Effects of Oral Hypoglycemic Agents and Diet on Protein Metabolism in Type 2 Diabetes

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OBJECTIVE — We tested whether oral hypoglycemic agents (OHA), gliclazide with or without metformin, during an isoenergetic (ISO) and then a low-energy diet (LED) improve the altered kinetics of whole-body protein metabolism in type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 13 type 2 diabetic patients (aged 51 ± 2 years, weight 110 ± 5 kg, BMI 41 ± 1 kg/m^2, fasting glucose [FSG] 11.5 ± 0.9 mmol/l) (means ± SEM) and 10 obese control subjects (48 ± 3 years, 98 ± 6 kg, 37 ± 2 kg/m^2, FSG 5.5 ± 0.3 mmol/l) consumed an ISO, 1.5 g · kg^{-1} · day^{-1} protein for a body weight corresponding to a BMI of 25 (BMI_{25}), a formula diet (7 days for obese control subjects, 15 days for diabetic patients), and then a 28-day LED with 50% of the energy of ISO but the same protein intake (101 ± 2 g/day). OHAs were given during ISO (days 8–15) and LED. On days 6–8 (and 12–14 for diabetic subjects) of ISO and 26–28 of LED, the 60-h oral ^{15}N-glycine method was used to obtain nitrogen flux (Q), synthesis (S), and breakdown (B). Muscle protein catabolism was estimated from N$^{\tau}$-methylhistidine (3MH) excretion.

RESULTS — During ISO with hyperglycemia, Q, and B adjusted for fat-free mass, sex, and age were higher and nitrogen balance and net endogenous protein synthesis (S-B) lower than in control subjects ($P < 0.05$). OHA decreased FSG (9 ± 1 mmol/l) and 3MH and increased plasma insulin–to–glucose ratio, nitrogen retention, and S-B to levels in control subjects. The change in S-B correlated with that in FSG ($r = -0.845$, $P = 0.001$) and in fasting plasma C-peptide ($r = 0.852$, $P = 0.0005$). With LED and OHA, weight decreased 6.3 kg, glycemia reached near-normal levels, and nitrogen equilibrium was maintained; Q decreased by 7%, S and B by 11% ($P < 0.05$) to values found in control subjects.

CONCLUSIONS — OHA during ISO corrected protein turnover in relation to glycemia and plasma C-peptide. The LED maintained protein homeostasis in obese control subjects and, in diabetes patients with OHA, normalized protein metabolism. These findings have implications for diet and OHA prescription.

Diabetes Care 23:1–8, 2000

The roles of insulin deficiency and treatment in protein homeostasis are well documented in type 1 diabetes (1–8) but insulin's role in type 2 diabetes remains uncertain. The American Diabetes Association recently concluded that there were insufficient data on which to make firm dietary protein recommendations (9), and one expert renewed the call for more research (10). We have reported that protein metabolism is accelerated in moderately hyperglycemic obese diabetic subjects when compared with an obese control group during a weight-maintaining diet with ample protein intake (11). These alterations were corrected with euglycemia from exogenous insulin (12, 13). However, euglycemia with a very-low-energy diet (VLED) did not completely reestablish nitrogen equilibrium in diabetic, in contrast to nondiabetic, subjects. This suggested that type 2 diabetic subjects have altered adaptive mechanisms for protein sparing, independent of the protein quality (11,12). When glycemia was normalized with insulin at the onset of the VLED, nitrogen balance was improved but equilibrium was still not achieved (13). The question remained whether protein metabolism and nitrogen balance could be corrected using the conventional approach to treatment that combines moderate energy restriction with oral hypoglycemic agents (OHA). We investigated the effects of OHA on protein metabolism during both isoenergetic feeding (ISO) and 4 weeks of energy restriction or low-energy diet (LED). With ISO, we aimed for the best possible glycemic control, whereas with LED, the goal was euglycemia.

RESEARCH DESIGN AND METHODS

Subjects

A total of 13 obese subjects with type 2 diabetes (7 women, 6 men) and 10 obese nondiabetic control subjects (9 women, 1 man) were admitted to the Clinical Investigation Unit of the Royal Victoria Hospital (Table 1). OHAs were stopped 1 week before admission. Consent was obtained according to the Institutional Human Ethics Committee. Clinical and laboratory evaluations showed no evidence of hepatic, cardiovascular, hematologic, renal or pulmonary dysfunction, or gout. The subjects performed two half-hour walks each day. The ISO diet was a weight-maintaining liq-

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Received for publication 15 March 1999 and accepted in revised form 22 September 1999.

