

Glucose Production, Utilization, and Cycling in Response to Moderate Exercise in Obese Subjects With Type 2 Diabetes and Mild Hyperglycemia

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The glucoregulatory and hormonal responses to moderate-intensity exercise (50% $\dot{V}O_{2\max}$ for 45 min) were examined in subjects with type 2 diabetes and mild hyperglycemia. We studied seven obese subjects with type 2 diabetes and seven lean and seven obese control subjects (fasting plasma glucose levels, 7.5 ± 0.5 , 4.8 ± 0.1 , and 5.2 ± 0.1 mmol/l, respectively). Glucose production, utilization, and cycling (flux between glucose and glucose-6-phosphate [G-6-P]) were measured with [$6\text{-}^3\text{H}$]glucose and [$2\text{-}^3\text{H}$]glucose using the constant specific-activity method. Insulin levels decreased normally during exercise in diabetic subjects. Plasma glucose levels decreased in diabetic subjects, but remained constant in control subjects. Basal glucose production was not different among groups and increased similarly during exercise. The decrease in plasma glucose in diabetic subjects was due to greater glucose utilization (867 ± 83 vs. 726 ± 143 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$; $P < 0.05$). This was a consequence of the mass effect of hyperglycemia, since glucose metabolic clearance increased similarly in all groups. Glucose cycling, expressed as a percentage of total glucose output (i.e., flux through G-6-P) was elevated at rest ($P < 0.01$), but decreased during exercise ($P < 0.01$). The catecholamine response to exercise was blunted in diabetic subjects, presumably indicating autonomic dysfunction. In conclusion, during moderate-intensity exercise in obese diabetic subjects with mild hyperglycemia, 1) insulin secretory responses were normally regulated; 2) glucose homeostasis was different from that in nondiabetic subjects because glucose levels decreased during exercise; 3) the decrease in plasma glucose was due to greater-than-normal rates of glucose utilization, which were sustained by hyperglycemia; and 4) elevated basal rates of glucose cycling decreased during exercise, presumably because exercise simultaneously lowered plasma glucose, was associated with a blunted catecholamine response, and accentuated an underlying defect in hepatic glucokinase activity in type 2 diabetes. *Diabetes* 47:1763–1770, 1998

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ECG, electrocardiogram; FFA, free fatty acid; G-6-P, glucose-6-phosphate; PDH, pyruvate dehydrogenase; RQ, respiratory quotient.

Although exercise is an important component of the treatment of type 2 diabetes, the metabolic responses to exercise in type 2 diabetes are not completely understood. In fact, not many studies have evaluated the acute metabolic response to exercise in subjects with type 2 diabetes (1–10). In most of these studies (1,2,4–8,10), plasma glucose levels declined during exercise, whereas they did not change from basal levels in weight-matched control subjects. The decrease in glucose was mainly attributed to a blunted rise in glucose production as compared with control subjects (2,8); however, in recent studies (7,9), a greater increase in glucose utilization has been described. Most of these studies have been carried out in subjects with type 2 diabetes and high plasma glucose levels (2,7–9); therefore, it is possible that hyperglycemia itself brought about secondary changes in the glucoregulatory response to exercise. The aim of the present study was to examine glucoregulatory and hormonal responses to exercise in subjects with type 2 diabetes and mild hyperglycemia to detect the early events. Furthermore, type 2 diabetic patients with mild hyperglycemia are also the individuals that benefit most from exercise. In addition to glucose production and utilization, glucose cycling, which is the flux of glucose that cycles through the hepatic glucose and glucose-6-phosphate (G-6-P) pools (11), was also measured. In the postabsorptive state as well as during exercise, when the net flux of glucose across the liver is toward glucose production, glucose cycling represents the amount of glucose that, after being dephosphorylated by glucose-6-phosphatase, does not reach the peripheral tissues but is cycled back into the liver. It has been postulated that changes in the rate of cycling could affect the availability of glucose to the contracting muscle during exercise (12). However, studies in normal subjects have failed to demonstrate an important role of glucose cycling in the regulation of glucose flux during higher-intensity exercise ($70\% \dot{V}O_{2\max}$) (12).

Elevated rates of glucose cycling at rest are an early feature of type 2 diabetes and may have a compensatory role in limiting the magnitude of glucose production and, therefore, the resulting hyperglycemia (13,14). The response of glucose cycling to exercise has not been evaluated in type 2 diabetes. In the present study, glucose turnover and cycling were determined at rest and during moderate exercise in obese subjects with type 2 diabetes and mild hyperglycemia, and in two groups of control subjects, an obese weight-matched group and a lean control group.

RESEARCH DESIGN AND METHODS

Subjects. The following groups of seven volunteers each were studied: lean nondiabetic subjects, obese nondiabetic subjects, and obese subjects with type 2 diabetes and mild hyperglycemia (fasting plasma glucose <10 mmol/l). Subjects were recruited based on absence of illness (other than diabetes), history and physical examination, routine blood and urine tests, and resting and standard exercise electrocardiogram (ECG) tests. Subjects with type 2 diabetes had a previous diagnosis of diabetes based on fasting plasma glucose levels repeatedly >7.8 mmol/l. The known duration of diabetes ranged from 2 to 5 years. No complications of diabetes were present. The clinical data of the three groups of subjects are shown in Table 1. Both obese control subjects and obese diabetic subjects were older than lean control subjects, whereas fitness levels (expressed as the percentage of the predicted $\dot{V}O_{2\max}$ values based on age and sex) were matched in all groups. Obese subjects, despite having a normal oral glucose tolerance test, had significantly higher plasma glucose and HbA_{1c} levels than lean control subjects. Subjects with type 2 diabetes had very mild hyperglycemia.

Protocol. The protocol was approved by the Human Subjects Review Committee of the University of Toronto. Informed consent was obtained from the subjects after the nature of each procedure was explained. Studies were performed in the Respiratory Research Laboratory of the Toronto Hospital. Subjects were studied after an overnight fast. To assess nitrogen balance for indirect calorimetry, urine was collected during the 24-h period before the study. The protocol consisted of 1) a basal period of 160 min, 2) 45 min of exercise at 50% $\dot{V}O_{2\max}$, and 3) 150 min of recovery from exercise. An 18-gauge cannula was inserted into a forearm vein for sampling. In the contralateral forearm, a 20-gauge cannula was inserted for the infusion of high-performance liquid chromatography-purified [2-³H]- and [6-³H]glucose. At $t = -160$ min, 66×10^6 dpm of a sterile equal mixture of [2-³H]- and [6-³H]glucose was injected as an intravenous bolus, followed by a constant intravenous infusion of 0.66×10^6 dpm/min. In the diabetic subjects, a larger bolus was administered according to glycemia (i.e., $66 \times [1 + (PG-5)/5 \times 0.6] \times 10^6$ dpm, where PG = plasma glucose in mmol/l) to achieve plateau specific activities in 120 min despite the slower glucose kinetics (15). At $t = 0$ min, the subjects began exercising on an ergometer bicycle under continuous ECG monitoring. After a 2-min warm-up period, the power output was kept fixed at $\sim 50\%$ $\dot{V}O_{2\max}$ (55.9 ± 2.9 , 59.4 ± 1.8 , and $58.1 \pm 1.5\%$ [NS], corresponding to workloads of 88.6 ± 10.1 , 64.3 ± 7.2 , and 65.7 ± 8.4 W [NS] in lean control, obese control, and obese diabetic subjects, respectively). At the onset of exercise, the tracer infusion was increased by 50% to prevent a fall in plasma glucose specific activity. Exercise was continued for 45 min, then stopped after a 2-min cool-down period. The original tracer infusion rate was reestablished and continued throughout the 150-min recovery period. Blood was sampled at the following time points: -40, -30, -20, -10, 0, 5, 10, 15, 25, 35, 45, 55, 65, 85, 105, 135, 165, 175, 185, and 195 min. Gas exchange measurements were taken 30 min before beginning exercise, throughout exercise, and during the last 30 min of recovery.

