more marked during exercise, when splanchnic lactate uptake in the diabetic group was 4–5-fold greater than in the controls. Similarly, pyruvate and glycerol uptake in the splanchnic region for the patients exceeded that of controls both at rest and during exercise by 2–4-fold. The increased uptake of substrates in the diabetic group was a consequence of elevated arterial concentrations (Table III) as well as an increase in fractional extraction by the splanchnic bed.

Splanchnic exchange of individual amino acids is presented in Table IX. As demonstrated previously (11), splanchnic uptake in the diabetics of endogenous glycogenic amino acids, particularly alanine, exceeded that of controls by 45–200%. The augmented uptake was a consequence of increased fractional extraction above that of controls in the case of alanine (62±5% vs. 41±8%, $P < 0.05$), threonine (28±4% vs. 15±4%, $P < 0.025$), and serine (36±4% vs. 18±8%, $P < 0.05$). Dur-

TABLE VIII

*Splanchnic Exchange of Substrates in Diabetic Patients and Controls at Rest and during Exercise**

			Exercise	
		Rest	10 min	40 min
Glucose output,	P	0.85±0.11	1.03±0.15	3.08±0.70
mmol/min	C	0.61±0.06	0.91±0.15	2.44±0.49
Lactate uptake,	P	0.34±0.10‡	1.26±0.29§	1.18±0.34§
mmol/min	C	0.18±0.02	0.23±0.03	0.26±0.07
Pyruvate uptake,	P	16±7	68±23‖	58±23
μmol/min	C	8±7	15±13	22±17
Glycerol uptake,	P	69±10§		238±75§
μmol/min	C	26±4		65±19
3-Hydroxybutyrate output, *μmol/min*	P	300±110		610±320
Acetoacetate output, *μmol/min*	P	280±70		200±70

* Data are given as means ±SE.
‡ Significantly different from the corresponding control value, $P < 0.05$.
§ Significantly different from the corresponding control value, $P < 0.01$.
‖ Significantly different from the corresponding control value, $P < 0.02$.

ing exercise the splanchnic uptake of alanine in the diabetics was slightly but not significantly greater than at rest, yet it clearly exceeded the corresponding value for controls by 45% ($P < 0.02$). As in the resting state, the augmented uptake of alanine during exercise was a consequence of increased fractional extraction in the diabetic group. Similarly, the splanchnic uptake of threonine, serine, proline, α-aminobutyrate, and phenylalanine during exercise was significantly greater in diabetics than controls (Table IX).

In the resting state splanchnic uptake and release of oleic acid (Table IV) were similar in both groups but during exercise the patients' uptake of oleic acid exceeded that of controls by 95% ($P < 0.1$).

A net splanchnic release of both 3-hydroxybutyrate and acetoacetate was demonstrable in the patient group at rest and during exercise (Table VIII). The effect of exercise on splanchnic ketone acid production was variable, depending on the presence or absence of ketosis in the resting state (see below).

Ketotic and nonketotic diabetics. As noted previously, four of the diabetic patients showed resting arterial 3-hydroxybutyrate levels above 2 mmol/liter (Table I). In Table X the arterial concentrations and splanchnic exchange of those substrates for which differences were observed between the ketotic and nonketotic subjects are shown. The poor metabolic control in the ketotic group was reflected by higher resting levels of glucose, FFA, and branched-chain amino acids (Table X). During exercise the arterial glucose level rose in all four ketotic patients, in contrast to the finding of unchanged or decreasing blood glucose in the nonketotic patients. The arterial FFA level and the splanchnic uptake of oleic acid also increased by approximately 50% during exercise in the ketotic patients, while in the nonketotic, as in the control subjects, the splanchnic oleic acid uptake decreased during exercise. The production of 3-hydroxybutyrate by the splanchnic region increased 2–3-fold during exercise in the ketotic patients, but remained unchanged in the nonketotic group. With respect to splanchnic and peripheral exchange of glucose, lactate, pyruvate, and glycerol, no differences were observed between the ketotic and nonketotic groups.