Abbreviations: 3MH, N$^{\tau}$-methylhistidine; B, protein breakdown; BMI_{25}, body weight corresponding to a BMI of 25; FFM, fat-free mass; FSG, fasting serum glucose; ISO, isoenergetic diet; LED, low-energy diet; OHA, oral hypoglycemic agent; Q, nitrogen flux; S, protein synthesis; S-B, net protein synthesis; VLED, very-low-energy diet.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Subject characteristics*

	Type 2 diabetic subjects	Obese control subjects
Sex (M/F)	6/7	1/9
Age (years)	51 ± 2	48 ± 3
Anthropometry		
Weight (kg)	110 ± 5	98 ± 6
Height (cm)	165 ± 2	162 ± 2
BMI (kg/m^2)	41 ± 1	37 ± 2
Waist-to-hip ratio		
Men	1.05 ± 0.04	1.02
Women	0.87 ± 0.03	0.82 ± 0.02
Waist circumference (cm)	122 ± 4	106 ± 3*
Body composition		
FFM (kg)	60 ± 3	52 ± 2
Body fat (%)	45 ± 2	45 ± 2
Diabetes data		
Duration of diabetes (years)	4.9 ± 1.1	—
Glycated hemoglobin (%)	12.2 ± 1.2	—
Fructosamine (μmol/l)	305 ± 19	—
FSG (mmol/l)	11.5 ± 0.9	5.5 ± 0.3*

Data are means ± SEM. Normal range for glycated hemoglobin, 4.6–8.5%; normal range for fructosamine, 230–300 μmol/l. *$P < 0.001$ vs. type 2 diabetes.

uid formula (Ensure, Ross Laboratories, Montréal), supplemented with casein-soy protein (Bariatrix International, Lachine, Québec, Canada) and a glucose polymer (Polycose, Ross Laboratories). It was taken 7 days by obese control and 15 days by diabetic subjects. Maintenance energy intake (2,582 ± 72 kcal/day) was calculated as 1.5 × REE (14), with 1.5 g protein · day^{-1} · kg^{-1} for a body weight corresponding to a BMI of 25 (BMI_{25}) (101 ± 2 g/day). Of the diet, 56% was as carbohydrate, 15% protein, and 29% fat. The LED was based on Boost (Mead Johnson Canada, Belleville, Ontario, Canada), a bran cereal (All Bran; Kellogg Canada, Etobicoke, Ontario, Canada), milk, and the same protein formula, to provide 50% of maintenance energy with the same protein intake (1,315 ± 72 kcal/day, 54 ± 1% carbohydrate, 31 ± 1% protein, and 18 ± 1% fat). Energy intakes were supplemented (50% glucose polymer, 50% soy oil) to meet energy losses in daily quantitative glycosuria. During the LED, a daily multivitamin–trace element supplement (Centrum Forte; Cyanamid Canada, Montréal) was given. Water intake was ≥1.5 l/day. Doses of gliclazide (Diamicron; Servier Canada, Laval, Québec, Canada) and metformin (Glucophage; Nordic Laboratories, Laval, Québec, Canada) were adjusted as dictated by frequent capillary glucose determinations (Accuchek II; Boehringer Mannheim, Montréal). As glycemia decreased during the LED, first metformin, then gliclazide doses were decreased. Daily 24-h urine collections were made. Weekly overnight fasting serum electrolytes, calcium, phosphate, uric acid, lipids, liver and kidney function tests, complete blood counts, and daily 24-h urine urea nitrogen, creatinine, and glucose were assayed in the hospital laboratory.

Procedures and analytical methods

^{15}N-glycine kinetic studies of protein metabolism were done on days 5–7 (and 13–15 for diabetic patients) of ISO, and on days 26–28 of LED. The method for protein turnover with ^{15}N-glycine given orally every 3 h over 60 h has been detailed earlier (11–13,15–20). Overnight-fasted venous blood was drawn on days 1 and 3 of each ^{15}N-glycine study (and during the day in some subjects) and weekly during the LED. Sample collection and processing methods have been detailed elsewhere (11–13). 3-hydroxybutyrate was analyzed by an enzymic method, glucose by the glucose oxidase, insulin and C-peptide by radioimmunoassay (most reagents from Linco Research, St. Charles, MO), by methods detailed elsewhere (13). Urine ammonium nitrogen was measured with a specific ion electrode (Orion Research, Cambridge, MA) and total nitrogen by chemiluminescence (Pyro-Chemiluminescent Nitrogen System; Antek, Houston, TX). Daily nitrogen balance was based on the known intake minus the measured daily urinary nitrogen, a factor for fecal nitrogen (70 mg N/g N intake), and other losses (5 mg/kg) (21). The 24-h urinary N$^{\tau}$-methylhistidine (3MH) excretion was measured by high-pressure liquid chromatography (22). Muscle protein breakdown was calculated as in Bilmazes et al. (23). Fat-free mass (FFM) was determined with a bioimpedance analyzer (BIA-103; RJL Systems, Detroit, MI) (24). FFM post-LED was estimated by subtracting FFM based on cumulative nitrogen loss.

