A Fasting-Induced Decrease in Plasma Glucose Concentration Does Not Affect the Insulin Response to Ingested Protein in People With Type 2 Diabetes

Asad Saeed, Sidney A. Jones, Frank Q. Nuttall, and Mary C. Gannon

We previously have reported that protein, on a weight basis, is just as potent as glucose in increasing the insulin concentration in people with type 2 diabetes. In people without diabetes, protein is only approximately 30% as potent as glucose in this regard. In the present study, we tested the hypothesis that the increased insulin responsiveness to protein in people with type 2 diabetes is due to the elevated plasma glucose concentration in these individuals. Seven male subjects with untreated type 2 diabetes were given 50 g protein in the form of very lean beef at 8 AM after an overnight fast. On another occasion, the same individuals were fasted for an additional 24 hours to lower their plasma glucose concentration to near the normal reference range. They were then given 50 g protein. The 8 AM glucose concentration was lower after 24 hours of additional fasting, as expected. After ingestion of the protein meal, there was an unexpected, modest increase in glucose concentration after an additional 24 hours of fasting that was not observed with only an overnight fast. Despite the approximately 15% lower plasma glucose concentration at the time of the protein meal, the insulin responses were nearly identical. Thus, the greater insulin response to ingested protein is not likely to be due merely to a higher initial glucose concentration.

IT HAS BEEN KNOWN for many years that protein ingestion or the administration of amino acids, orally or intravenously, stimulate an increase in the serum insulin concentration in people with and without mild type 2 diabetes mellitus. This effect is probably mediated directly by an effect of amino acids on the β cells and indirectly through an incretin mechanism.[1]

We previously have reported that 50 g protein given in the form of very lean beef was just as potent as 50 g glucose in increasing the circulating insulin concentration in obese subjects with type 2 diabetes mellitus.[2] However, in normal subjects, protein was only 28% as potent as glucose.[3]

Why protein is relatively more potent in people with type 2 diabetes is unknown. It could be due to a higher than normal ambient glucose concentration. The glucose concentration is known to modulate the effect of nonglucose insulin secretagogues, such as gastric inhibitory polypeptide (GIP), glucagon like peptide-1,7-36-amide (GLP-1-[7-36]-amide), and fructose. For instance, an increase in glucose concentration of greater than 1 mmol/L above a normal fasting glucose concentration has been reported to be required for GIP to potentiate glucose-stimulated insulin secretion in nondiabetic people.[4,5]

The dietary protein-stimulated incretins, if any, are not known. Cholecystokinin (CCK) and/or GLP-1 (7-36) amide are possible candidates.[6,7]

We, as well as others, have shown that even short-term starvation considerably decreases the plasma glucose in people with type 2 diabetes mellitus. On average, a decrease of between 20% to 40% can be expected, irrespective of the initial overnight fasting glucose concentration.[8] Therefore, we decided to determine if a decrease in fasting glucose resulting from short-term starvation would modify the exaggerated insulin response in subjects with type 2 diabetes.

MATERIALS AND METHODS

Seven male subjects with untreated, type 2 diabetes mellitus (total glycohemoglobin $< 11\%$) were studied in a Special Diagnostic and Treatment Unit (SDTU). All subjects met the American Diabetes Association criteria for the diagnosis of type 2 diabetes mellitus.[9] Their mean age was 67 years (range, 50 to 80); mean body mass index (BMI), 30 kg/m^2 (range, 22 to 37); and mean total glycohemoglobin (tGHb) 8.9% (range, 7.8% to 10.1%) (reference range, 4.6% to 6.6%). Thyroid, renal, and liver function tests were within the reference range. Written informed consent was obtained from all subjects, and the study was approved by the Minneapolis Department of Veterans Affairs Medical Center and the University of Minnesota Committees on Human Subjects. None of the subjects was treated with either oral hypoglycemic agents or insulin before or during the study. The subject profiles are given in Table 1.