DISCUSSION

In theory, the calculation of systemic and regional exchange of substrates performed in the present study requires a steady state. However, since it is not feasible to attain an absolute steady state in studies of this type, it is noteworthy that the rate at which the arterial concentrations changed during exercise was quite low; for lactate, alanine, glycerol, and FFA it did not exceed 2%·min^{-1}. Considering the mean circulation times of the leg and splanchnic vascular beds, this change in arterial concentration introduces a maximum error in the calculations of regional exchange of no more than 3–4%

TABLE IX

*Splanchnic Exchange of Amino Acids at Rest and during Exercise in Diabetic Patients and Controls**

	Rest		Exercise (40 min)	
	Diabetes	Controls	Diabetes	Controls
		μmol/min		
Taurine	2.4±1.4	0.3±1.0	−4.2±4.7	0.1±0.3
Threonine	24.8±4.7‡	9.8±2.6	16.0±3.0‖	7.8±1.8
Serine	34.3±4.9§	11.6±4.6	15.3±1.7§	6.8±1.5
Proline	22.5±9.9	5.8±3.0	13.9±3.1§	1.7±2.1
Citrulline	−5.1±3.7	−5.6±3.3	−9.4±3.7	−7.2±2.6
Glycine	25.2±8.3	33.9±16.1	11.2±4.3	4.0±4.3
Alanine	94.3±12.0‖	63.5±12.7	107.0±15.1‡	72.9±10.8
α-Aminobutyrate	5.1±2.3	0.3±1.2	1.0±0.4§	−1.9±0.7
Valine	9.5±10.6	−1.5±6.4	−3.3±1.6	−5.6±3.0
Cystine	10.4±6.6	8.0±3.6	−1.4±0.9	3.5±5.0
Methionine	3.5±0.6	2.2±0.5	2.9±0.5	2.1±0.7
Isoleucine	1.1±2.5	−0.1±0.8	−1.6±0.9	−3.0±1.7
Leucine	6.5±5.3	0.9±1.6	−4.9±1.3	−5.8±3.4
Tyrosine	8.3±1.6	9.6±1.5	6.4±0.7	5.1±0.6
Phenylalanine	5.7±2.4	4.0±1.0	4.1±0.8§	0.6±0.7

* Data are presented as means ±SE.
‡ Significantly different from the corresponding control value, $P < 0.05$.
§ Significantly different from the corresponding control value, $P < 0.02$.
‖ Significantly different from the corresponding control value, $P < 0.1$.

TABLE X

*Arterial Concentration and Splanchnic Exchange of Substrates in the Resting State and during Exercise in Ketotic and Nonketotic Diabetic Subjects**

	Rest		Exercise (40 min)	
	Ketotic	Nonketotic	Ketotic	Nonketotic
Arterial concentration, *mmol/liter*				
3-Hydroxybutyrate	2.58±0.26	0.30±0.17	2.35±0.20	0.24±0.17
Acetoacetate	0.95±0.23	0.12±0.06	0.82±0.28	0.16±0.06
Glucose	22.4±3.7	12.4±1.9	26.5±6.3	10.7±2.1
FFA	0.99±0.09	0.59±0.04	1.54±0.15	0.63±0.15
Valine	0.43±0.06	0.28±0.02	0.42±0.05	0.26±0.02
Leucine	0.29±0.05	0.14±0.01	0.28±0.04	0.14±0.01
Isoleucine	0.15±0.03	0.06±0.01	0.15±0.03	0.06±0.01
Splanchnic exchange, *μmol/min*				
3-Hydroxybutyrate	−440±250	−190±60	−1,140±650	−220±160
Acetoacetate	−440±100	−160±20	−320±150	−110±20
FFA‡	244±37	182±7	368±26	100±15

* Data are given as the means ±SE.

‡ Estimated from [^{14}C]oleic acid uptake: see Methods.

of the estimated values. This error appears to be of little consequence in relation to other, nonsystematic errors inherent in the procedures employed. With regard to the measurements of oleic acid turnover, it is noteworthy that a stable level of oleic acid specific activity (Fig. 1) was achieved in each subject due to the rapid turnover of plasma FFA, thus validating calculations of oleic acid turnover and regional exchange.

Previous studies have established that in normal man glucose uptake by working muscle rises in response to exercise (4, 7). For the present group of diabetic patients with moderate to severe hyperglycemia, the uptake of blood glucose by the exercising leg muscles rose 16–18-fold during exercise, a response no different from that of controls. Since all patients had required insulin therapy for more than 4 yr, they presumably had little if any remaining β-cell function. Although recent studies have suggested that long-standing diabetics may retain some β-cell function, as indicated by measurements of C-peptide reactivity (28), even in normal subjects insulin secretion is diminished by exercise (29). The present findings thus support the previously advanced hypothesis that uptake of glucose by contracting muscle is not dependent upon the availability of increased amounts of insulin (4).