Statistical methods

Analysis of variance (ANOVA) with repeated measures and paired and unpaired *t* tests were done using Primer Biostatistics (McGraw-Hill, Montréal). Protein kinetic responses to treatment during ISO and LED were compared by ANOVA simple factorial, with age, FFM, and sex as covariates. Stepwise multiple regression analysis (SPSS Windows release 6.0; SPSS, Chicago) was done to identify variables contributing to changes in kinetic parameters. Significant differences ($P < 0.05$) were identified by the Bonferroni adjustment. Linear correlations were calculated using the Pearson correlation coefficients. Data are presented as means ± SEM.

RESULTS

— The subject characteristics are presented in Table 1 and physiologic responses in Table 2. There were no significant untoward effects experienced or abnormal values of the clinical laboratory tests (not shown). Urinary creatinine did not change significantly during ISO or LED in either group (data not shown). The small weight change during ISO without OHA was accounted for by increased urine volume due to glycosuria. Weight loss during LED was 5.8 ± 0.3% for diabetic patients and 5.2 ± 0.3% for obese control subjects, and body fat decreased ($P < 0.001$) from 45.2 ± 2.1 to 41.8 ± 2.3% in diabetic patients and from 46.4 ± 2.0 to 43.4 ± 2.0% in control subjects. FFM did not change significantly with OHA and/or LED. The doses of gliclazide were decreased progressively by the end of LED in the 10 diabetic patients still receiving it. The metformin given to nine diabetic patients during ISO was discontinued during LED in five and decreased in the others. Of the diabetic patients, three received no medication by the end of LED. Glycated hemoglobin was 10.5 ± 1.4% at the end of

Table 2—*Selected responses to ISO and LED*

			LED (week)			
	ISO	ISO + OHA	1	2	3	4
Cumulative weight change (kg)						
Diabetic subjects	−1.0 ± 0.4*	−0.2 ± 0.2†	−2.0 ± 0.2‡	−3.3 ± 0.2§	−5.1 ± 0.3‖	−6.3 ± 0.4#
Obese control subjects	−0.02 ± 0.2†	—	−1.5 ± 0.2‡	−2.9 ± 0.2§	−4.0 ± 0.3¶	4.8 ± 0.4**
Cumulative urine volume (l)						
Diabetic subjects	21.6 ± 0.7*	18.5 ± 0.6†	—	—	—	—
Glycosuria (mmol/day)						
Diabetic subjects	411 ± 78*	36 ± 10†	1.6 ± 0.4‡	2.2 ± 0.8‡	1.5 ± 0.5‡	0.9 ± 0.3‡
Medication						
Gliclazide (mg)	—	265 ± 28 (13)	227 ± 30 (12)	235 ± 34 (10)	202 ± 36 (10)	182 ± 36 (10)
Metformin (g)	—	1.9 ± 0.1 (9)	1.6 ± 0.2 (6)	1.3 ± 0.3 (6)	1.6 ± 0.2 (4)	1.6 ± 0.2 (4)
Serum urea (mmol/l)						
Diabetic subjects	5.1 ± 0.4*	4.4 ± 0.3†	5.3 ± 0.3‡	5.3 ± 0.3‡	5.7 ± 0.4‡	5.5 ± 0.3‡
Obese control subjects	5.0 ± 0.3*†	—	5.5 ± 0.4*‡	5.3 ± 0.4*‡	5.4 ± 0.4*‡	5.5 ± 0.4*‡
FSG (mmol/l)						
Diabetic subjects	12.5 ± 0.8*	9.1 ± 0.8†	7.3 ± 0.4§	6.4 ± 0.4§‖	6.1 ± 0.4§‖	5.8 ± 0.4‖
Obese control subjects	5.2 ± 0.2‡¶	—	4.9 ± 0.3¶	5.3 ± 0.3‡	5.1 ± 0.2‡¶	5.0 ± 0.2‡‖¶
Plasma free fatty acids (μmol/l)						
Diabetic subjects	995 ± 81*	952 ± 78*	879 ± 77*	899 ± 95*	835 ± 82*	901 ± 64*
Obese control subjects	863 ± 32*	—	907 ± 96*	957 ± 80*	840 ± 90*	1,028 ± 117*
Blood 3-hydroxybutyrate (μmol/l)						
Diabetic subjects	93 ± 17*	89 ± 21*	NA	213 ± 74*	NA	436 ± 157†
Obese control subjects	33 ± 6*	—	NA	107 ± 21*	NA	150 ± 37*
Fasting plasma insulin (pmol/l)						
Diabetic subjects	210 ± 16*	240 ± 28*	150 ± 12†	156 ± 13†	130 ± 13†	120 ± 10†
Obese control subjects	177 ± 16*	—	130 ± 8†	122 ± 7†	129 ± 13†	124 ± 10†
Insulin/glucose						
Diabetic subjects	17.1 ± 1.5*	29.4 ± 4.8†	21.6 ± 3.0†	24.2 ± 1.7†‡	20.8 ± 1.6†‡	21.9 ± 2.3†‡
Obese control subjects	34.6 ± 4.0†	—	27.6 ± 2.6†‡	23.5 ± 1.6‡	25.7 ± 2.7‡	25.4 ± 2.9‡
Fasting plasma C-peptide (pmol/l)						
Diabetic subjects	995 ± 119*	1,019 ± 142*	848 ± 93*†	894 ± 117*†	842 ± 88*†	815 ± 86†
Obese control subjects	649 ± 76‡	—	495 ± 65‡§	440 ± 53§	482 ± 66‡§	511 ± 76‡§
C-peptide/insulin						
Diabetic subjects	4.4 ± 0.5*	4.7 ± 0.5*	5.4 ± 0.5*†	5.6 ± 0.5*†	6.6 ± 0.5†	6.1 ± 0.4†
Obese control subjects	3.8 ± 0.3*‡	—	3.8 ± 0.4‡	3.6 ± 0.4‡	3.8 ± 0.4‡	4.1 ± 0.6‡