Laboratory methods and calculations. Plasma glucose was measured on a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Immunoreactive insulin levels were determined using Pharmacia radioimmunoassay kits (Pharmacia AB, Uppsala, Sweden). The insulin assay had a lower detection limit of 22 pmol/l and a 41% cross-reactivity with proinsulin. The C-peptide levels were determined using a kit from Novo Nordisk (Novo Research Institute, Bagsvaerd, Denmark). Glucagon assays were performed using a COOH-terminal specific antibody (16). Other parameters that were measured included cortisol (17), epinephrine and norepinephrine (18), lactate, alanine, glycerol, β -hydroxybutyrate (19), and free fatty acids (FFAs) (20). Plasma radioactivity from [2-³H]- and [6-³H]glucose was determined after deproteinization with Ba(OH)₂ and ZnSO₄, passage through ion exchange columns, and subsequent evaporation. An aliquot of the column eluate

was submitted to the dimedone procedure (21) to isolate the radioactivity originating from [6-³H]glucose. The radioactivity of [2-³H]glucose was calculated as the difference between total radioactivity and that of [6-³H]glucose, corrected for recovery. Recovery was obtained by running aliquots of [6-³H]glucose in each assay, and was $\sim 95\%$. Aliquots of the infused [6-³H]glucose and [2-³H]glucose mixture and of the labeled glucose infusate were assayed together with the plasma samples. The intra-assay coefficients of variation were 2.5% for the radioactivity from [6-³H]glucose and 3.5% for the radioactivity from [2-³H]glucose; the interassay coefficients of variation were 6.5% for the radioactivity from [6-³H]glucose and 11% for the radioactivity from [2-³H]glucose. Total glucose output (rate of appearance evaluated with [2-³H]glucose) and glucose production (net glucose output; rate of appearance evaluated with [6-³H]glucose) were calculated with a modified form of Steele's equation (22), which takes into account the extra tracer infused during exercise (23). Data were smoothed according to the optimal segments method (24), using the optimal error algorithm (25). Glucose cycling was evaluated from the difference between total glucose output and glucose production. Gas exchange was measured using a PK Morgan Exercise System (Morgan Scientific Instruments, Dallas, TX). Protein oxidation was estimated from the urinary nitrogen (26) production rate. The nonprotein respiratory quotient was calculated and the carbohydrate and lipid oxidation rate determined (27). Nonoxidative glucose disposal was determined from the difference between the rate of glucose disappearance and glucose oxidation during rest and recovery. During exercise, glucose oxidation exceeds glucose disposal because of muscle glycogen oxidation (28). After ~ 3 -4 min of submaximal exercise, a steady state is reached, at which time the energy for muscle contraction is provided by oxidation of substrates (28).

Statistical analysis. Data are presented as means \pm SE. Differences between treatments were analyzed using one-way analysis of variance for repeated measures within each period. Differences between periods within each treatment were analyzed using two-way analysis of variance for repeated measures. This was followed, if appropriate, by Tukey's *t* test. Pearson's *r* values were determined using linear regression analysis. Statistical calculations were performed using SAS software (Statistical Analysis System, Cary, NC).

RESULTS

The respiratory quotients (RQs) were 0.82 ± 0.02 , 0.76 ± 0.02 , and 0.82 ± 0.02 (NS) in the basal state in lean control, obese control, and obese diabetic subjects, respectively. They rose to 0.86 ± 0.02 , 0.86 ± 0.02 , and 0.86 ± 0.03 during exercise and declined during recovery to 0.76 ± 0.12 , 0.74 ± 0.01 , and 0.78 ± 0.01 , respectively. ($P < 0.05$, obese diabetic vs. obese nondiabetic subjects). Basal heart rates were 65 ± 5 , 79 ± 3 , and 72 ± 4 beats/min in lean control, obese control, and obese diabetic subjects, respectively. During exercise, these rose to 137 ± 6 , 142 ± 3 , and 132 ± 3 beats/min, respectively, and returned to basal levels during recovery; there was no significant difference among groups.

Basal glucose levels were mildly elevated in obese diabetic subjects (Fig. 1). Glucose levels were slightly, but not significantly, higher in obese than in lean nondiabetic subjects. During exercise and recovery, plasma glucose did not change

TABLE 1
Subject characteristics

	Lean control subjects	Obese control subjects	Obese diabetic subjects
<i>n</i>	7	7	7
Sex (M/F)	4/3	2/5	3/4
Age (years)	$39.9 \pm 2.0^*$	49.1 ± 4.5	46.6 ± 1.2
BMI (kg/m ²)	$23.2 \pm 1.1^*$	32.8 ± 2.0	32.8 ± 1.8
$\dot{V}O_{2\max}$ (ml/min)	$2,681 \pm 310$	$2,145 \pm 213$	$2,305 \pm 203$
$\dot{V}O_{2\max}$ (% predicted)§	113.9 ± 26.6	108.8 ± 11.6	116.9 ± 4.1
Fasting glucose (mmol/l)	4.3 ± 0.1	5.0 ± 0.1	$7.5 \pm 0.8^\ddagger$
HbA _{1c} (%)	4.5 ± 0.2	5.0 ± 0.1	$6.6 \pm 0.6^\ddagger$

Data are means \pm SE. * $P < 0.05$ vs. obese control, obese diabetic; † $P < 0.05$ vs. lean control; ‡ $P < 0.05$ vs. obese control, lean control; §percent of the predicted $\dot{V}O_{2\max}$ values based on age and sex.

