Effect of Composition of Mixed Meals—Low- Versus High-Carbohydrate Content—on Insulin, Glucagon, and Somatostatin Release in Healthy Humans and in Patients With NIDDM

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Effects of a low-carbohydrate high-fat meal (LCM) versus a high-carbohydrate low-fat meal (HCM) on insulin, glucagon, and somatostatin release in nonobese healthy volunteers and in subjects with mild non-insulin-dependent diabetes mellitus (NIDDM) were compared. The meals were isocaloric. In the LCM, 26% of energy was supplied by carbohydrate, 51% by fat, and 23% by protein, whereas corresponding figures for HCM were 62, 22, and 16%. Hormonal responses were expressed as incremental areas over baseline. In healthy volunteers the HCM had a significantly greater effect on the insulin response than did the LCM. In contrast, in the diabetic group the insulin response to HCM was markedly impaired and was of the same magnitude as that to LCM. Glucagon release was significantly augmented after LCM in the nondiabetic as well as in the diabetic group, despite a pronounced and concomitant hyperglycemia in the latter. Moreover, HCM tended to stimulate glucagon release but only in the diabetic subjects. LCM and HCM induced a significant and sustained elevation of somatostatin levels in both groups; these responses were not significantly influenced by glucose tolerance or composition of meal. In conclusion, the present study suggests that at least two islet dysfunctions—decreased insulin response and enhanced glucagon release—characterize mild NIDDM. With a high-carbohydrate meal, a severe impairment of insulin secretion and a slight pardoxical glucagon release are observed. With a low-carbohydrate fat-rich meal, β-cell responsiveness seems to be intact, but α-cell secretion is enhanced.
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In healthy men and animals, glucose stimulates insulin and somatostatin[1-3] and suppresses glucagon secretion.[4] Unlike glucose, amino acids enhance the release of all three hormones.[5] Glucose and amino acids exhibit a synergistic effect on insulin[6] and possibly an additive one on somatostatin secretion,[7] whereas amino acid–stimulated glucagon release is decreased by glucose.[4] In patients with non-insulin-dependent diabetes mellitus (NIDDM), insulin and somatostatin response to glucose are decreased compared with normals,[2] whereas amino acid–induced glucagon secretion is exaggerated.[4]

The response of islet hormones to a standardized mixed meal (~45% of energy supplied by carbohydrate, 35% by fat, and 20% by protein) is in accordance with the above findings. Thus, in healthy subjects a standardized mixed meal augments insulin, glucagon, and somatostatin secretion.[4,8] In patients with NIDDM, insulin response is decreased, and the glucagon secretion is inappropriately elevated. In these patients, in contrast to the response to glucose, somatostatin response to a mixed meal seems to be intact.[8]

The impact of variation in the meal composition on the secretory resonse of the endocrine pancreas has not been fully evaluated. In this study we have compared effects of a high-carbohydrate low-fat meal (HCM) versus low-carbohydrate high-fat meal (LCM) on the response of insulin, glucagon, and somatostatin in healthy subjects and nonobese patients with mild NIDDM.

MATERIALS AND METHODS

The study comprised six healthy volunteers and six subjects with mild NIDDM according to WHO criteria.[9] The weight of the six healthy volunteers (according to the Metropolitan Life Insurance Tables, 1983) was 100 ± 3.4% and that of the NIDDM group was 102 ± 6.6% of normal weight. The ages of the two groups were 39.8 ± 2.3 and 55.2 ± 2.3

yr, respectively. The studies were performed early in the morning after an overnight fast with subjects resting in supine position. Each subject received two standard breakfasts (300 kcal each) in random order. The meals were eaten seated. A period of 3–14 days elapsed between consecutive experiments.

In the low-carbohydrate meal, 26% of energy was supplied by carbohydrate, 51% by fat, and 23% by protein (200 ml whole milk, one slice of crisp bread, 5 g butter, 20 g ham, 20 g cheese). In the high-carbohydrate meal, 62, 22, and 16% of energy came from carbohydrate, fat, and protein, respectively (150 ml low-fat milk, 20 g white bread, 25 g dark bread, 5 g butter, 15 g ham, 22 g jam). The nutritional content of each meal was calculated according to Tables of the National Food Administration (Statens Livsmedelsverk, Stockholm, Sweden, 1974). The fiber content of both types of breakfast was low. Breakfast was ingested in 5 min, and venous blood samples were obtained 20 min before the meal, at the beginning of the meal (time zero) and at 20-min intervals for 120 min thereafter.