Data are means ± SEM or means ± SEM (*n* for diabetic subjects receiving medication). Calculations are by ANOVA with repeated measures. Data with a common symbol are not significantly different, both between diabetic and control subjects at all time points and during treatment within each group. Normal range for serum urea, 2.1–7.5 mmol/l. NA, not available.

LED. Glucose decreased ($P < 0.05$) at 7 days of ISO with OHA and to euglycemia by 4 weeks of LED. Insulin did not change with OHA during ISO and was not different from control subjects; in both groups it decreased significantly during LED. The insulin-to-glucose ratio was significantly greater in obese control versus diabetic subjects without OHA but not with OHA nor during LED. C-peptide was significantly higher in diabetic than control subjects during ISO, did not change with OHA, remained elevated versus control subjects during LED, and decreased slightly but significantly by week 4. During ISO, the ratio of C-peptide to insulin did not differ between groups. By week 3 of LED, it increased in diabetic subjects and was higher throughout LED versus control subjects, in whom it did not change.

Glucose and insulin before daytime feedings in two control subjects, with ISO and LED, neither changed nor showed diet-related differences (Fig. 1). In diabetic patients, glucose was significantly elevated during ISO with or without OHA, and highest at 11:00. Insulin was higher at 17:00 than at 08:00 during ISO without treatment and higher at all times vs. 08:00, during ISO with OHA. During LED, insulin was higher ($P < 0.05$) at 11:00 compared with other times of day (Fig. 1).

In diabetic patients, daily nitrogen balance (Fig. 2) was not different from zero during ISO (0.03 ± 0.4 g N/day), but less than in control subjects (1.2 ± 0.2 g N, $P < 0.05$), then became positive with OHA (1.8 ± 0.3 g N/day; $P < 0.05$), and not different from control subjects. Cumulative 7-day nitrogen retention was enhanced with OHA from 2.6 ± 3.1 to 11.8 ± 2.4 g N, $P < 0.05$, becoming the same as in control subjects, 9.0 ± 1.6 g N. Cumulative nitrogen retention over the last 3 days of ISO was greater in control subjects (3.6 ± 0.5) vs. diabetic patients (0.2 ± 1.2 g N, $P = 0.03$) but not different with OHA (5.3 ± 1.0 g N). During ISO, the change in cumulative ni-