The release of lactate and pyruvate from the exercising leg was substantially larger in the patient group than for controls. The relative work loads were kept closely similar in the two groups (59% of maximal oxygen uptake for diabetics, 56% for controls). Moreover, all patients and subjects were actively employed at the time of the study and participated in physical activities to approximately the same extent. It thus appears unlikely that the greater lactate release in the diabetic group was due to differences in either relative work load or physical training. It is noteworthy that the estimated leg glucose oxidation (see below) was similar in the two groups, suggesting that the diabetic state and the associated hyperglycemia favors glucose utilization along the glycolytic pathway.

The measurements of oxygen and substrate uptake by the exercising leg muscles allow a direct estimate of the contribution made by each fuel to the total oxidative metabolism. Data for FFA were estimated by assuming the fractional uptake of oleic acid to be valid for all FFA, the validity of this assumption having been established previously (15, 26). Fig. 2 presents the estimated oxidation of glucose and FFA at 40 min of exercise. After correction for release of glycolytic end products (lactate, pyruvate, and alanine) the contribution of glucose to total oxidative metabolism in the patient group is estimated at 28%, which agrees closely with the corresponding value for controls (25%). In contrast, FFA uptake accounts for 39% of the oxidative metabolism in the patients, as compared to 27% in controls. In addition, oxidation of 3-hydroxybutyrate may account for a further 4% of the total metabolism. Thus, the combined FFA-ketone contribution to oxidation in the diabetic group exceeds that of controls by 60%. These calculations are supported by the observation of a lower respiratory exchange ratio in the patient group (0.80±0.01) than in the control group (0.85±0.02, $P < 0.05$). The substrate balance data also reveal that whereas blood-borne substrates account for no more than 52%

of the total oxidative metabolism of the leg in healthy controls, the corresponding value for the diabetic patients is more than 70%. The greater dependence of the diabetics on blood-borne substrates is in agreement with the observation of decreased availability of muscle glycogen in diabetic subjects with comparable degrees of hyperglycemia to those of the present study (30).

The patients' splanchnic glucose output in the resting state was slightly but not significantly greater than that of controls. In Table XI the contribution of glucose precursors to total glucose output is presented. In agreement with previous studies (11), the total splanchnic uptake of glucose precursors was augmented (80% greater than control), indicating an increased rate of hepatic gluconeogenesis. During exercise this difference became even more marked. Total glucose output rose to approximately the same extent in both groups. However, splanchnic uptake of glucose precursors increased markedly during exercise in the diabetics, so that at 40 min precursor uptake was more than threetimes greater in the diabetics than in controls and could account for 27% of glucose output vs. 9% for controls (Table XI). The current findings thus indicate that in diabetes mellitus a greater fraction of the total splanchnic glucose output during exercise may derive from hepatic gluconeogenesis. Supporting this conclusion is the observation of increased oxygen consumption by the splanchnic area in the diabetic group, both at rest and during exercise (Table II). It should be noted, however, that extrahepatic tissues may contribute to net splanchnic precursor exchange. Consequently, the above calculations of precursor contributions to gluconeogenesis must be viewed as estimates.

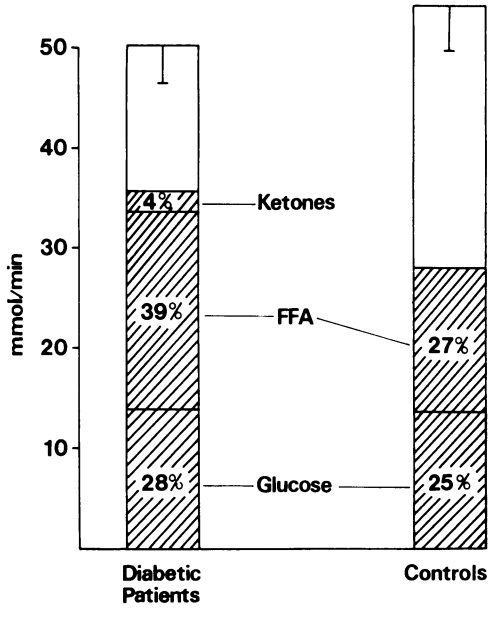

FIGURE 2 Leg uptake of oxygen and substrates during exercise in diabetic patients and controls. The height of the bars represents the mean±SE of oxygen uptake for both legs. The cross-hatched areas indicate substrate uptake expressed in oxygen equivalents. Glucose values have been corrected for release of lactate, pyruvate, and alanine. The percent values represent the proportion of total leg oxygen uptake accountable for by oxidation of each substrate.