Each subject was studied twice in random order, determined by the flip of a coin. During each study period, they were admitted to the SDTU, which is similar to a clinical research center (CRC). They were given a standardized meal (55% carbohydrate, 30% fat, 15% protein) at 6 PM and a snack at 8 PM. After an overnight fast of 12 hours, an indwelling catheter was inserted into a peripheral vein. During the sampling period, the catheter was kept patent with intravenous saline. On one occasion, the subjects were given 50 g protein after an overnight fast. On the other occasion, they were starved for an additional 24 hours (total of 36 hours, with water only, ad libitum) and then given 50 g protein. Protein was ingested in the form of very lean (6.5% fat) hamburger. The meat was browned in a nonstick pan and placed in a refrigerator until it was to be served. Cooking was completed by placing the meat in a commercial microwave oven for 30 seconds. The beef was ingested over a 10-minute period beginning at 8 AM. Blood samples were obtained at 7:30 AM, 7:45 AM, 8 AM, and 8:15 AM and then every 15 minutes for 2 hours, every half hour for the next 3 hours, and then at hourly intervals until the end of the 8-hour study. The samples were processed for glucose, insulin, C-peptide, triglycerides, plasma urea nitrogen (PUN), creatinine, uric acid, nonesterified fatty acids (NEFA), alpha-amino nitrogen (AAN), and glucagon determinations.

Plasma glucose was determined by a glucose oxidase method using

From the Metabolic Research Laboratory, Section of Endocrinology, Metabolism, and Nutrition, Minneapolis VA Medical Center, Minneapolis; and the Departments of Medicine and Food Science and Nutrition, University of Minnesota, Minneapolis, MN.

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Address reprint requests to Mary C. Gannon, PhD, Director, Metabolic Research Laboratory (111G), VA Medical Center, One Veterans Dr, Minneapolis, MN 55417.

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Table 1. Patient Characteristics

Patient No.	Age (yr)	Height Inches (cm)	Weight Pounds (kg)	BMI (kg/m^2)	tGHb	Length of Diabetes Mellitus	Concomitant Diseases	Medications
1	76	63.5 (161)	213 (96)	37	8.7	12 mo	History of alcoholic hepatitis, liver function tests currently within normal limits	Sertraline
2	79	73 (185)	174 (78)	23	8.3	24 mo	Prostate cancer, history of hiatal hernia	Goserelin
3	50	70 (178)	170 (77)	24	10.9	1 mo	Hypercholesterolemia	None
4	57	70 (178)	234 (105)	33	10.1	24 mo	Mild peripheral neuropathy	Lisinopril
5	59	72 (183)	266 (120)	36	9.0	New	None	None
6	72	72 (183)	170 (77)	23	7.8	20 mo	Hypertension, Paget's disease	Lisinopril Simvastatin
7	70	72 (183)	234 (105)	31	8.8	8 mo	Hypertension, asthma, psoriasis	Methotrexate Albuterol
Mean	66 ± 4			30 ± 2	9.1 ± .4			
Range	50-79			23-37	7.8-10.9			

a Beckman glucose analyzer with an O_2 electrode (Beckman Instruments, Fullerton, CA). Serum immunoreactive insulin and C-peptide were measured using a standard double antibody radioimmunoassay (RIA) method using kits produced by Incstar (Stillwater, MN). Glucagon was determined by RIA using 30 K antiserum purchased from Health Sciences Center (Dallas, TX). AAN was determined by the method of Goodwin.[10] Serum NEFAs were determined enzymically using a kit purchased from Wako Chemicals (Dallas, TX). Triglycerides and urea nitrogen were determined using an EktaChem Analyzer (Eastman Kodak, Rochester, NY).

The various integrated 8-hour area responses were calculated using the averages of the 7:30 AM, 7:45 AM, and 8 AM values as a baseline and a computer program based on the trapezoid rule.[11] Statistics were determined using Student's *t* test for paired variates with the Statview 4.1 program (Abacus Concepts, Berkeley, CA) for the Macintosh computer (Apple Computer, Cupertino, CA). A *P* value of less than .05 was the criterion for significance. Data are presented as means ± SEM.

