EFFECT OF ORAL AMINO ACID ON COUNTERREGULATORY RESPONSES AND
COGNITIVE FUNCTION DURING INSULIN-INDUCED HYPOGLYCEMIA IN NON-
DIABETIC AND TYPE 1 DIABETIC PEOPLE

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ABSTRACT

Objective. Amino acids (AA) stimulate glucagon responses to hypoglycaemia, and may be utilized by the brain. Aim of study was to assess the responses to hypoglycemia in nondiabetic and type 1 diabetic subjects after ingestion of AA mixture.

Research Design and Methods. 10 nondiabetic and 10 diabetic type 1 subjects were studied on three different occasions during intravenous insulin (2 mU/kg/min) + variable glucose for 160 minutes. In two studies, clamped hypoglycemia (plasma glucose 47 mg/dl for 40 min) was induced and either oral placebo (P) or AA mixture (42g) was given at +30 min. In the third study AA were given, but euglycemia was maintained.

Results. Plasma glucose and insulin were no different in the hypoglycemia studies both with P and AA (p>0.2). After AA, plasma AA concentration increased to levels observed after mixed meal (2.4±0.13 vs P study 1.7±0.1 mmol/l, p=0.02). During clamped euglycemia, AA resulted in transient increase in glucagon concentrations, which returned to basal by the end of study. During clamped hypoglycemia, glucagon response was sustained and increased more in AA studies vs P in nondiabetics and diabetics (p<0.05), but other counterregulatory hormones and total symptom score were not different. Beta-OH-butyrate was less suppressed after AA (200±15 vs 93±9 μmol/L, p=0.01). Among the cognitive tests administered, the following indicated less deterioration after AA than P: Trail-making B, PASAT (2 sec), Digit span forward, Stroop colored words and verbal memory tests (nondiabetics), and Trail-Making B, Digit span backwards and Stroop color tests (diabetics).

Conclusions. Oral amino acids improve cognitive function in response to hypoglycemia, and enhance the response of glucagon in nondiabetic and in diabetic subjects.
In Type 1 diabetes mellitus (T1DM) there is not only failure of the pancreatic β-cell, but also severe impairment of the α-cell in terms of response to hypoglycemia (1,2). Indeed, adequate glucagon secretory response to falling plasma glucose concentration which, along with adrenaline response, is normally the primary line of defense against hypoglycemia (3), is lost within a few months of diabetes onset (2). This process appears unavoidable, irreversible and unrecoverable in the natural history of the disease (4) contributing to impaired glucose counterregulation and risk of severe hypoglycemia (5). As a result, in T1DM, the therapeutic goal of near-normoglycemia with intensive insulin treatment may carry the burden of greater frequency of hypoglycemia as indicated by the DCCT (6).

Several attempts have been made to reduce the risk of hypoglycemia in intensively treated T1DM. The efforts have been substantially focused in two directions. The first has been to define more rational and physiological strategies of insulin replacement (7-9). The second has been to explore the possibility of inducing a recovery of glucagon response to hypoglycemia. Indeed, the finding that a number of amino acids may stimulate glucagon release from pancreatic α-cells (10) has prompted a series of studies to test the hypothesis that amino-acid stimulation may sustain glucagon secretion in response to hypoglycemia in T1DM (11-13). The majority of studies have been conducted by giving amino acids intravenously as a mixture (11), or as dipeptide (alanine and glutamine) (12) or as a single aminoacid (alanine) (13), with conflicting results. Our group has recently shown that i.v. alanine is able to partially restore glucagon response to insulin-induced hypoglycemia (13). Surprisingly, to the best of our knowledge, no study so far has examined whether amino-acids given orally, may stimulate glucagon response during insulin-induced hypoglycemia in T1DM. This hypothesis might be interesting, as previous studies have shown that the failure of glucagon response to hypoglycemia in T1DM recovers after food intake (14,15). This has led to the hypothesis that it is the protein component of the meal which induces such an effect (15). In fact oral alanine may improve glucose recovery from hypoglycemia, mediated by a sustained increase in plasma glucagon in T1DM (16,17). In addition to the potential effect on recovery of glucagon responses, amino acids might also serve as a substrate alternative to glucose for the brain and therefore limit cognitive dysfunction during hypoglycemia (18 as do lactate (19) and β-hydroxybutyrate (20).