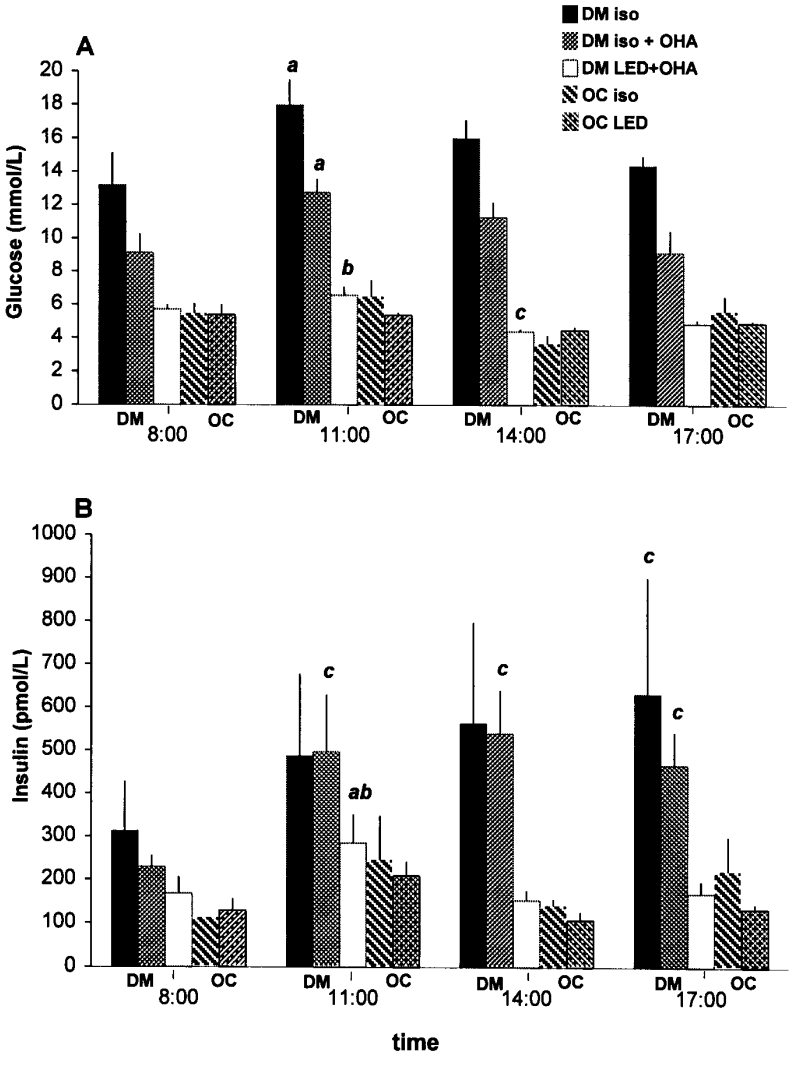

Figure 1—*Plasma glucose (*A*) and insulin excursions (*B*) from samples taken before meals in five diabetic and two control subjects at day 7 of ISO without OHA in diabetic and control subjects and at day 7 of OHA in diabetic patients, and at day 27 of LED in both diabetic and control subjects.* a, P < *0.05 vs. 08:00 and 17:00;* b, P < *0.05 vs. 14:00 and 17:00;* c, P < *0.05 vs. 08:00, all within the same study in the same group. Data are means ± SEM.*

trogen balance over the last 3 days without OHA compared with OHA correlated ($r = -0.728$, $P = 0.0004$) with the change in FSG as an effect of treatment. At the onset of LED, nitrogen balance decreased in both groups, but remained in equilibrium and not different between groups throughout (Fig. 2). Cumulative nitrogen balance during LED was −3.4 ± 10.3 g N in diabetic patients (−106 ± 322 g lean tissue) and 4.7 ± 6.1 g N (147 ± 191 g lean tissue) in control subjects ($P = 0.507$).

The kinetic parameters of protein metabolism are shown in Fig. 3. At all times, nitrogen flux (Q), synthesis (S), and breakdown (B) correlated positively with FFM. By simple factorial ANOVA, with FFM, sex, and age as covariates, rates of whole-body Q in g/day were significantly higher ($P = 0.006$) in hyperglycemic diabetic patients than in control subjects during ISO but not with OHA therapy. With 4 weeks of LED, Q decreased significantly in diabetic patients by 7%, to values not different from control subjects. S was greater ($P < 0.05$) in diabetic patients than in control subjects during ISO with OHA, whereas B was greater in diabetic patients without and with OHA. The LED was associated with a decrease of 11% in S and of 11% in B ($P < 0.05$) to values no longer different from control subjects. However, the changes in S and B with OHA were such that S-B was increased significantly in diabetic patients during ISO. After LED, S-B did not differ from that of control subjects. In control subjects, LED was associated with a slight decrease in S and S-B.

We performed stepwise multiple regression analysis with Q, S, B, S-B, and their changes in response to OHA with or without LED as dependent variables, and with sex, FSG, insulin, C-peptide, initial FFM, presence of diabetes, and cumulative weight loss as independent variables (Table 3). In diabetic patients without OHA and in control subjects, a large portion of the variation in Q and in B was explained by sex and FSG; that in S by sex; and that in S-B by the cumulative weight loss of the first week and FSG. When analysis was done with values taken from diabetic patients with OHA and control subjects, the majority of the variation in Q, S, and B was explained by FFM and the presence of diabetes, and half of that in net synthesis was accounted for by insulin concentrations. Because FFM and of course sex did not substantially change during the course of the study, 85% of the change in S-B in response to OHA during ISO was explained by the changes in FSG and in plasma C-peptide.

Post-LED, one-third of the variation in Q, S, and B was explained by plasma C-peptide and no variables explained the variation in S-B. For the variation in the changes in Q, S, and B, ~30% was explained by the change in weight; 85% of the variation in the changes in S-B were explained by changes in C-peptide and FSG.

Urinary 3MH excretion was higher in diabetic compared with control subjects during ISO (Table 4). With OHA, it decreased significantly, to a value no longer different from control subjects. With LED, it decreased by 13% ($P < 0.05$) in both groups to comparable values. These significant differences were not affected whether excretion was expressed on per day, per millimole of creatinine, or per kilogram of FFM. Muscle protein breakdown was greater in hyperglycemic diabetic patients compared with control subjects and decreased significantly with OHA to values no longer different from control subjects. With LED, it decreased ($P < 0.05$) in both groups to comparable values. Percent contribution of muscle protein breakdown to whole-body protein breakdown did not differ between control subjects and diabetic patients with or without OHA during ISO. It became significantly lower with

LED in diabetic patients compared with values during ISO while hyperglycemic, though not during OHA treatment. In control subjects the percent did not decrease significantly with LED.