The study was designed to detect a change in insulin concentration of 10 μU/mL and a change in net insulin area of 84 μU hr/mL, setting a type II error at .2 (80% power) with a .05 level of significance.

RESULTS

Glucose

After an overnight, 12-hour fast, the mean 8 AM fasting blood glucose concentration was 142 ± 11 mg/dL (Fig 1). After ingestion of 50 g protein, the mean glucose concentration decreased continually and reached a nadir of 107 ± 7 mg/dL 7 hours after the meal.

When food was withheld for an additional 24 hours, the mean 7:30 to 8 AM fasting blood glucose had decreased to 121 ± 12 mg/dL. In contrast to the continuous decrease in glucose concentration when protein was ingested after an overnight 12-hour fast, protein ingestion after an additional 24 hours of starvation resulted in an initial transient increase in glucose concentration. This was followed by a more rapid, continuous decrease, which reached a nadir of 97 ± 9 mg/dL 6 hours after the meal. The respective net 8-hour glucose area responses using the initial values as baseline were –166 ± 39 mg · h/dL and –62 ± 36 mg · h/dL, respectively. The difference

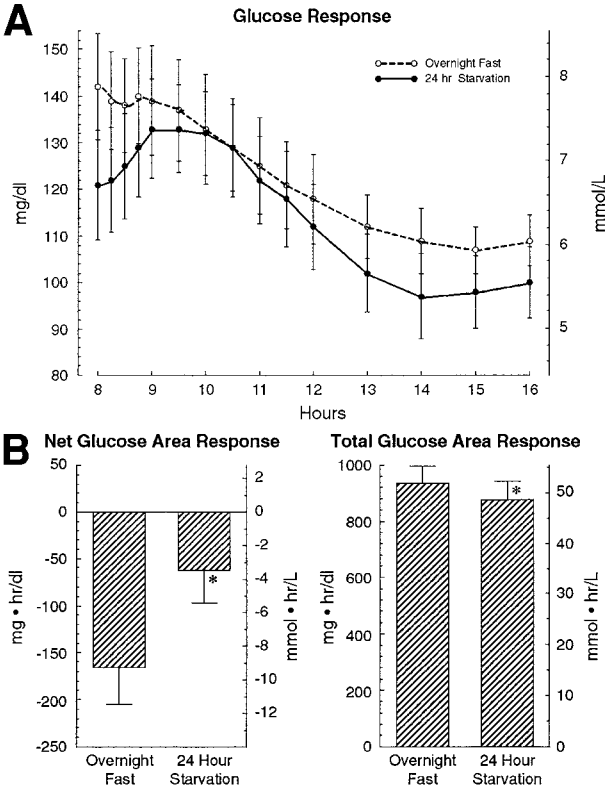

Fig 1. (A) Plasma glucose response to 50 g beef protein in 7 men with untreated type 2 diabetes. (○) Response after overnight fast; (●) response after an additional 24-hour period of starvation. (B) Net glucose area response integrated over 8 hours using the overnight fasting glucose concentration as baseline. (C) Total glucose area response integrated over 8 hours using zero as baseline. *Statistically different from the overnight fasting value (*P* ≤ .05).