TABLE XI

Balance of Gluconeogenic Substrates across the Splanchnic Vascular Bed at Rest during 40 min Exercise in Patients with Diabetes Mellitus and in Controls

	Rest		Exercise	
	Diabetics	Controls	Diabetics	Controls
Glucose production, *mmol/min*	0.85	0.61	3.08	2.44
Uptake of				
Lactate*	0.17	0.09	0.59	0.13
Pyruvate*	0.01	0.01	0.03	0.01
Glycerol*	0.04	0.01	0.12	0.03
Amino acids*‡	0.12	0.08	0.09	0.05
Total	0.34	0.19	0.83	0.22
Total uptake/glucose production, %	40	31	27	9

* Expressed as glucose equivalents in millimoles per minute.
‡ Sum of splanchnic uptakes of threonine, serine, proline, glycine, alanine, cystine, methionine, tyrosine, and phenylalanine.

The augmented precursor uptake in the patient group was a result of increased splanchnic fractional extraction above that of controls in the case of lactate, pyruvate, and alanine (Fig. 2). In addition, increased availability of all substrates, particularly lactate and glycerol (Table III), contributed to the rise in precursor uptake. Inasmuch as a greater peripheral output of lactate was observed in the diabetics, the overall balance data indicate augmented recycling of glucose via the Cori cycle in the diabetic group.

As to the factors responsible for the augmented hepatic uptake of precursors in the diabetics during exercise, it is noteworthy that in the diabetic group plasma glucagon levels were higher than in the controls (Table III). Absolute or relative hyperglucagonemia has been reported previously in diabetics in the resting state (31, 32). In contrast, previous data have shown that insulin concentrations fall in normal subjects to less than 5 μU/ml with exercise (4). Thus it would appear unlikely that differences in insulin concentration between patients and controls are of primary importance in this context. The possibility remains that differences in the concentration of other hormones, such as epinephrine, norepinephrine, and growth hormone (33, 34), may have contributed to the greater hepatic precursor uptake in the diabetics.

In contrast to the changes in peripheral substrate uptake and in splanchnic uptake of gluconeogenic precursors, which were fairly uniform for the entire group of diabetics, the effects of exercise on arterial glucose and FFA concentrations and on splanchnic exchange of ketone acids and FFA differed markedly in the ketotic and nonketotic diabetics (Table X). A rise in arterial glucose and FFA and in splanchnic uptake of FFA and output of 3-hydroxybutyrate was observed in the ketotic group (Table X). The ratio of splanchnic release of 3-hydroxybutyrate to acetoacetate rose from 1:1 in the resting state to 3:1 during exercise, suggesting a more reduced state of mitochondrial adenine nucleotides. This finding is in agreement with the increase in the $NADH/NAD^+$ ratio observed in association with stimulation of ketogenesis (35).

The role of increased lipolysis in the ketogenic response to exercise is indicated by the rise in arterial FFA and splanchnic FFA uptake, observed in the ketotic group only (Table X). Furthermore, a quantitative comparison between splanchnic FFA uptake and ketone body production in the ketotic patients reveals that ketogenesis was the most important pathway of hepatic FFA metabolism, the splanchnic ketone body output corresponding to as much as 85% of the FFA uptake at rest and 93% during exercise. These values for the nonketotic diabetics were 45% at rest and 78% during exercise. For healthy individuals in the resting state 31% of the splanchnic FFA uptake is reported to be converted to ketone bodies (36).

In the resting state utilization of ketone acids by the leg in the diabetics (Table VI) accounted for no more than 10% of the total splanchnic production (Table VIII). The liver released 3-hydroxybutyrate and acetoacetate in about equal amounts, while only acetoacetate was utilized in skeletal muscle, suggesting that 3-hydroxybutyrate was preferentially used in other tissue(s). During exercise, the utilization of 3-hydroxybutyrate by the leg rose more than the splanchnic production, resulting in a decreasing arterial level of 3-hydroxybutyrate in all diabetic subjects. In fact, almost 85% of the splanchnic output of 3-hydroxybutyrate and 53% of total ketone acid production could be accounted for by uptake to the exercising legs, indicating that during exercise, muscle tissue becomes a major site of ketone body disposal. In contrast to the effects of exercise on 3-hydroxybutyrate metabolism, the uptake of acetoacetate observed at rest reverted to a net release, suggesting that during exercise oxidation of 3-hydroxybutyrate to acetoacetate occurs faster than overall ketone acid oxidation.