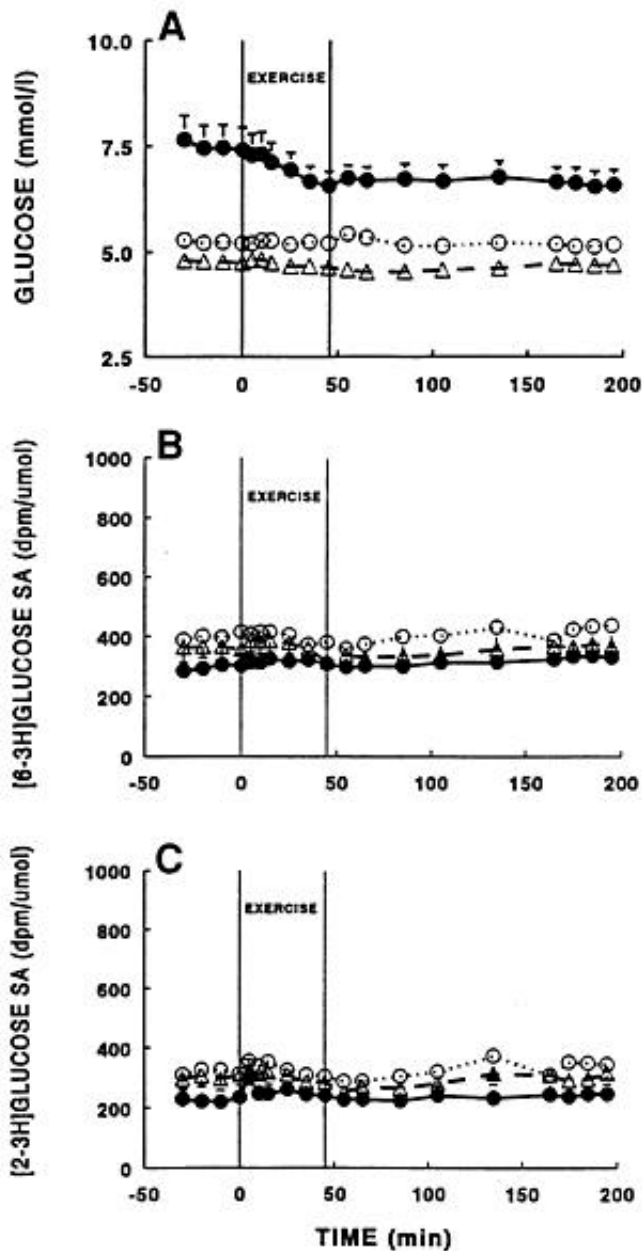


FIG. 1. Plasma glucose levels (A), plasma specific activity (SA) of [6-³H]glucose (B), and plasma SA of [2-³H]glucose (C) in lean nondiabetic subjects (—△—), obese nondiabetic subjects (··O··), and obese subjects with type 2 diabetes and mild hyperglycemia (—●—) during rest ($t = -40$ to 0 min), exercise at $50\% \text{Vo}_{2\text{max}}$ ($t = 0$ – 45 min), and recovery ($t = 45$ – 195 min). Data are means \pm SE. Significances are described in the text.

from basal levels in lean or obese control subjects. In obese diabetic subjects, glucose levels gradually declined by 1 mmol/l during exercise and remained lower than basal levels during recovery. Specific activities of [6-³H]- and [2-³H]glucose were maintained within $\pm 15\%$ of basal levels (Fig. 1). The basal levels were lower in obese diabetic subjects because the radioactivity of the tracer infusates was lower. This was due to random differences among tracer batches.

Basal insulin levels (Table 2) were greater in obese control and diabetic subjects than in lean control subjects ($P < 0.05$ vs. obese control subjects, $P < 0.001$ vs. obese diabetic sub-

jects). In lean control subjects, insulin levels were close to the detection limit of the assay. During exercise and recovery, the insulin levels did not change from basal levels in lean control subjects. In obese control and obese diabetic subjects, there was a nonsignificant trend toward a decline in insulin levels during exercise. This was followed by a rebound in insulin levels during recovery, which was significant ($P < 0.001$) in obese nondiabetic subjects. The C-peptide mirrored the insulin levels; however, the rebound in C-peptide was significant only in obese diabetic subjects (Table 3).

There was no significant difference in basal glucose turnover among groups (Fig. 2). Basal glucose clearance was lower in obese diabetic subjects than in the two control groups ($P < 0.05$). Glucose production and utilization increased approximately twofold during exercise and decreased during recovery in all groups. During exercise, glucose production was similar in all groups; however, glucose utilization in obese diabetic subjects was greater than that in lean control subjects ($P < 0.05$) and tended to be greater than that in obese nondiabetic subjects, although the difference failed to reach statistical significance ($P = 0.066$). The rise in glucose clearance during exercise was not significantly different among groups.

Basal lactate levels (Fig. 3) were similar in all groups, whereas basal alanine levels were greater in obese diabetic subjects than in lean control subjects ($P < 0.05$). During exercise, lactate and alanine increased to levels that were greater in diabetic subjects than in the control subjects. However, only the differences in lactate between obese diabetic subjects and obese nondiabetic subjects and in alanine between obese diabetic subjects and lean subjects reached significance ($P < 0.01$ and $P < 0.001$, respectively).

There was no significant difference in glycerol, FFAs, and β -hydroxybutyrate among groups throughout the experiments. Basal glycerol levels were 81 ± 13 , 117 ± 10 , and 98 ± 13 $\mu\text{mol/l}$ in lean control, obese control, and obese diabetic subjects, respectively. Plasma glycerol rose to peak levels of 197 ± 30 , 244 ± 29 , and 187 ± 36 $\mu\text{mol/l}$, respectively, at the end of exercise and returned to basal levels during recovery. Basal FFA levels were 687 ± 97 , 822 ± 93 , and 814 ± 96 $\mu\text{mol/l}$ in lean control, obese control, and obese diabetic subjects, respectively. FFA levels declined at the onset of exercise (10-min values of 620 ± 91 , 645 ± 65 , and 688 ± 84 $\mu\text{mol/l}$ for lean control subjects, obese control subjects, and obese diabetic subjects, respectively), but increased at the end of exercise and peaked 5 min into recovery (1141 ± 180 , 1359 ± 90 , and 1390 ± 156 $\mu\text{mol/l}$, respectively). Basal β -hydroxybutyrate levels were 92 ± 34 , 77 ± 11 , and 90 ± 20 $\mu\text{mol/l}$ in lean control, obese control, and obese diabetic subjects. At the onset of exercise, β -hydroxybutyrate levels declined (10 min values of 66 ± 34 , 32 ± 6 , and 48 ± 8 $\mu\text{mol/l}$, respectively) and then rose until the end of recovery (346 ± 78 , 247 ± 25 , and 261 ± 52 $\mu\text{mol/l}$, respectively).

There was no significant difference in basal glucagon, cortisol, or epinephrine levels among groups (Table 3). Basal norepinephrine levels were greater in lean subjects than in the two obese groups ($P < 0.05$). The glucagon levels increased slightly during exercise and remained more elevated during recovery. However, this increase was significant ($P < 0.05$) only in obese nondiabetic and diabetic subjects. The cortisol response to exercise appeared to be greater in obese nondiabetic and diabetic subjects than in lean control subjects.