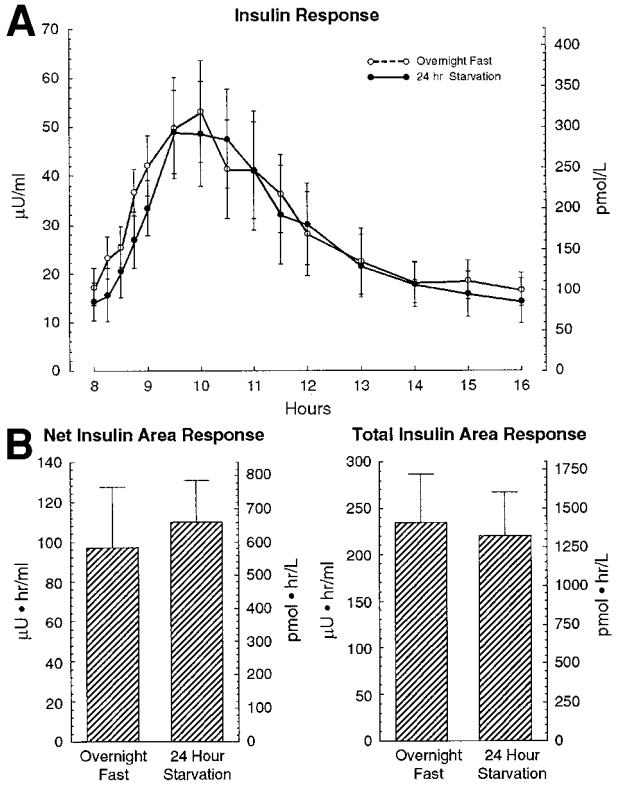

Fig 2. (A) Serum insulin response to 50 g beef protein in 7 men with untreated type 2 diabetes. (○) Response after overnight fast; (●) response after an additional 24-hour period of starvation. (B) Net insulin area response integrated over 8 hours using the overnight fasting glucose concentration as baseline. (C) Total insulin area response integrated over 8 hours using zero as baseline.

was statistically significant. The respective total areas under the curve were 934 ± 63 and 876 ± 67 mg · hr/dL. This also was statistically significant.

Insulin

The mean initial insulin concentration was 17 ± 3.9 μU/mL after the overnight fast (Fig 2). After ingestion of 50 g protein, it increased to a peak of 53 ± 11 μU/mL 2 hours after the meal. It then gradually decreased back to the initial value by the end of the 8-hour study period.

When the subjects were starved for an additional 24 hours, the mean initial insulin concentration was 14 ± 4 μU/mL. After protein ingestion, it increased to a peak of 49 ± 10 μU/mL in 1.5 hours. Thereafter, it decreased gradually back to the baseline concentration at the end of the study period. The respective 8-hour net insulin area responses were 97 ± 31 μU · h/mL and 110 ± 21 μU · h/mL. The 2 insulin areas were not different statistically. The respective total insulin areas were 234 ± 53 and 220 ± 47 μU · h/mL. They also were not different statistically.

C-Peptide

The C-peptide response generally corresponded to the insulin response, both with and without the additional 24-hour period of starvation (Table 2).

AAN

As expected, protein ingestion resulted in an increase in the AAN concentration. It reached a maximum in 2 hours after the meal and had returned to the basal concentration at 6 hours. Except for a slight difference in the peak, which was higher after the overnight fast, the AAN curves with and without an additional 24 hours of starvation were essentially identical (Fig 3). The respective net 8-hour AAN areas were 6.19 ± 0.64 and 5.42 ± 1.02 mg · h/dL and the respective total AAN areas 36 ± 0.94 and 35 ± 1.17 mg · h/dL. These areas were not statistically significant.