With regard to the "beneficial" effects of exercise on diabetes mellitus, the present results demonstrate that the utilization of glucose, FFA, and ketone bodies by skeletal muscle is much increased by exercise. However, it is noteworthy that in ketotic diabetics, exercise is associated with augmented arterial levels of glucose and FFA, as well as a rise in splanchnic ketone body production (Table X). Furthermore, in both ketotic and nonketotic diabetics, exercise increases the uptake of gluconeogenic precursors to double the rate observed in the resting state and to levels 3–4 times greater than in exercising healthy controls (Table XI). Thus with respect to ketogenesis as well as gluconeogenesis, short-term exercise, particularly in ketotic patients, may be viewed as intensifying rather than ameliorating the diabetic state. However, these data should not be interpreted as implying a clinically deleterious effect of exercise on the diabetic state, especially since all nonketotic subjects showed falling levels of blood glucose during exercise.

With regard to the effects of diabetes on the metabolic changes in exercise, the overall response to short-term (40 min) exercise in the diabetic group is in many respects strikingly similar to that observed in normal subjects during prolonged periods (4 h) of exercise (6). In both circumstances, as compared to short-term (40 min) exercise in healthy controls, the contribution of FFA to total oxidative fuel consumption by the leg is increased by 60%. The utilization of gluconeogenic precursors by the splanchnic bed is also increased by two-fold or more. In addition there is a consistent uptake of the branched-chain amino acids by the leg, where they may serve as an auxiliary fuel. These similarities thus suggest that as compared to the normal state, diabetes may exert an accelerating influence on the metabolic adaptation to exercise.

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REFERENCES

1. Marble, A., and R. M. Smith. 1936. Exercise in diabetes mellitus. *Arch. Intern. Med.* **58**: 577–588.
2. Joslin, E. P. 1959. The treatment of diabetes mellitus. *In* The Treatment of Diabetes Mellitus. E. P. Joslin, H. F. Root, P. White, and A. Marble, editors. Lea & Febiger, Philadelphia, Pa. 10th edition. 243–300.
3. Maehlum, S., and E. D. R. Pruett. 1973. Muscular exercise and metabolism in male juvenile diabetics. II. Glucose tolerance after exercise. *Scand. J. Clin. Lab. Invest.* **32**: 149–153.
4. Wahren, J., P. Felig, G. Ahlborg, and L. Jorfeldt. 1971. Glucose metabolism during leg exercise in man. *J. Clin. Invest.* **50**: 2715–2725.
5. Jorfeldt, L., and J. Wahren. 1970. Human forearm muscle metabolism during exercise. V. Quantitative aspects of glucose uptake and lactate production during prolonged exercise. *Scand. J. Clin. Lab. Invest.* **26**: 73–81.