TABLE 2
Insulin and C-peptide levels

Time	Immunoreactive insulin (pmol/l)			C-peptide (pmol/l)		
	Lean control subjects	Obese control subjects	Obese diabetic subjects	Lean control subjects	Obese control subjects	Obese diabetic subjects
Basal						
-20	28 ± 1	80 ± 11	116 ± 31	0.18 ± 0.03	0.52 ± 0.08	0.61 ± 0.11
0	27 ± 1	78 ± 13	116 ± 31	0.16 ± 0.03	0.47 ± 0.08	0.62 ± 0.12
Exercise						
5	29 ± 2	73 ± 10	108 ± 29	0.20 ± 0.04	0.50 ± 0.08	0.64 ± 0.10
10	33 ± 4	72 ± 10	120 ± 27	0.19 ± 0.03	0.47 ± 0.08	0.62 ± 0.10
15	33 ± 4	75 ± 11	122 ± 23	0.20 ± 0.04	0.45 ± 0.08	0.64 ± 0.10
25	34 ± 4	68 ± 10	114 ± 24	0.21 ± 0.04	0.44 ± 0.08	0.64 ± 0.09
35	29 ± 2	66 ± 10	106 ± 24	0.20 ± 0.03	0.41 ± 0.08	0.61 ± 0.10
45	28 ± 2	61 ± 8	100 ± 24	0.18 ± 0.03	0.42 ± 0.08	0.55 ± 0.09
Recovery						
55	34 ± 4	124 ± 15	149 ± 29	0.21 ± 0.04	0.59 ± 0.08	0.66 ± 0.10
65	29 ± 2	106 ± 13	149 ± 36	0.17 ± 0.03	0.57 ± 0.08	0.66 ± 0.11
85	24 ± 1	85 ± 18	111 ± 28	0.14 ± 0.02	0.49 ± 0.09	0.56 ± 0.11
135	24 ± 1	74 ± 10	89 ± 20	0.13 ± 0.02	0.44 ± 0.07	0.52 ± 0.11
175	25 ± 2	68 ± 12	98 ± 26	0.14 ± 0.02	0.44 ± 0.09	0.49 ± 0.10
195	24 ± 2	67 ± 10	101 ± 25	0.14 ± 0.02	0.39 ± 0.08	0.49 ± 0.09

Data are means ± SE. Significances are reported in the text.

However, the difference was not significant. The epinephrine response to exercise was increased in obese control subjects ($P < 0.001$ vs. lean control subjects) and decreased in obese diabetic subjects ($P < 0.05$ vs. lean control subjects). The norepinephrine response to exercise was decreased in obese diabetic subjects ($P < 0.01$ vs. lean control subjects).

Carbohydrate oxidation tended to be lower and lipid oxidation tended to be higher in obese nondiabetic subjects

than in the other two groups. However, the differences were not significant (Table 4). During exercise, both carbohydrate and lipid oxidation increased as expected, and the increase in carbohydrate oxidation was greater than that in lipid oxidation, as reflected by increased RQs as compared with basal levels. As expected, during exercise, carbohydrate oxidation was greater than tracer-determined utilization of plasma glucose, indicating net oxidation of glycogen. The differences

TABLE 3
Plasma level of counterregulatory hormones

Time	Basal		Exercise			Recovery			
	-20	0	10	35	45	55	85	175	195
Glucagon (pg/ml)									
Lean control subjects	250 ± 55	253 ± 57	263 ± 58	271 ± 58	284 ± 61	290 ± 66	286 ± 74	258 ± 59	243 ± 55
Obese control subjects	261 ± 60	270 ± 62	299 ± 72	299 ± 71	307 ± 69	313 ± 70	313 ± 73	267 ± 70	279 ± 72
Obese diabetic subjects	201 ± 50	198 ± 48	204 ± 49	235 ± 54	240 ± 61	283 ± 78	278 ± 80	221 ± 61	203 ± 52
Cortisol (nmol/l)									
Lean control subjects	171 ± 25	171 ± 25	218 ± 22	276 ± 28	268 ± 30	248 ± 30	193 ± 22	138 ± 30	116 ± 17
Obese control subjects	102 ± 17	116 ± 19	157 ± 33	262 ± 44	356 ± 64	408 ± 44	279 ± 30	138 ± 14	130 ± 8
Obese diabetic subjects	141 ± 25	149 ± 17	207 ± 36	361 ± 64	422 ± 64	400 ± 72	284 ± 47	152 ± 17	132 ± 11
Epinephrine (pmol/l)									
Lean control subjects	284 ± 53	350 ± 80	628 ± 127	825 ± 161	849 ± 134	457 ± 151	313 ± 67	347 ± 86	305 ± 81
Obese control subjects	368 ± 66	570 ± 142	1,183 ± 127	1,178 ± 105	1,200 ± 167	696 ± 147	535 ± 142	477 ± 161	554 ± 167
Obese diabetic subjects	231 ± 80	406 ± 184	299 ± 47	551 ± 86	632 ± 142	456 ± 105	303 ± 65	188 ± 39	191 ± 33
Norepinephrine (nmol/l)									
Lean control subjects	3.0 ± 0.6	3.6 ± 0.5	8.9 ± 2.2	8.3 ± 1.7	9.6 ± 2.0	3.8 ± 0.9	2.9 ± 0.5	2.5 ± 0.4	2.7 ± 0.7
Obese control subjects	2.1 ± 0.4	2.1 ± 0.5	7.5 ± 1.8	8.1 ± 1.4	7.7 ± 1.6	3.1 ± 0.7	2.7 ± 0.7	1.9 ± 0.4	2.0 ± 0.4
Obese diabetic subjects	2.2 ± 0.2	2.2 ± 0.2	4.2 ± 0.4	5.8 ± 0.3	5.7 ± 0.6	3.3 ± 0.6	2.1 ± 0.4	1.9 ± 0.3	2.3 ± 0.3

Data are means ± SE. Significances are reported in the text.