Table 2. Metabolite Concentrations

	C-Peptide		Triglyceride		Urea Nitrogen		Uric Acid	
Time	Overnight	24 Hours+	Overnight	24 Hours+	Overnight	24 Hours+	Overnight	24 Hours+
8:00 AM	0.71 ± 0.1	0.60 ± 0.1	208 ± 50	239 ± 58	14 ± 0.6	15 ± 1.0	6.0 ± 0.3	6.5 ± 0.3
8:15	0.77 ± 0.1	0.67 ± 0.1	227 ± 56	258 ± 63	14 ± 0.6	15 ± 0.8	6.1 ± 0.3	6.7 ± 0.4
8:30	0.84 ± 0.1	0.71 ± 0.1	222 ± 55	246 ± 59	14 ± 0.6	15 ± 0.8	6.2 ± 0.3	6.8 ± 0.3
8:45	0.98 ± 0.1	0.86 ± 0.1	209 ± 51	236 ± 58	14 ± 0.6	15 ± 1.0	6.3 ± 0.3	6.8 ± 0.3
9:00	1.08 ± 0.1	0.97 ± 0.1	210 ± 52	232 ± 57	14 ± 0.6	16 ± 1.2	6.4 ± 0.3	7.0 ± 0.3
9:30	1.24 ± 0.1	1.16 ± 0.2	223 ± 52	234 ± 61	15 ± 0.7	18 ± 1.4	6.4 ± 0.3	7.0 ± 0.3
10:00	1.28 ± 0.2	1.25 ± 0.2	225 ± 54	240 ± 65	16 ± 0.8	19 ± 1.4	6.3 ± 0.3	7.2 ± 0.5
10:30	1.14 ± 0.2	1.29 ± 0.2	237 ± 54	233 ± 56	17 ± 0.9	19 ± 1.5	6.2 ± 0.3	6.7 ± 0.4
11:00	1.14 ± 0.2	1.27 ± 0.2	246 ± 58	250 ± 61	18 ± 0.8	20 ± 1.4	6.2 ± 0.3	6.8 ± 0.4
11:30	1.00 ± 0.2	1.19 ± 0.2	256 ± 67	258 ± 64	18 ± 0.8	20 ± 1.5	6.1 ± 0.3	6.5 ± 0.4
12:00 PM	0.96 ± 0.2	1.02 ± 0.2	264 ± 70	248 ± 62	18 ± 0.8	21 ± 1.4	6.1 ± 0.3	6.6 ± 0.3
1:00	0.89 ± 0.2	0.90 ± 0.1	260 ± 72	224 ± 53	18 ± 0.8	21 ± 1.4	6.0 ± 0.3	6.5 ± 0.3
2:00	0.76 ± 0.1	0.68 ± 0.1	366 ± 73	220 ± 50	18 ± 0.8	20 ± 1.6	6.0 ± 0.3	6.5 ± 0.4
3:00	0.70 ± 0.1	0.68 ± 0.1	242 ± 65	225 ± 59	17 ± 0.9	20 ± 1.5	6.0 ± 0.3	6.6 ± 0.3
4:00	0.64 ± 0.1	0.66 ± 0.1	234 ± 65	215 ± 56	17 ± 0.8	19 ± 1.6	6.0 ± 0.3	6.6 ± 0.3
Net area	1.94 ± 0.71	2.59 ± 0.45	272 ± 126	−37 ± 56	22 ± 2.3	34 ± 4.1*	0.73 ± 0.38	1.93 ± 0.55*
Total area	7.21 ± 1.1	7.24 ± 0.45	1,181 ± 487	1,810 ± 448	131 ± 5.8	149 ± 10.4*	48 ± 2.2	52 ± 2.8

*$P \leq .05$ compared with overnight fasted values.

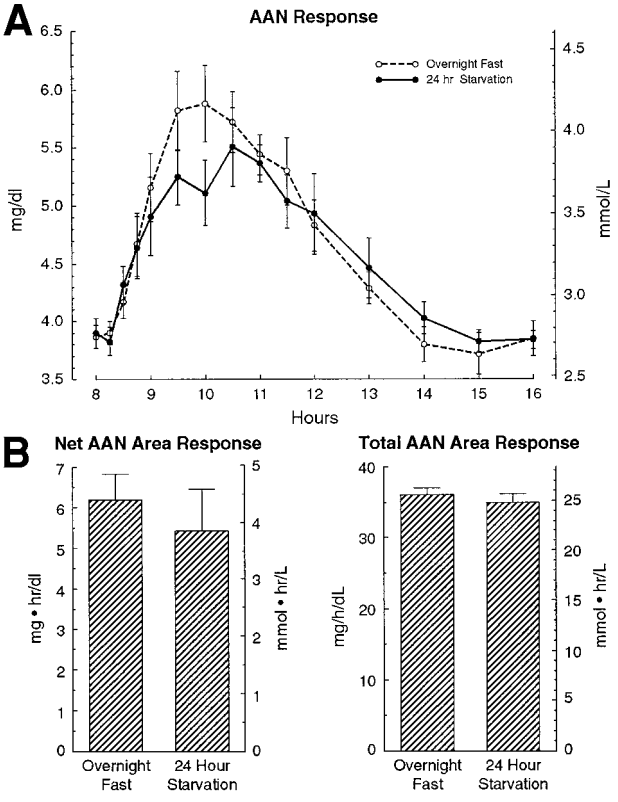

Fig 3. (A) Plasma AAN response to 50 g beef protein in 7 men with untreated type 2 diabetes. (○) Response after overnight fast; (●) response after an additional 24-hour period of starvation. (B) Net AAN area response integrated over 8 hours using the overnight fasting glucose concentration as baseline. (C) Total AAN area response integrated over 8 hours using zero as baseline.