CONCLUSIONS — This study shows that abnormalities of protein balance associated with moderate hyperglycemia in type 2 diabetes are significantly diminished by the addition of OHA. Our results indicate that diabetic subjects respond to low-energy diets with a generous protein content in a manner similar to obese control subjects such that the differences in protein metabolism between diabetic and obese control subjects become negligible. During moderate hyperglycemia, nitrogen balance was significantly less in the diabetic subjects. That this is "abnormal" in this specific setting is supported by the significant increase in nitrogen retention and net endogenous protein synthesis associated with OHA that produced considerable improvement in glycemia. There was no significant within-group change in Q, S, or B attributable to OHA. Although B remained more elevated, Q was no longer elevated and S became more elevated than in the control subjects. The concurrent decrease in B and increase in S were responsible for the significantly greater S-B, equivalent to 17 g/day of protein retention, 28% (4.8 g/day) of which was due to the decrease in muscle protein breakdown. OHA treatment caused a 27% decrease in glycemia and a 93% decrease in glycosuria without changes in fasting insulin or C-peptide (Table 2). The fasting insulin/glucose ratio increased 72%. The OHA significantly improved daytime premeal glycemia, again with comparable insulin levels (Fig. 1). In addition to a relative increase in insulin secretion attributable to gliclazide, there may have been enhanced peripheral insulin sensitivity (25,26).

Previous studies of OHA reported no change in postabsorptive rates of proteolysis using ^{13}C-leucine (27,28) and were interpreted to suggest that even limited amounts of circulating insulin are sufficient to prevent protein loss in type 2 diabetes. Our results, over the whole day, do not confirm such results. At least some of the OHA effects on protein metabolism we found would be analogous to their effects on glucose and result from relatively enhanced insulin secretion, especially in the portal vein, influencing hepatic amino acid metabo-

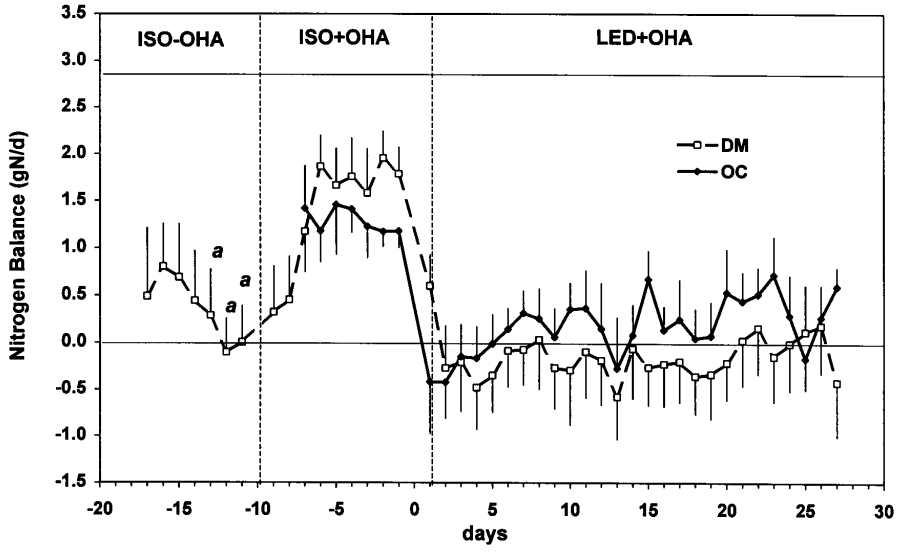

Figure 2—*Daily nitrogen balance during ISO for 7 days in control subjects and 16 days in diabetic patients and for 27 days of LED.* a, $P < 0.05$ *vs. ISO in diabetic patients with treatment and in control subjects. Data are means ± SEM.*