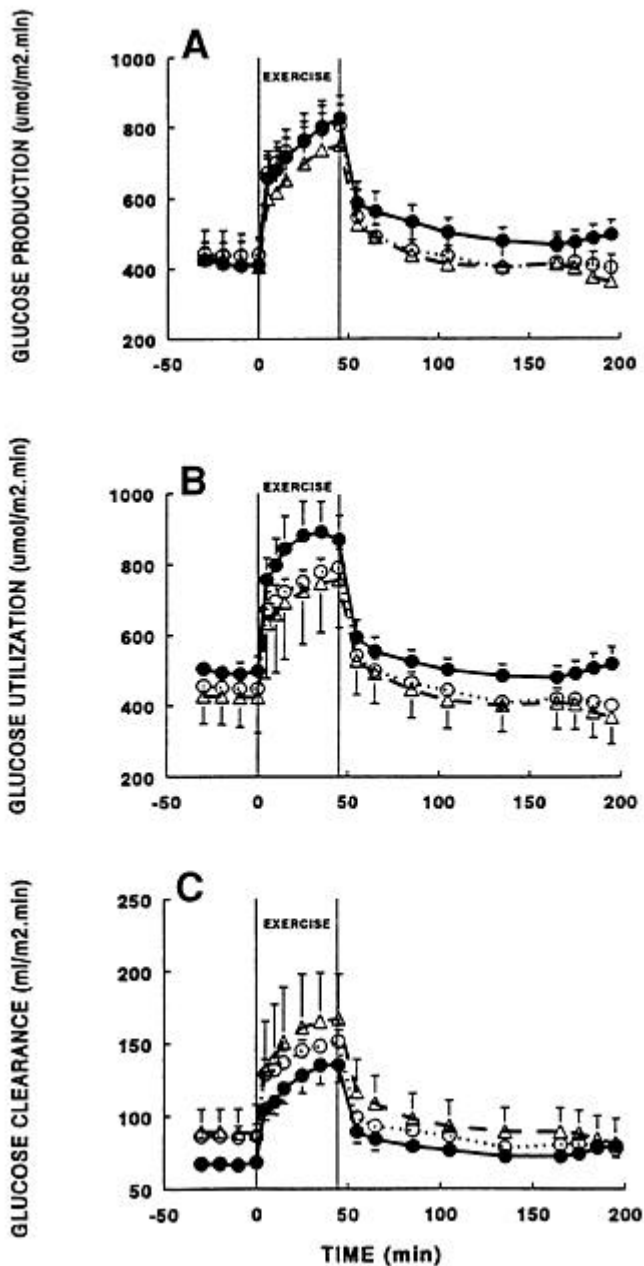


FIG. 2. Glucose production (A), utilization (B), and clearance (C) in lean nondiabetic subjects ($--\Delta--$), obese nondiabetic subjects ($\cdot\cdot\circ\cdot\cdot$), and obese subjects with type 2 diabetes and mild hyperglycemia ($—\bullet—$) during rest ($t = -40$ to 0 min), exercise at $50\% \text{Vo}_{2\text{max}}$ ($t = 0-45$ min), and recovery ($t = 45-195$ min). Data are means \pm SE. Significances are described in the text.

between carbohydrate oxidation and glucose utilization during exercise (estimate of glycogen oxidation) (9) were 1955 ± 455 , $1,300 \pm 245$, and $1,747 \pm 691 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in lean control, obese control, and obese diabetic subjects (NS). Nonoxidative glucose disposal was not significantly different among groups (Table 5).

Figure 4 illustrates the glucose cycling rate expressed in absolute values and as a percentage of total glucose output (glucose flux through glucose-6-phosphatase). Basal glucose cycling (expressed in absolute values) was most elevated in obese diabetic subjects ($P < 0.01$ vs. lean control subjects), intermediate in nondiabetic obese subjects (NS vs. obese

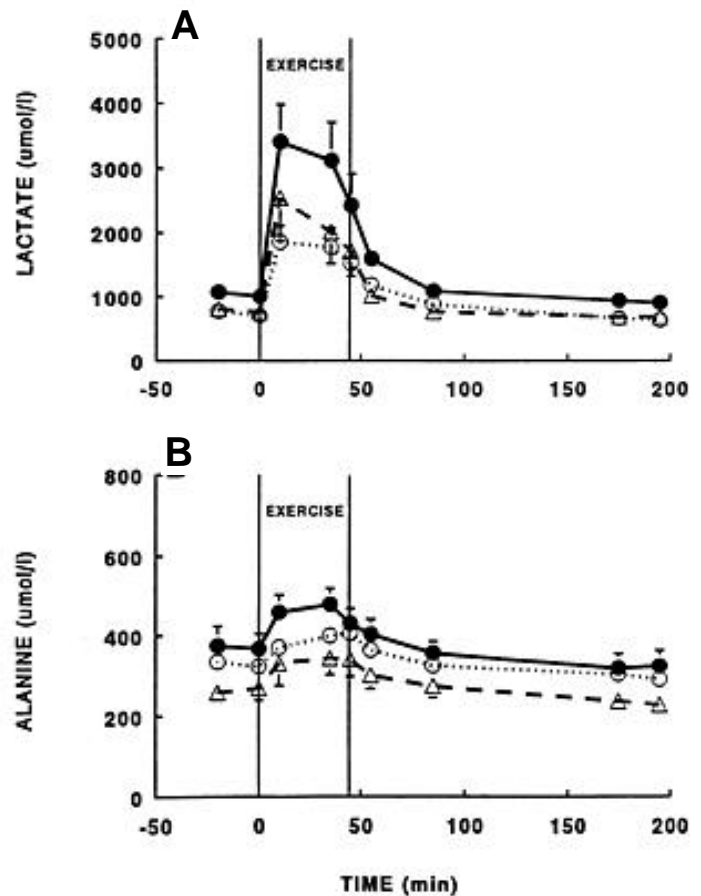


FIG. 3. Plasma lactate (A) and alanine levels (B) in lean nondiabetic subjects ($--\Delta--$), obese nondiabetic subjects ($\cdot\cdot\circ\cdot\cdot$), and obese subjects with type 2 diabetes and mild hyperglycemia ($—\bullet—$) during rest ($t = -40$ to 0 min), exercise at $50\% \text{Vo}_{2\text{max}}$ ($t = 0-45$ min), and recovery ($t = 45-195$ min). Data are means \pm SE. Significances are described in the text.

diabetic subjects or lean control subjects), and lowest in nondiabetic lean individuals. Glucose cycling increased during exercise ($P < 0.001$) and declined during recovery in both nondiabetic groups. In obese diabetic subjects, it did not change during exercise or recovery. Figure 4B shows that glucose cycling, expressed as the percentage of total glucose output, was greater ($P < 0.01$) in obese diabetic subjects than in the two nondiabetic groups. During exercise and recovery, glucose cycling, expressed as a percentage of total glucose output, did not change from basal levels in obese or lean nondiabetic subjects; however, it decreased in obese diabetic subjects ($P < 0.01$).

Basal glucose cycling, expressed in absolute values or as a percentage of total glucose output, correlated with the prevailing plasma glucose levels ($r = 0.443$ and 0.356 , respectively; $P < 0.001$), but not with BMI. Glucose cycling, expressed as a percentage of total glucose output, correlated with the prevailing plasma glucose level ($r = 0.413$, $P < 0.01$), but not with the catecholamine concentrations in obese diabetic subjects.