Glucagon

The initial glucagon concentration was higher after the additional 24-hour period of starvation (Fig 4). After protein ingestion, with and without the additional 24 hours of starvation, it increased as expected and reached a peak in 1.5 hours. At each time point in the study, the glucagon concentration was higher after the additional 24-hour starvation period. However, the respective 8-hour net glucagon area responses were 1,099 ± 170 and 1,091 ± 194 pg · h/mL, ie, they were essentially identical. The total 8-hour glucagon area response after the additional 24-hour starvation period was statistically significantly higher (3,763 ± 385 *v* 3,092 ± 253 pg · h/mL, respectively).

PUN

The initial mean PUN concentration was slightly lower after the overnight fast. The increase in response to protein also was modestly less, and the incremental area response was statistically significantly less than after the subjects had been starved for an additional 24 hours (Table 2).

Uric Acid

The initial mean uric acid concentration also was lower when the subjects were only fasted overnight. The increase in response to the ingested protein was also modestly less than when the subjects were starved for a further 24 hours. The incremental area responses were statistically significantly different (0.7 ± 0.4 *v* 1.9 ± 0.6) (Table 2).

NEFA

Throughout the 8-hour study period, the mean NEFA concentration was higher after the subjects were starved for an additional 24 hours (Fig 5). After protein ingestion, the NEFA concentration after the overnight fast and after the additional 24 hours of starvation decreased initially, as expected, and reached a nadir at 2 hours. Thereafter, they both increased. The concentration in the subjects after the 12-hour, overnight fast crossed the baseline by 5 hours and was higher by the end of the study compared with the basal concentration. When the subjects were starved for an additional 24 hours, this did not occur. However, the final concentration reached was nearly the same in both groups. The respective 8-hour net NEFA area responses were –298 ± 354 and –1,311 ± 489 mEq · h/L. This did not reach statistical significance. The respective 8-hour total NEFA areas were 4,998 ± 391 and 6,570 ± 455 mEq · h/L without and with the additional 24 hours of starvation. This difference was statistically significant.

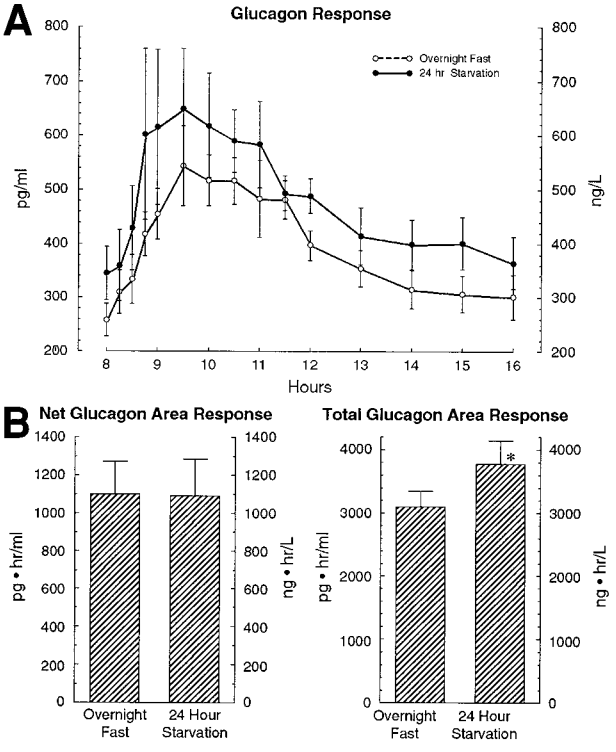

Fig 4. (A) Plasma glucagon response to 50 g beef protein in 7 men with untreated type 2 diabetes. (○) Response after overnight fast; (●) response after an additional 24-hour period of starvation. (B) Net glucagon area response integrated over 8 hours using the overnight fasting glucose concentration as baseline. (C) Total glucagon area response integrated over 8 hours using zero as baseline. *Statistically different from the overnight fasting value ($P \leq .05$).