Assays. Blood samples were collected in 5-ml plastic tubes (Vacutainer, Becton Dickinson Vacutainer System Europe, Meylan, France) containing EDTA (0.048 ml, 0.34 M). Aprotinin (1000 IU) was added to the tubes used for measuring glucagon and somatostatin (Bayer, Leverkusen, West Germany). Plasma was separated by centrifugation at +4°C and was stored at −22°C. Blood glucose was measured by a glucose oxidase method.[10] Insulin was determined by radioimmunoassay (RIA) as previously described.[11] C peptide was determined by RIA with commercially available kits (Novo, Bagsvaerd, Denmark). Glucagon RIA was based on the method of Faloona and Unger[12] using antibody 30 K. Somatostatin was extracted and concentrated from 2 ml plasma as previously described.[1,2] Briefly, somatostatin was extracted from plasma using Vycor glass (Mesh 140, Code 7930, Corning Glass International, Corning, New York), which was activated by heating to 600°C for 1 h. Fifty milligrams of Vycor glass was added to 2 ml plasma and mixed by rotation for 30 min. After centrifugation, the supernatant was removed, and

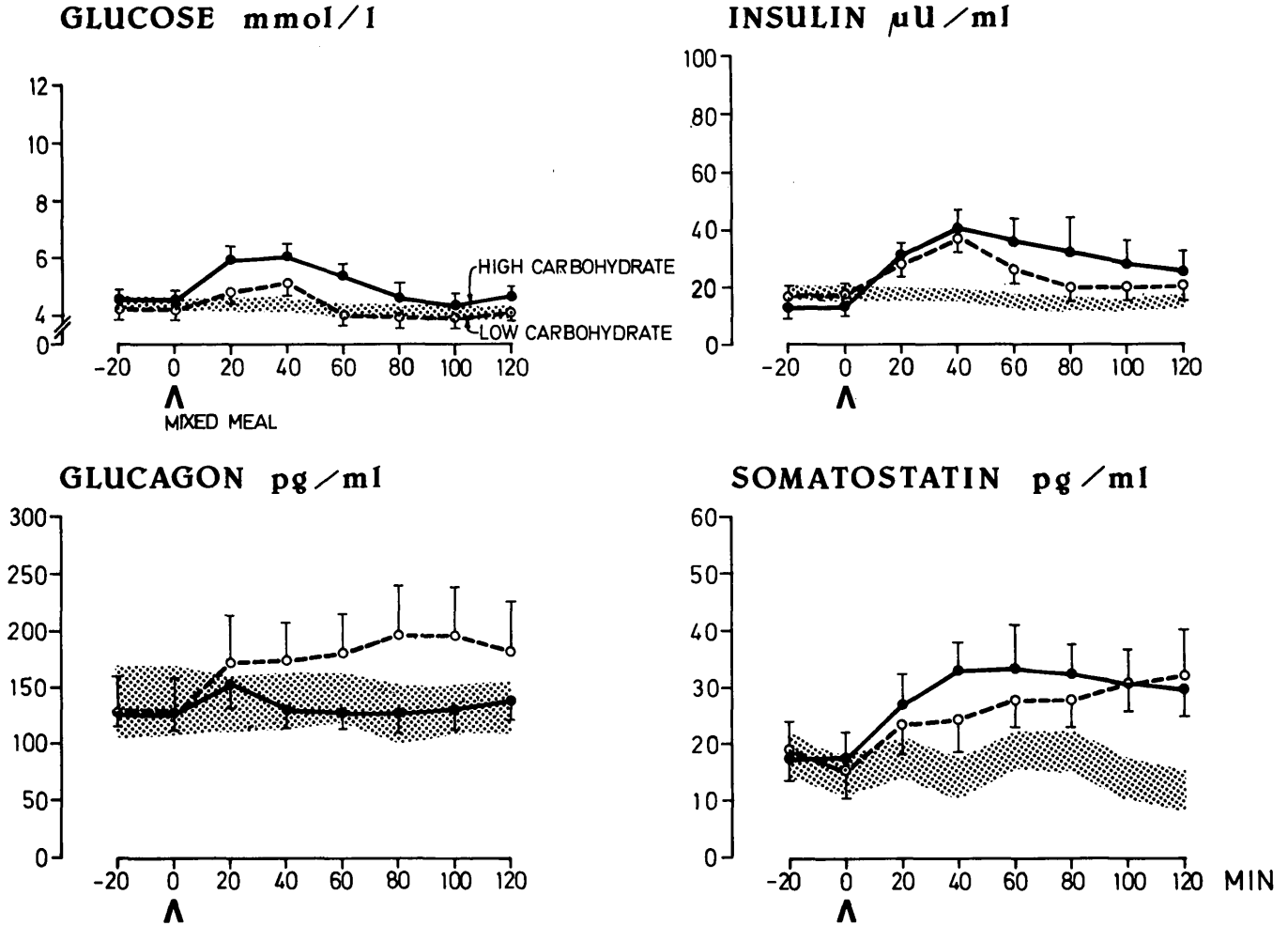

FIG. 1. Glucose and hormonal pattern after high- and low-carbohydrate meal in healthy subjects (N = 6). Shaded area *shows control experiments performed on another group of 6 healthy subjects receiving saline only. Results are expressed as mean* ± SEM.

the glass was washed with 3 ml H_2O followed by 2 ml HCl. After removal of the HCl the somatostatin was eluted from the glass with 1 ml 80% methanol, and after evaporation the residue was dissolved in 250 μl assay buffer. The aliquots of 100 μl were assayed in duplicate as previously described. A standard amount of somatostatin-14 (200 pg in 2 ml plasma) was extracted in parallel to the samples and served, after appropriate dilutions, as the standards in the assay. This procedure corrected for losses of somatostatin during the extraction procedure, which ranged between 50 and 65% for known added amounts (6–160 pg).