DISCUSSION

In the present study, 45 min of moderate exercise at $50\% \text{Vo}_{2\text{max}}$ did not affect plasma glucose in obese or lean nondiabetic subjects; however, the plasma glucose levels

TABLE 4
Substrate oxidation

	Basal	Exercise	Recovery
Carbohydrate ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)			
Lean control subjects	40.2 ± 10.2	499.1 ± 80.4*	11.2 ± 8.2†
Obese control subjects	12.1 ± 6.8	369.8 ± 44.0*	5.4 ± 2.8
Obese diabetic subjects	44.6 ± 10.8	470.1 ± 131.9*	31.1 ± 7.1‡
Lipid ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)			
Lean control subjects	26.1 ± 5.0	175.9 ± 24.9*	40.1 ± 7.5
Obese control subjects	42.9 ± 4.9	146.9 ± 29.4*	45.1 ± 1.6
Obese diabetic subjects	29.3 ± 6.4	135.3 ± 29.1*	42.0 ± 2.3

Data are means ± SE and refer to the last 30 min of each experimental period (basal resting state, exercise, and recovery). * $P < 0.001$, exercise vs. basal or recovery (carbohydrate, lipid); † $P < 0.01$ vs. basal; ‡ $P < 0.05$ vs. obese control subjects.

decreased in obese diabetic subjects. The glucose decline in this group was due to greater rates of glucose utilization, whereas glucose production was not different from that of control subjects. The rate of glucose cycling (flux between glucose and G-6-P) was elevated at rest but failed to increase during exercise in obese diabetic subjects.

During exercise, the greater glucose utilization in diabetic subjects versus control subjects did not result in greater carbohydrate oxidation, presumably because of greater glucose conversion to lactate and alanine due to reduced activity of pyruvate dehydrogenase (PDH). In fact, one might argue that glucose utilization was greater in diabetic subjects to normalize glucose oxidation despite reduced PDH activity. Glucose production, although not different from other groups, failed to match glucose utilization during exercise in diabetic subjects, presumably because a further rise in glucose production was prevented by hyperglycemia and/or hyperinsulinemia. However, plasma insulin levels decreased during exercise in this group, as they did in obese nondiabetic subjects. In lean control subjects, insulin levels did not decrease, consistent with previous observations in subjects with low basal insulin levels (9,29). The decrease in insulin levels in diabetic subjects contrasted with previous observations that showed no change in insulin levels during exercise in type 2 diabetic subjects (2,3,8,10), but is in accordance with results of other studies (1,7,9).

The reasons for the variability in the insulin response to exercise in type 2 diabetic individuals are unclear. However, normal insulin responses were mostly found in subjects who were mildly hyperglycemic (as in the present study), underwent prolonged exercise (1), had normal catecholamine responses (7,9), and were physically fit (7). A training program increasing $\text{Vo}_{2\text{max}}$ accentuated the decrease in insulin levels during exercise in nondiabetic subjects (10). Although in most previous studies (2,3,8) the levels of physical fitness were matched between diabetic and control subjects, $\text{Vo}_{2\text{max}}$ tended to be low in both groups, which could possibly have accentuated an impaired insulin response in the diabetic subjects.

Glucagon levels were comparable among groups and are minimally affected by moderate exercise in humans, as

TABLE 5
Glucose disposal

	Basal	Recovery
Oxidative ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)		
Lean control subjects	223.5 ± 56.8	62.1 ± 45.5*
Obese control subjects	67.1 ± 37.9	29.9 ± 15.5
Obese diabetic subjects	247.9 ± 60.0	172.7 ± 39.4†
Nonoxidative ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)		
Lean control subjects	262.4 ± 70.0	402.1 ± 56.4
Obese control subjects	380.9 ± 46.9	379.0 ± 30.1
Obese diabetic subjects	245.3 ± 79.9	322.9 ± 33.4

Data are means ± SE and refer to the last 30 min of basal and recovery periods. * $P < 0.01$ vs. basal; † $P < 0.05$ vs. obese control.

described previously (2). The cortisol response to exercise tended to be greater in the two obese groups, in accordance with hyperactivity of the hypothalamopituitary adrenal axis in obesity (30,31). The increased epinephrine response to exercise in obese nondiabetic subjects concurs with results of previous studies in obese rats (32). The decreased catecholamine responses to exercise in type 2 diabetic subjects agree with results from some (10), but not all, previous studies in type 2 diabetes (7,9), and probably indicate autonomic dysfunction. Because catecholamines have not been shown to be important regulators of glucose turnover during moderate exercise (33–36), we feel it unlikely that the attenuated rise in catecholamines could explain the failure of glucose production to match glucose utilization in subjects with type 2 diabetes.

A decrease in plasma glucose levels during moderate exercise has been described in all (2,4,7,8,10) but one (9) of the previous studies in subjects with type 2 diabetes and moderate hyperglycemia. In the studies in which insulin levels failed to decrease during exercise (2,8), the plasma glucose decline in type 2 diabetes was due to a blunted increase in glucose production, whereas in the studies in which insulin levels decreased, greater than normal rates of glucose utilization were found (7,9). The different results may also be due in part to the old tracer methods used in previous studies (2,8), which may be associated with overestimation of glucose production at rest and underestimation of glucose production during exercise (7,37).

In all (1,4–6) but one (3) of the studies carried out in subjects with type 2 diabetes and mild hyperglycemia, plasma glucose levels decreased during exercise, similar to those in subjects with moderate hyperglycemia; however, the mechanism of this decrease has not been previously investigated. The results of the present study suggest that plasma glucose decreased as a consequence of the greater-than-normal increase in glucose utilization, which appeared to be due to the residual mild hyperglycemia, because glucose clearance increased similarly to control subjects.

The elevation in basal glucose cycling in the presence of normal rates of glucose production agree with results from previous studies in mildly hyperglycemic diabetic subjects (13,14,38). The correlation between resting glucose cycling and the prevailing plasma glucose suggests that in type 2 diabetes, elevated rates of glucose cycling are, in part, substrate dependent. This concurs with the notion that in the fasting state, the rate of glucose cycling is determined by the flux

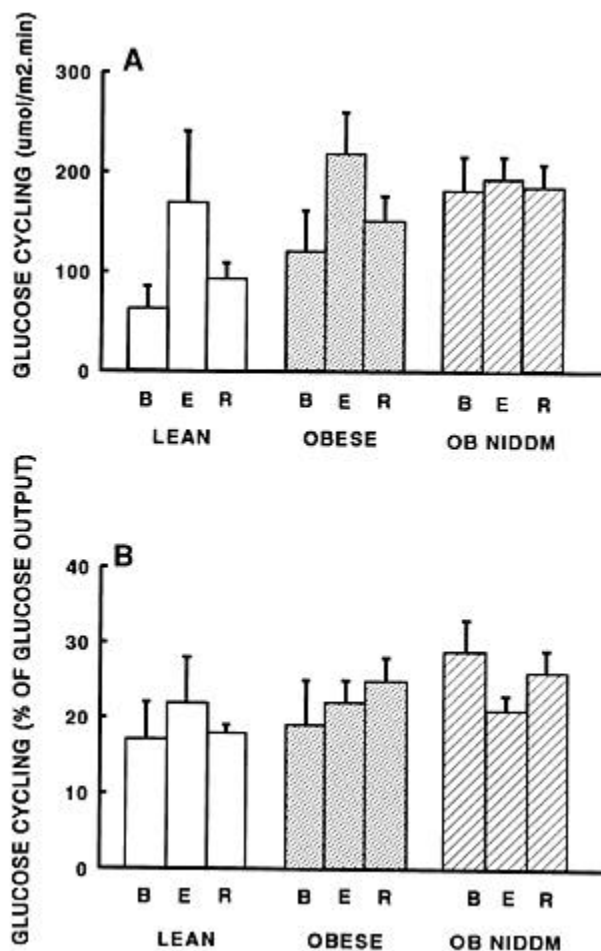


FIG. 4. Glucose cycling expressed in absolute values (**A**) and as a percentage of total glucose output (**B**) in lean nondiabetic subjects (\square), obese nondiabetic subjects (\boxtimes), and obese subjects with type 2 diabetes and mild hyperglycemia (OB NIDDM) (\boxplus) during the last 30 min of each experimental period: basal resting state (B), exercise (E), and recovery (R) during rest ($t = -40$ to 0 min), exercise at 50% $\dot{V}O_{2max}$ ($t = 0-45$ min), and recovery ($t = 45-195$ min). Data are means \pm SE. Significances are described in the text.