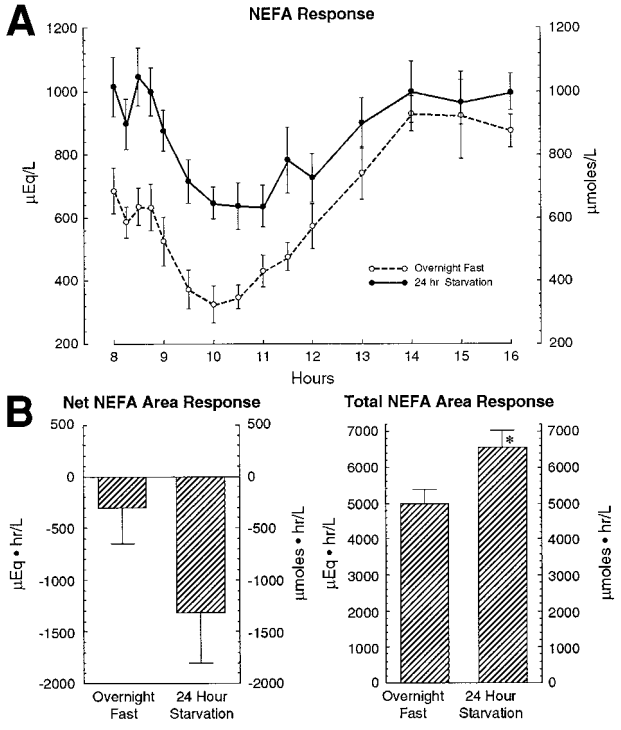

Fig 5. (A) Plasma NEFA response to 50 g beef protein in 7 men with untreated type 2 diabetes. (○) Response after overnight fast; (●) response after an additional 24-hour period of starvation. (B) Net NEFA area response integrated over 8 hours using the overnight fasting glucose concentration as baseline. (C) Total NEFA area response integrated over 8 hours using zero as baseline. *Statistically different from the overnight fasting value ($P \leq .05$).

Triglycerides

The integrated mean triglyceride concentration was modestly lower after the additional 24 hours of starvation, but this was not statistically significant (Table 2). There was a tendency for the concentration to increase in response to the ingested protein in the subjects fasted overnight, as noted previously,[12] but not when they were starved for an additional 24 hours. The incremental area response also was greater when the subjects were not starved for the additional 24 hours, but this did reach statistical significance due to the large variance (Table 2) (1,881 ± 487 *v* 1,810 ± 448, overnight and an additional 24-hour starved, respectively).

Creatinine

The initial serum creatinine concentrations were similar. They increased slightly after protein ingestion (data not shown). However, the incremental area responses were nearly identical in the 2 groups.

Quantitative Urine Data

The 8-hour quantitative urine data are shown in Table 3. The means for all of the data were less after the additional 24 hours of starvation. However, only the sodium excretion was statistically significantly less.

DISCUSSION

The glucose concentration being monitored by the β cells is the primary determinant of their insulin secretory activity. The other known insulin secretagogues, such as amino acids, fructose, fatty acids, and ketoacids play only a minor role. This may not be the case in people with type 2 diabetes mellitus. In a previous study from our laboratory, ingestion of 50 g beef protein resulted in an increase in insulin concentration that was just as great as that after ingestion of 50 g glucose.[2] Indeed, the insulin response to protein was 3.5-fold greater than the response in nondiabetic people ingesting a similar amount of protein.[13] The reason for this difference in insulin response remains unexplained, but has been attributed to an impairment in glucose, but not amino acid, sensing in the β cells of people with type 2 diabetes.[14,15] However, data we have obtained indicate that not only is the response to protein intact, but that it synergizes with administered glucose in stimulating insulin secretion.[2] This suggested that the ambient glucose concentration may affect the β-cell response to protein ingestion either by a direct effect of amino acids on insulin secretion or an indirect effect through protein-stimulated incretin hormone release.