Tyrosine-1-somatostatin (a gift from Dr. A. Arimura, Tulane University, New Orleans) was labeled with ^{125}I and used as tracer. The antiserum used against somatostatin-14 (R1 41E) was raised in our laboratory. Its specificity was validated previously.[13] The antiserum was used in a final dilution of 1:56,000. The limit of detection of somatostatin-14 was 0.5 pg per assay tube. The interassay coefficient of variation was 13.5% (N = 8), and the intra-assay coefficient of variation was 6% (N = 12).

The method of extraction of somatostatin-like immunoreactivity using Vycor glass was compared with a method of acid ethanol extraction.[14] Mean basal plasma levels in nine normal subjects were 19.9 ± 1.8 (SEM) for the Vycor glass method and 16.5 ± 2.3 for the ethanol extraction method ($P > .05$).

Statistics. Results are expressed as mean ± standard error of the mean (SEM). Significance was tested using the Student's t test (two tailed) for paired and unpaired samples.

RESULTS

Healthy subjects (*Figure 1, Table 1*). The ingestion of both types of mixed meal was followed by hyperglycemia and by increased release of insulin and C peptide. However, the hyperglycemic effect of the LCM was transient (0–60 min area 41.7 ± 9.3 mmol/L, $P < .05$ and 60–120 min area 9.0 ± 18.2, NS). As expected, the HCM stimulated insulin and C-peptide release to a greater extent ($P < .025$) than did the LCM.

Only the LCM significantly stimulated glucagon secretion; the incremental areas were 7315 ± 2266 pg · ml^{-1} · 120 min^{-1} ($P < .02$) for the LCM but only 298 ± 269 pg · ml^{-1} · 120 min^{-1} ($P < .1$) for the HCM.

Basal somatostatin levels varied from 1.9 to 36 μg/ml. The two test meals augmented somatostatin levels to approximately the same extent.

Diabetic subjects (*Figure 2, Table 1*). Test meals increased blood glucose, insulin, and C-peptide levels. Hyperglycemia was considerably more pronounced with HCM than with LCM (incremental area 257.3 ± 48.4 vs. 70.0 ± 19.5 mmol · L^{-1} · 120 min^{-1}, $P < .005$), whereas C-peptide response was only moderately larger in HCM experiments ($P < .02$). Moreover, insulin responses were similar with both types of meals.