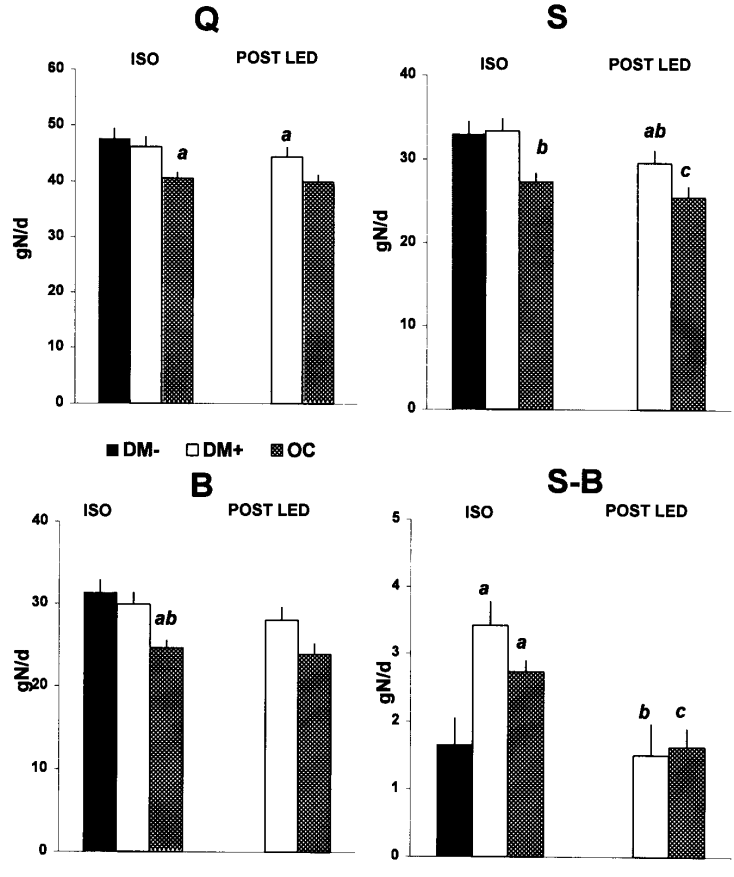

Figure 3—*Whole-body protein kinetics during ISO and after 28 days of LED. Data are grams of nitrogen per day, means ± SEM. Results were obtained by simple factorial ANOVA for between groups, with FFM, sex, and age as covariates for Q, S, and B, and by paired* t *test for within groups.* a, $P < 0.05$ *vs. corresponding values in diabetic patients during ISO without OHA;* b, $P < 0.05$ *vs. corresponding value in diabetic patients during ISO with OHA;* c, $P < 0.05$ *vs. corresponding value in control subjects during ISO.*

Table 3—*Multiple linear regression analyses with kinetics of protein metabolism as dependent variables*

Dependent variable	ISO (diabetic subjects without OHA and control subjects)			ISO (diabetic subjects with OHA and control subjects)			Post-LED		
	Variable	r^2	*P*	Variable	r^2	*P*	Variable	r^2	*P*
Q	Sex	0.557	<0.0001	FFM	0.653	<0.0001	C-peptide	0.369	0.0045
	FSG	0.654	<0.0001	Sex	0.735	<0.0001			
S	Sex	0.388	0.0015	FFM	0.527	0.0001	C-peptide	0.333	0.0077
				Insulin	0.681	<0.0001			
				Diabetes	0.751	<0.0001			
B	Sex	0.421	0.0008	FFM	0.535	0.0002	C-peptide	0.348	0.0062
	FSG	0.558	0.0003	Diabetes	0.642	0.0001			
S-B	Cumulative weight loss at day 7	0.483	0.0002	Insulin	0.465	0.0146			
	FSG	0.604	0.0001						

All variables are shown in the order of entry (n = 23).

lism, as well as a peripheral effect on protein catabolism. The increase in C-peptide was a significant independent factor in the increase in S-B. At the hepatic level, insulin has both direct and "indirect" effects, with decreased gluconeogenesis potentially contributed to by altered substrate presentation (29). In the case of myofibrillar protein, as inferred by 3MH, there was a 10% decrease in catabolism. This method does not discriminate the type of muscle from which the product is derived (striated versus gastrointestinal smooth muscle, for example). Nonetheless, given the magnitude of the overall improvement in S-B, there must have been a substantial effect on tissues other than muscle, particularly the liver.

We cannot attribute the entire effect on protein metabolism to a putative effect of increased portal vein insulin. Successful metformin monotherapy is associated with a decrease in (peripheral) insulin (30). Its major glycemic effect is from inhibition of hepatic glucose output, directly and by enhancement of insulin suppression of gluconeogenesis (30). While metformin enhances glucose disposal in vitro, in humans the lowered glycemia and/or decreased insulin and its effects on insulin receptors, and weight loss, all have similar independent effects (30,31). Our results with OHA during ISO are most likely due to inhibition of gluconeogenesis by direct effects of metformin and portal insulin, with a contribution of decreased muscle protein catabolism, perhaps related to the enhanced effects of the same levels of insulin, with or without direct peripheral effects of the agents. Clearly, studies of insulin sensitivity of protein metabolism are required for each agent separately.

Because the subjects of the current study were similar in body composition (Table 1), it is unlikely that the observed initial protein retention with OHA was due to prior depletion of body protein, as occurs in protein-energy malnutrition (32). Nor is this response explainable by low protein intake before the study (data not shown). This response is consistent with our previous findings (12,13) in which intensive insulin therapy was associated with a greater decrease in B than in S, leading to greater S-B. This change in nitrogen balance and turnover with treatment is more sensitive than estimates of body composition, and confirms the presence of abnormalities of nitrogen metabolism during moderate hyperglycemia. Our findings also suggest that maintenance of body composition and nitrogen equilibrium are at the "cost" of more rapid protein turnover, and require sufficient energy and protein intakes (12,13). Insufficient intakes do not support nitrogen equilibrium (11–13,33,34). The positive nitrogen balance observed in the present study is therefore most likely explained by the combination of OHA treatment with the higher protein intake, individualized for each subject. The division of the meals into six equal portions may also have potentiated the nitrogen retention during ISO with OHA, and likewise, have contributed to the slightly positive nitrogen balance observed in the control subjects (35,36).