Therefore, the major aim of this study was to compare the insulin response to ingested protein before and after an additional 24 hours of starvation, ie, after a starvation-induced decrease in glucose concentration. After an additional 24 hours of starvation, the mean ambient glucose concentration decreased by 21 mg/dL, representing a decrease of approximately 15%.

After beef protein ingestion, the plasma glucose concentration continued to decrease in the overnight fasted subjects, as expected.[12] In the same subjects starved for an additional 24 hours, protein ingestion resulted in a small, transient increase in glucose concentration. It then declined at a faster rate than when the subjects were fasted only overnight. Nevertheless, the insulin response was essentially identical in both groups. Thus, the 15% reduction in the ambient glucose concentration resulting from an additional 24 hours of starvation did not effect the insulin secretory response. This was partially confounded by the transient increase in glucose concentration in response to

Table 3. 8-Hour Integrated Urine Data

	Volume (mL)	Creatinine (mg)	Urea (mg)	Uric Acid (mg)	Sodium (matoms)	Potassium (matoms)	Glucose
Overnight	1,546 ± 196	648 ± 66	5,469 ± 323	384 ± 32	119 ± 8	42 ± 4	0
24-h fast	1,193 ± 183	615 ± 61	4,989 ± 558	258 ± 44	59 ± 7*	30 ± 5	0

*Indicates statistically significantly different ($P \leq .05$) compared with overnight fasted values.

the protein ingested when the subjects had been starved for an additional 24 hours. The small increase in glucose may have itself stimulated insulin secretion. It is unclear why the glucose concentration initially increased in response to protein ingestion after an additional 24 hours of starvation. It may have been due to the higher NEFA concentration in the subjects after the additional 24-hour period of starvation (Fig 5). This could have resulted in a decreased glucose oxidation rate consequent to the NEFAs substituting for glucose as a fuel. NEFAs are known to be oxidized in proportion to their concentration in the circulation.[16,17] However, it does not explain the more rapid decrease in glucose concentration beginning 1.5 hours after protein ingestion. The NEFA concentration was still higher during that time period.

The glucagon concentration was higher and thus, potentially could have resulted in an increase in gluconeogenesis. However, the glucagon concentration also remained elevated later in the time course, even though the glucose concentration decreased more rapidly than when the subjects were fasted overnight (Figs 1 and 4). Alternatively, availability of exogenous amino acids absorbed after the protein ingestion added to an already elevated endogenous gluconeogenic pool (lactate, alanine, glycerol) may have caused a substrate-driven increase in gluconeogenesis. Although the concentration of other gluconeogenic substrates was not measured, this is an unlikely explanation. Generally, gluconeogenesis is not substrate driven.[12,18-22] The amino acid concentration represented by the AAN concentration also was not different between the 2 groups of subjects. Thus, the mechanism remains unknown.

We previously reported that ingestion of beef results in an increase in uric acid concentration. Most likely it is due to metabolism of purines present in the beef muscle cells.[12] In the present study, the uric acid concentration was elevated, and the response to protein was modestly greater after the subjects had starved for an additional 24 hours. This may have been due to an elevation in "ketoacids." Starvation-induced ketosis is known to result in an elevation in serum uric acid concentration at least initially.[23]

The higher PUN concentration when the subjects were starved for an additional 24 hours is due to a decreased excretion of urea since the amount of urea excreted during the 8 hours of the study was less. A modest increase in urea formation, however, cannot be ruled out. The additional 24 hours of starvation did not significantly affect the triglyceride concentration.

In summary, starvation for an additional 24 hours, which resulted in a 15% decrease in the initial morning glucose concentration, did not affect the quantitative insulin response to ingestion of 50 g beef protein. Thus, the difference in ambient glucose concentration in these subjects with untreated type 2 diabetes was not important in determining the insulin secretory response to ingested protein.

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