The LCM markedly augmented glucagon release (5443 ± 705 pg · ml^{-1} · 120 min^{-1}, $P < .001$) in presence of significant hyperglycemia (70.0 ± 19.5 mmol · L^{-1} · 120 min^{-1}, $P < .02$). The HCM also tended to stimulate glucagon re-

TABLE 1
Effects of low and high carbohydrate meal (LCM and HCM) on levels of glucose, insulin, C peptide, glucagon, and somatostatin (mean ± SEM)

	Healthy subjects (N = 6)			NIDDM (N = 6)		
	LCM	HCM	P values*	LCM	HCM	P values*
Blood glucose						
Basal† (mmol/L)	4.3 ± 0.2	4.5 ± 0.1		6.7 ± 0.6	6.2 ± 0.6	
Stimulated‡ (mmol · L^{-1} · 120 min^{-1})	9.0 ± 18.2	84.3 ± 22.2	<.005	70.0 ± 19.5	257.3 ± 48.4	<.005
Insulin						
Basal (μU/ml)	16.7 ± 1.7	13.2 ± 2.2		15.8 ± 2.0	14.7 ± 2.2	
Stimulated (μU · ml^{-1} · 120 min^{-1})	943.3 ± 373.9	2108.0 ± 631.2	<.025	1813.0 ± 479.1	2277.0 ± 635.7	NS
C peptide						
Basal (pmol/ml)	0.55 ± 0.09	0.56 ± 0.07		0.72 ± 0.13	0.62 ± 0.18	
Stimulated (pmol · ml^{-1} · 120 min^{-1})	42.5 ± 10.6	94.9 ± 21.4	<.02	70.0 ± 15.7	98.8 ± 23.9	<.02
Glucagon						
Basal (pg/ml)	134.5 ± 28.7	134.8 ± 16.0		123.7 ± 17.9	120.5 ± 15.6	
Stimulated (pg · ml^{-1} · 120 min^{-1})	7315 ± 2266	298 ± 269	<.02	5443 ± 705	1185 ± 451	<.005
Somatostatin						
Basal (pg/ml)	15.4 ± 5.4	17.1 ± 4.1		21.1 ± 4.4	23.0 ± 8.4	
Stimulated (pg · ml^{-1} · 120 min^{-1})	1129.1 ± 257.9	15.32 ± 319.8	NS	1923.0 ± 556.1	2005.3 ± 495.7	NS

NS, not significant.
*Comparison LCM vs. HCM.
†Basal levels are means of two determinations (−20 and 0 min).
‡Glucose and hormonal responses are calculated as incremental areas during 0–120 min.