through glucokinase, which is opposite to the net flux of glucose across the liver. Because glucokinase activity tends to be reduced in diabetes (39), a high flux through this enzyme can be sustained only by a high substrate (glucose) flow. We have shown in depancreatized dogs (40), and recently in type 2 diabetes (41), that elevated basal rates of glucose cycling were normalized by acute insulin infusion. In depancreatized dogs, this was due to the combination of two effects of insulin: 1) the normalization of plasma glucose and 2) a reduction of in vivo activity of glucose-6-phosphatase (40). The effect of a selective decrease in plasma glucose has not been studied. However, selective induction of hyperglycemia increased glucose cycling in nondiabetic and diabetic rats (39), but failed to significantly affect the absolute rate of cycling in nondiabetic humans (42,43), although cycling, expressed as a percentage of total glucose output, increased with hyperglycemia (42,43). In collaborative studies, we have recently shown that in type 2 diabetes, selective hyperglycemia fails to increase glucose cycling, whether expressed in absolute rates or as a percentage of total glucose output (41).

It is therefore unlikely that the effect of exercise on glucose cycling in the diabetic subjects in the present study was mediated only by the decrease in plasma glucose.

Exercise is associated with an increase in the glucagon-to-insulin ratio, and consequently with increased in vivo activity of glucose-6-phosphatase. This presumably drives glucose cycling (flux through glucokinase) by simultaneously increasing total glucose output and decreasing the hepatic content of G-6-P. The pancreatic hormone response to exercise was not affected by diabetes in our study, although the catecholamine response was reduced. Previous studies have shown that high-dosage epinephrine infusions resulting in plasma levels of $\sim 2,000$ pmol/l only minimally increased glucose cycling (43). However, a combined effect of the blunted rise in catecholamines with that of the decrease in plasma glucose cannot be discounted, and the lack of correlation between glucose cycling and catecholamine levels during exercise may simply reflect the low number of observations and the variability of the catecholamine assay. Alternatively, or in addition, the hormonal response to exercise might selectively accentuate a defect in glucokinase in diabetes, which, when combined with the decrease in plasma glucose, could result in decreased glucose cycling. Defective glucokinase activity has been reported in experimental diabetes (44,45) and in a group of type 2 diabetic individuals (46), and is also consistent with the findings of our collaborative studies in type 2 diabetes (41).

It has been postulated that during exercise, a decrease in the percentage of glucose that, after being dephosphorylated by glucose-6-phosphatase, is cycled back into the liver could increase the availability of glucose to the contracting muscle (12). However, our results showed that glucose cycling, expressed as a percentage of glucose output, was not affected by moderate-intensity exercise in nondiabetic control subjects. In the diabetic subjects, the percentage of glucose cycled back into the liver slowly decreased during exercise; however, this decrease failed to match glucose production with the greater-than-normal increase in glucose utilization. These results indicate that glucose cycling does not play a major role in the regulation of glucose flux during exercise, in accordance with previous studies in nondiabetic subjects undergoing higher-intensity exercise (70% $\dot{V}O_{2max}$ for 60 min).

In conclusion, in obese subjects with type 2 diabetes and mild hyperglycemia, we found the following. First, although insulin secretory responses are normally regulated, plasma glucose levels decreased during moderate exercise, due to greater than normal rates of glucose utilization. These were sustained by the mass effect of hyperglycemia. Second, elevated basal rates of glucose cycling (expressed as a percentage of total glucose output) decreased during exercise, presumably because exercise simultaneously lowers plasma glucose, is associated with an attenuated catecholamine response and perhaps accentuates a defect in glucokinase activity in type 2 diabetes.