Table 4—*Urinary 3MH and estimated rates of muscle protein breakdown*

	ISO	ISO + OHA	LED
3MH (μmol/day)			
Diabetic subjects	222 ± 21*	199 ± 20†	174 ± 17‡
Obese control subjects	166 ± 11†		142 ± 8‡
3MH (μmol · mmol^{-1} creatinine · day^{-1})			
Diabetic subjects	16.1 ± 0.6*	14.9 ± 0.5†	13.5 ± 0.6‡
Obese control subjects	14.1 ± 0.5†		13.1 ± 0.4‡
3MH (μmol/kg FFM)			
Diabetic subjects	3.6 ± 0.2*	3.3 ± 0.2†	2.9 ± 0.2‡
Obese control subjects	3.1 ± 0.1†		2.8 ± 0.1‡
Muscle protein B (g/kg FFM)			
Diabetic subjects	0.91 ± 0.04*	0.83 ± 0.05†	0.72 ± 0.02‡
Obese control subjects	0.78 ± 0.03‡		0.68 ± 0.02‡
Muscle B as % whole-body B			
Diabetic subjects	28 ± 2*	26 ± 2*†	25 ± 2†
Obese control subjects	27 ± 2*		24 ± 1*†

Data are means ± SEM. Data with a common symbol are not different from one another, both between groups and between treatment results within each group.

With the LED, nitrogen equilibrium was maintained throughout, both in control and diabetic subjects. This result confirms the need for high protein intake when energy is restricted, as a requirement for maintaining lean body mass while selectively mobilizing fat (37). Furthermore, in the diabetic patients, it shows that near-normal metabolic control achieved by diet with OHA therapy normalized not only nitrogen balance but the kinetics of protein metabolism (Fig. 3, Table 4). The duration of the LED, and the 5–6% weight loss, virtually all as fat, make these observations relevant to practice. That the decreased energy intake and fat loss contributed considerably to the normalized metabolic status is confirmed by the requirement to progressively decrease and even discontinue the OHA. The present results define clinically relevant conditions in which selective fat loss without compromise of protein metabolism or lean tissue mass occur in both control and diabetic subjects. This result is in contrast to that seen with VLED (11–13,34).

In the hyperglycemic diabetic patients the increased excretion of 3MH indicates greater catabolism of myofibrillar proteins. This result is consistent with findings in both type 1 (2,3,6,38) and type 2 diabetes (38). OHA caused a 32% reduction in 3MH to levels similar to those of the control subjects during ISO. The further small but significant decrease in 3MH with LED in both groups (Table 3) translated into a smaller proportion of whole-body protein breakdown attributable to muscle than in diabetic patients while they were hyperglycemic. This result suggests a shift in proteins contributing to catabolism toward other tissues and exogenous protein. This shift occurred in a setting of substantially lower fasting insulin (Table 2) and probably lower postprandial increases, consistent with increased sensitivity of muscle protein catabolism to insulin.

The consensus has hitherto been that abnormalities of protein metabolism are less sensitive to insufficient insulin action than those of glucose. A greater deficiency of insulin secretion and/or action has been presumed necessary before important consequences on protein metabolism are found. The present study tends to refute this notion, inasmuch as abnormalities of protein metabolism are present even with mild hyperglycemia. FSG explained partly the variation in nitrogen flux and protein breakdown otherwise accounted for by sex; the sex effect reflects the contributions of lean tissue and of protein intake, which was adjusted to body mass. Furthermore, it is conceivable that there is a "dose-response" relationship between plasma glucose levels and the derangement of protein metabolism. The threshold for abnormal S-B appears to be at a fasting glycemia between 6 and 7 mmol/l (39).

Finally, our data confirm the need for increased (>1 g/kg body weight) dietary protein during moderate energy restriction in both nondiabetic and diabetic obese persons. The present study strongly supports the use of a treatment of type 2 diabetes that consists of OHA, moderate energy restriction, and generous protein intake, not only for its beneficial effects on glucose and lipids but because it is able to correct several aspects of whole-body protein metabolism.

Acknowledgments — This study was supported by grants from the Canadian Diabetes Association and by Servier Canada, Inc.

The authors wish to thank Madeleine Giroux, Anoma Gunasekara, Marie Lamarche, and Ginette Sabourin for their excellent technical assistance; Marla Myers, PDt, and Mary Shingler, RN, for their clinical support; and Josie Plescia for her secretarial expertise.

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