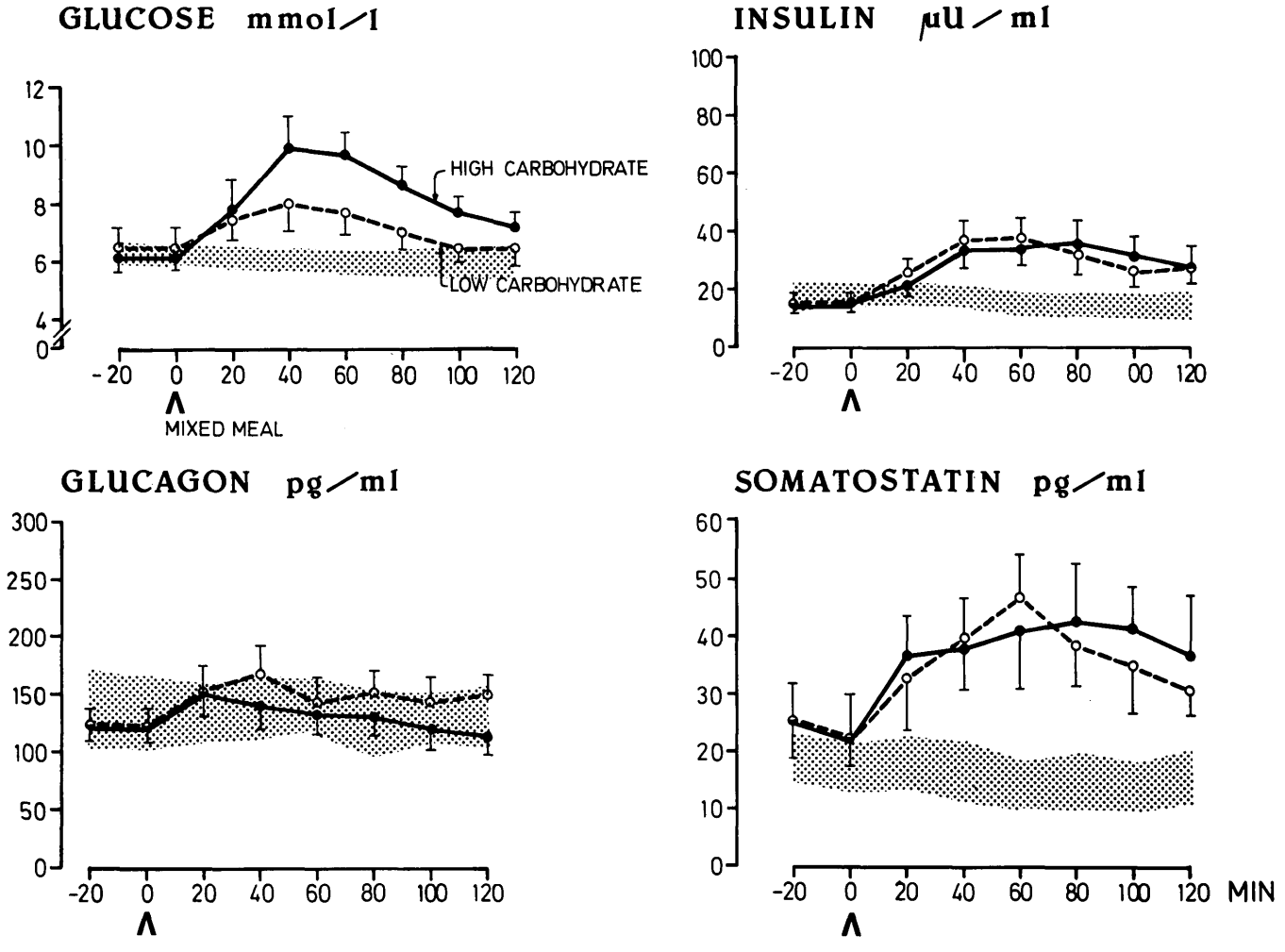

FIG. 2. Glucose and hormonal pattern after high- and low-carbohydrate meal in patients with mild NIDDM (N = 6). Shaded area *shows control experiments performed on another group of 6 NIDDM patients with similar degree of hyperglycemia. Results are expressed as mean ± SEM.*

lease (1185 ± 451 pg · ml^{-1} · 120 min^{-1}, .1 > *P* > .05), although pronounced hyperglycemia occurred (257.3 ± 48.4 mmol · L^{-1} · 120 min^{-1}, *P* < .001).

Basal somatostatin concentration varied from 7.5 to 48 µg/L. The increase in somatostatin levels was similar after the two test meals used.

DISCUSSION

We confirm that insulin and C-peptide release after HCM is impaired in nonobese subjects with mild NIDDM.[4] Thus, with HCM, peripheral insulin and C-peptide levels increased to the same extent in controls and diabetic subjects, although hyperglycemia was much more pronounced in the latter group (84.3 ± 22.2 vs. 257.3 ± 48.4 mmol · L^{-1} · 120 min^{-1}). In contrast, with the LCM the hyperglycemia in the diabetic subjects was accompanied by a more pronounced insulin and C-peptide response compared with controls (1813.0 ± 479.1 vs. 943.8 ± 373.9 µU insulin · ml^{-1} · 120 min^{-1} and 70.0 ± 15.7 vs. 42.5 ± 10.6 pmol C peptide · ml^{-1} · 120 min^{-1}).