6. Ahlborg, G., P. Felig, L. Hagenfeldt, R. Hendler, and J. Wahren. 1974. Substrate turnover during prolonged exercise in man: splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. *J. Clin. Invest.* **53**: 1080–1090.
7. Sanders, C. A., G. E. Levinson, W. H. Abelmann, and N. Freinkel. 1964. Effect of exercise on the peripheral utilization of glucose in man. *New Engl. J. Med.* **271**: 220–225.
8. Vranic, M., and G. A. Wrenshall. 1969. Exercise, insulin and glucose turnover in dogs. *Endocrinology.* **85**: 165–171.
9. Vranic, M., N. Kovacevic, and G. A. Wrenshall. 1970. Exercise and glucose turnover in the normal, partially and totally depancreatized dogs on matched intraportal insulin infusion. *Fed. Proc.* **29**: 314. (Abstr.)
10. Rowell, L. B., E. J. Masoro, and M. J. Spencer. 1965. Splanchnic metabolism in exercising man. *J. Appl. Physiol.* **20**: 1032–1037.
11. Wahren, J., P. Felig, E. Cerasi, and R. Luft. 1972. Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. *J. Clin. Invest.* **51**: 1870–1878.
12. Bradley, S. E. 1948. Measurements of hepatic blood flow. *Methods Med. Res.* **1**: 199–204.
13. Rowell, L. B., K. B. Kraning, II, T. O. Evans, J. W. Kennedy, J. R. Blackmon, and F. Kusumi. 1966. Splanchnic removal of lactate and pyruvate during prolonged exercise in man. *J. Appl. Physiol.* **21**: 1773–1783.
14. Jorfeldt, L., and J. Wahren. 1971. Leg blood flow during exercise in man. *Clin. Sci. (Oxf.).* **41**: 459–473.
15. Hagenfeldt, L., and J. Wahren. 1968. Human forearm muscle metabolism during exercise. II. Uptake, release and oxidation of individual FFA and glycerol. *Scand. J. Clin. Lab. Invest.* **21**: 263–276.
16. Huggett, A. S. G., and D. A. Nixon. 1957. Use of glucose oxidase, peroxidase, and *o*-dianisidine in determination of blood and urinary glucose. *Lancet.* **2**: 368–370.
17. Wahren, J. 1966. Quantitative aspects of blood flow and oxygen uptake in the human forearm during rhythmic exercise. *Acta Physiol. Scand.* **67**: *Suppl.* **269**: 1–92.
18. Bücher, T., R. Czok, W. Lamprecht, and E. Latzko. 1962. Pyruvat. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim/Bergstrasse, Germany. 253–259.
19. Wieland, O. 1962. Glycerin. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim/Bergstrasse, Germany. 211–214.
20. Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymic determination of D(−)-β-hydroxybutyric acid and acetoacetic acid in blood. *Biochem. J.* **82**: 90–96.
21. Spackman, D. A., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30**: 1190–1206.
22. Aguilar-Parada, E., A. M. Eisentraut, and R. H. Unger. 1969. Pancreatic glucagon secretion in normal and diabetic subjects. *Am. J. Med. Sci.* **257**: 415–419.
23. Hagenfeldt, L. 1966. A gas chromatographic method for the determination of individual free fatty acids in plasma. *Clin. Chim. Acta.* **13**: 266–268.
24. Drabkin, D. L. 1950. Measurement of O_2-saturation of blood by direct spectrophotometric determination. *Methods Med. Res.* **2**: 159–161.
25. Drabkin, D. L., and J. H. Austin. 1935. Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *J. Biol. Chem.* **112**: 51–65.
26. Hagenfeldt, L., J. Wahren, B. Pernow, and L. Räf. 1972. Uptake of individual free fatty acids by skeletal muscle and liver in man. *J. Clin. Invest.* **51**: 2324–2330.
27. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition.
28. Block, M. B., R. L. Rosenfield, M. E. Maķo, D. F. Steiner, and A. H. Rubenstein. 1973. Sequential changes in beta-cell function in insulin-treated diabetic patients assessed by C-peptide immunoreactivity. *N. Engl. J. Med.* **288**: 1144–1148.
29. Franckson, J. R. M., R. Vanroux, R. Leclercq, H. Brunengraber, and H. A. Ooms. 1971. Labelled insulin catabolism and pancreatic responsiveness during long-term exercise in man. *Horm. Metab. Res.* **3**: 366-373.
30. Roch-Norlund, A. E., J. Bergström, H. Castenfors, and E. Hultman. 1970 Muscle glycogen in patients with diabetes mellitus. Glycogen content before treatment and the effect of insulin. *Acta Med. Scand.* **187**: 445–453.
31. Unger, R. H., E. Aguilar-Parada, W. Müller, and A. M. Eisentraut. 1970. Studies of pancreatic alpha cell function in normal and diabetic subjects. *J. Clin. Invest.* **49**: 837–848.
32. Wise, J. K., R. Hendler, and P. Felig. 1973. Evaluation of alpha-cell function by infusion of alanine in normal, diabetic and obese subjects. *N. Engl. J. Med.* **288**: 487–490.
33. Hansen, Aa. P. 1970. Abnormal serum growth hormone response to exercise in juvenile diabetics. *J. Clin. Invest.* **49**: 1467–1478.
34. Hansen, Aa. P. 1971. Normalization of growth hormone hyperresponse to exercise in juvenile diabetics after normalization" of blood sugar. *J. Clin. Invest.* **50**: 1806–1811.
35. Löffler, G., F. Matschinsky, and O. Wieland. 1965. Über den Mechanismus der gesteigerten Ketonkörperbildung. II. Redox-Status des DPN der isolierten Rattenleber bei Durchströmung mit Fettsäuren. *Biochem. Z.* **342**: 76–84.
36. Havel, R. J., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49**: 2017–2035.