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REFERENCES

- Koivisto V, DeFronzo R: Exercise in the treatment of type 2 diabetes. *Acta Endocrinol* 105 (Suppl. 262):107-116, 1984
- Minuk HL, Vranic M, Marliss EB, Hanna AK, Albisser AM, Zinman B: Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. *Am J Physiol* 240:E458-E464, 1981
- Jenkins AB, Furler SM, Bruce DG, Chisholm DJ: Regulation of hepatic glucose output during moderate exercise in non-insulin dependent diabetes. *Metabolism* 37:966-969, 1988
- Hubinger A, Franzen A, Gries FA: Hormonal and metabolic response to physical exercise in hyperinsulinemic and nonhyperinsulinemic type 2 diabetics. *Diabetes Res* 4:57-61, 1987
- Berrish TS, Elliott CE, Cooper BG, Reed JW, Orskov H, Alberti KGMM, Walker M: The role of plasma non-esterified fatty acids during exercise in type 2 diabetes mellitus. *Diabet Med* 10:152-158, 1993
- Paternostro-Bayles M, Wing RR, Robertson RJ: Effect of life-style activity of varying duration on glycemic control in type 2 diabetic women. *Diabetes Care* 12:34-37, 1989
- Martin IK, Katz A, Wahren J: Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am J Physiol* 269:E583-E590, 1995
- Kang J, Robertson RJ, Hagberg JM, Kelley DE, Goss FL, DaSilva SG, Suminski RR, Utter AC: Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabetes Care* 19:341-349, 1996
- Colberg SR, Hagberg JM, McCole SD, Zmuda JM, Thompson PD, Kelley DE: Utilization of glycogen but not plasma glucose is reduced in individuals with NIDDM during mild-intensity exercise. *J Appl Physiol* 81:2027-2033, 1996
- Schneider SH, Khachadurian AK, Amorosa FH, Gavras H, Fineberg SE, Ruderman NB: Abnormal glucoregulation during exercise in type 2 (non-insulin-dependent) diabetes. *Metabolism* 36:1161-1166, 1987
- Karlander S, Roovete A, Vranic M, Efendic S: Glucose and fructose 6-phosphate cycle in humans. *Am J Physiol* 251:E530-E536, 1986
- Weber JM, Klein S, Wolfe RR: Role of the glucose cycle in control of net glucose flux in exercising humans. *J Appl Physiol* 68:1815-1819, 1990
- Efendic S, Karlander S, Vranic M: Mild type II diabetes markedly increases glucose cycling in the postabsorptive state and during glucose infusion irrespective of obesity. *J Clin Invest* 81:1953-1961, 1988
- Efendic S, Wajngot A, Vranic M: Increased activity of the glucose cycle in the liver: early characteristic of type 2 diabetes. *Proc Natl Acad Sci U S A* 82:2965-2969, 1985
- Searle GL, Strisower EH, Chaikoff IL: Glucose pool and glucose space in the normal and diabetic dog. *Am J Physiol* 176:190-194, 1954
- Faloon GR, Unger RH: Glucagon. In *Methods of Hormone Radioimmunoassay*. Jaffe BM, Berman HR, Eds. New York, Academic, 1974, p. 317-330
- Murphy BEP: Measurement of various steroids in body fluids by competitive protein-binding radioassay. *J Clin Endocrinol Metab* 27:973-990, 1967
- Sole MJ, Hussein MN: A simple specific radioenzymatic assay for the simultaneous measurement of picogram quantities of norepinephrine, epinephrine, and dopamine in plasma tissues. *Biochem Med* 18:301-307, 1977
- Lloyd B, Burren J, Smythe P, Alberti KGMM: Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-OH-butylate. *Clin Chem* 24:1724-1729, 1978
- Ho RJ: Radiochemical assay of long-chain fatty acids using ⁶³Ni as tracer. *Anal Biochem* 36:105-113, 1970
- Dunn A, Katz J, Golden S, Chenoweth M: Estimation of glucose turnover and recycling in rabbits using various ³H and ¹⁴C glucose labels. *Am J Physiol* 230:1159-1162, 1976
- deBodo RC, Steele R, Altszuler N, Dunn A, Bishop JS: On the hormonal regulation of carbohydrate metabolism: studies with ¹⁴C glucose. *Recent Prog Horm Res* 19:445-488, 1963
- Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic euglycemic glucose clamp: comparison of labelled and unlabelled glucose infusate. *Diabetes* 36:914-924, 1987
- Finegood DT, Bergman RN: Optimal segments: a method for smoothing tracer data to calculate metabolic fluxes. *Am J Physiol* 244:E472-E479, 1983
- Bradley DC, Steil GM, Bergman RN: Quantitation of measurement error with optimal segments: basis for adaptive time course smoothing. *Am J Physiol* 264:E902-E911, 1993
- Hawk PB, Oser BL, Summerson WH (Eds.): *Practical Physiological Chemistry*. Toronto, Blakiston, 1947, p. 814-822
- Lusk G: Animal calorimetry: analysis of the oxidation of mixtures of carbohydrate and fat. *J Biol Chem* 59:41-42, 1924
- Ferrannini E: The theoretical bases of indirect calorimetry: a review. *Metabolism* 37:287-301, 1988
- Cochran B, Marbach EP, Poucher R, Steinberg T, Gwinup G: Effect of acute muscular exercise on serum immunoreactive insulin concentration. *Diabetes* 15:838-841, 1966
- Marin P, Amemiya T, Andersson B, Jern S, Bjornorp P: Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism* 41:882-886, 1992
- Pagano G, Trovati M, Martiny W, Airoldi A, Cantino G, Pisu E, Lenti G: Metabolic and hormonal changes during exercise in healthy, diabetic and obese subjects. *Acta Diabetol* 16:19-26, 1979
- Balkan B, Strubbe JH, Bruggink JE, Steffens AB: Overfeeding-induced obesity in rats: insulin sensitivity and autonomic regulation of metabolism. *Metabolism* 42:1509-1518, 1993
- Wasserman DH, Lickley HLA, Vranic M: Interactions between glucagon and other counterregulatory hormones during normoglycemic and hypoglycemic exercise. *J Clin Invest* 74:1404-1413, 1984
- Wasserman DH, Williams PE, Lacy DB, Bracy D, Cherrington AD: Hepatic nerves are not essential to the increase in hepatic glucose production during muscular work. *Am J Physiol* 259:E195-E203, 1990
- Wasserman DH, Williams PE, Lacy DB, Goldstein RE, Cherrington AD: Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. *Am J Physiol* 256:E500-E509, 1989
- Wasserman DH, Spalding JS, Lacy DB, Colburn CA, Goldstein RE, Cherrington AD: Glucagon is a primary controller of hepatic glycogenolysis and gluconeogenesis during muscular work. *Am J Physiol* 257:E108-E117, 1989
- Hother-Nielsen O, Beck-Nielsen H: On the determination of basal glucose production rate in patients with type 2 (non-insulin-dependent) diabetes using primed-continuous 3-³H-glucose infusion. *Diabetologia* 33:603-610, 1990
- Pigon J, Giacca A, Ostenson CG, Vranic M, Efendic S: Normal hepatic insulin sensitivity in mild, lean non-insulin dependent diabetic patients. *J Clin Endocrinol Metab* 81:3702-3708, 1996
- Rossetti L, Giaccari A, Barzilai N, Howard K, Sebel G, Hu M: Mechanism by which hyperglycemia inhibits hepatic glucose production in conscious rats. *J Clin Invest* 92:1126-1134, 1993
- Shi ZQ, Giacca A, Fisher S, Vidal H, van de Werve G, Vranic M: Importance of substrate changes in the decrease of hepatic glucose cycling during insulin infusion and declining glycemia in the depancreatized dog. *Diabetes* 43:1284-1290, 1994
- Mevorach M, Giacca A, Aharon Y, Hawkins M, Shamon H, Rossetti L: Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. *J Clin Invest* 102:744-753, 1998
- Bell PM, Firth RG, Rizza RA: Effects of hyperglycemia on glucose production and utilization in humans. *Diabetes* 35:642-648, 1986
- Miyoshi H, Shulman GI, Peters EJ, Wolfe MH, Elahi D, Wolfe RR: Hormonal control of substrate cycling in humans. *J Clin Invest* 81:1545-1555, 1988
- Iynedjian PB, Gjinosci A, Renold AE: Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. *J Biol Chem* 263:740-744, 1988
- Barzilai N, Rossetti L: Role of glucokinase and glucose-6-phosphatase in the acute and chronic regulation of hepatic glucose fluxes by insulin. *J Biol Chem* 268:25019-25025, 1993
- Caro JF, Triester S, Patel VK, Tapscott EB, Leggett Frazier N, Dohm GL: Liver glucokinase: decreased activity in patients with type II diabetes. *Horm Metab Res* 27:19-22, 1995

Author Queries (please see Q in margin and underlined text)

Q1: In sentence beginning “The aim of the present study,” okay to change “early” to primary, as you seem to be distinguishing these events from secondary events?

Q2: In the sentence beginning “FFA levels declined,” have the groups been listed in the correct order with respect to their values?

Table 5: Please cite ‡ in table or delete.

Q3: Please spell out MCR.

Q4: Did you mean “number” by “n”, if not please provide spell-out.

Ref 1: Please provide supplement number.

Ref. 18: Is the journal the same as the current Biochemical and Molecular Medicine (Biochem Mol Med)?

Ref. 26: Please list editors.

Ref. 41: Has this article been published yet? If not, it would be cited as “unpublished observation.”