This study does not precisely answer why the insulinogenic effect of the HCM but not of the LCM is severely impaired in NIDDM. We believe that this is mainly due to the difference in the content of carbohydrate and fat in the meals. The protein content of the meals was similar (23 and 16% of energy in LCM and HCM, respectively) and is probably of minor importance in this respect. One can speculate that in patients with NIDDM, the increased amount of fat in the meal normalizes β-cell responsiveness by enhancing the effect of carbohydrates on the release of gastric inhibitory polypeptide (GIP), which in turn augments insulin response.[15]

In the diabetic subjects, HCM compared with LCM had a somewhat stronger effect on C-peptide release. The difference between the meals was not significant when insulin

responses were evaluated. This discrepancy is probably because insulin responses showed more pronounced variation than the corresponding C-peptide responses.

Because it appears that the β-cell responsiveness is normal in diabetic subjects challenged by LCM, other factors must be responsible for augmented hyperglycemia after such a meal. Probably the most important role is played by insulin resistance. We have not evaluated insulin sensitivity in the subjects participating in this study. However, we have previously shown that even normal-weight patients with the same degree of hyperglycemia as the diabetic subjects investigated in this study exhibit considerable insulin resistance.[16] A second factor that may be of importance is the significant glucagon release seen in NIDDM patients after LCM, despite concomitant hyperglycemia. The importance of inappropriate secretion of glucagon is supported by recent studies suggesting that even a marginal increase in glucagon levels is sufficient to provoke sustained hyperglycemia and enhanced glucose production in moderately insulin-deficient humans.[17]

In the diabetic group, even the HCM tended to stimulate glucagon release despite marked hyperglycemia. The hyperresponsiveness of the α-cells to a test meal in patients with NIDDM and pronounced hyperglycemia (fasting blood glucose >10 mmol/L) has been previously documented.[18] Because our patients had only mild hyperglycemia, it is evident that the abnormality of α-cell secretion is also an early and perhaps specific feature of NIDDM. Such an idea is further supported by the finding that when obese subjects with NIDDM were made normoglycemic by overnight administration of insulin, the glucagon response to a protein meal was the same as when they were hyperglycemic, whereas in subjects with IDDM, overnight insulin restored the response to normal.[19]

There is ample evidence that a mixed meal stimulates somatostatin release in normal humans.[1,8,15,20,21] The present study confirms and extends these observations by demonstrating that in healthy subjects the meals containing 26 or 62% carbohydrates increased peripheral somatostatin concentrations to the same extent. This finding is somewhat unexpected in view of the observations of Penman et al.[22] They compared effects of isocaloric (520 kcal) and isovolumetric quantities of carbohydrate, protein, and fat on somatostatin secretion in normal humans and concluded that fat and protein are more potent stimuli than are carbohydrates for somatostatin release. The reason for these differences is unclear.

In subjects with NIDDM, a mixed meal stimulated somatostatin secretion, as described by Conlon et al.,[8] whereas in another study no effect was seen.[20] The carbohydrate content of these meals was 35 and 48%, respectively. We found that in the diabetic subjects, mixed meals induced significant and sustained somatostatin secretion, regardless of low- (26%) or high- (62%) carbohydrate content. In search of an explanation for this discrepancy it may be relevant that in Conlon's study,[8] as well as in our study, somatostatin was measured in extracted plasma, whereas Vinik et al.[20] used unextracted plasma. Measurements in unextracted plasma include determination of macroglobulins,[8] which is primarily an artifact. The fasting plasma concentrations of somatostatin-like immunoreactivity reported here are comparable with those reported by other investigators using the same[1,22] or other[8,14,23–26] extraction procedures but are severalfold lower than those reported for unextracted plasma.[20,27]

In the present study we followed glucose and hormonal levels 2 h after the meal. Because at the end of the experiment glucose returned to prestimulatory levels with the LCM and were still somewhat increased with the HCM, it is possible that the insulin and somatostatin responses would be more pronounced with the HCM if the complete secretory responses were measured. However, the pattern of insulin and somatostatin responses (onset and peak) was almost identical with the two meals, which implies that our observation period is most likely representative.

In conclusion, the present study demonstrates decreased insulin release and enhanced glucagon release after a mixed meal in subjects with mild NIDDM. The magnitude of these two dysfunctions depends on the composition of the meal. With a high-carbohydrate low-fat meal a severe impairment of insulin secretion is predominantly observed. With a low-carbohydrate high fat meal, β-cell function seems to be intact, whereas α-cell responsiveness is markedly enhanced. In subjects with NIDDM, somatostatin response was of the same magnitude with both meals used.

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