The aim of the present study was to examine the effects of oral administration of a mixture of amino acids on hormonal counterregulatory responses, particularly glucagon, as well as responses of symptoms and cognitive function during insulin-induced hypoglycemia in people with T1DM compared to non-diabetic subjects.

RESEARCH DESIGN AND METHODS

Subjects. Institutional Review Board approval was obtained for these studies. Ten healthy nondiabetic volunteers (five men, age 32±7 years, BMI 23±2 kg/m², C-peptide 1.2±0.2 nmol/l, HbA1c 4.6±0.3% - Mean±SD) were studied along with 10 subjects with type 1 diabetes on long-term intensive insulin treatment (six men, age 30±8 years, diabetes duration 17±7.8 years, BMI 22.7±1.8 kg/m², HbA1c 7.4±1.0% - Mean±SD). At the time of the study, all type 1 diabetic subjects were free of any detectable microangiopathic complication and were negative at the screening for autonomic neuropathy, as judged on the basis of a standard battery of cardiovascular tests (21).

Design of studies. The study was carried out according to the Helsinki declaration after obtaining written informed consent by all subjects. All non-diabetic and diabetic volunteers were studied on three different occasions in random, computer-generated sequence, at 2-3 week intervals, with the modified hyperinsulinemic glucose clamp technique used to either maintain euglycemia, or induce hypoglycemia. In diabetic subjects, care was taken to avoid preprandial, postprandial and nocturnal blood glucose <72 mg/dl over the week prior to studies as previously reported (22). Briefly, the glycaemic targets were blood glucose 110–130 mg/dl in the fasting state, before meals and at
bedtime, and blood glucose 140–180 mg/dl 2 h after meals. Blood glucose was measured in the fasting state, before meals and two hours after meals, and three times a week at 03:00 am. In addition, blood glucose was measured whenever subjects believed their sugar was low. Patients were advised to decrease or increase the dose of basal insulin if fasting blood glucose was repeatedly below 110 mg/dl or above 130 mg/dl, and to decrease or increase the dose of rapid-acting insulin at meals if the 2-h post-prandial blood glucose was repeatedly below 140 mg/dl or above 180 mg/dl. Adjustments of rapid-acting insulin dose were made according to the carbohydrate content of meals. Four subjects had six values of blood glucose between 55 and 72 mg/dl in the week prior to the study which caused the postponing of the study to the next week. Finally, in order to avoid hypoglycemia the day before the experiment, the total daily insulin dosage was cut by 20% and patients were asked to contact one investigator by phone to receive advice on insulin doses.

On the morning of the studies, all non-diabetic and diabetic subjects were admitted to the General Clinical Research Center of Department of Internal Medicine at ~07:00 h. A hand vein of the non-dominant arm was cannulated retrogradely and maintained in a hot box (~60° C) for sampling of arterialized-venous blood (23). A superficial vein of the ipsilateral arm was also cannulated for infusion of insulin and glucose (see below). The two veins were maintained patent by means of 0.9% NaCl infusion (0.5 ml/min). In the diabetic subjects, an i.v. infusion of human regular insulin (diluted to 1 U/ml in 2 ml of the subject's blood and 0.9% NaCl to a final volume of 100 ml) was begun at ~07:30 h in a feedback fashion, using a syringe pump (Harvard Apparatus, Ealing, South Natick, Mass., USA), according to an algorithm described previously (24), in order to reach the target plasma glucose of 100 mg/dl by 08:30 h.

In all studies at time 09:00 h, (time 0 min), both in non-diabetic and diabetic subjects intravenous insulin at the rate of 2 mU.Kg⁻¹.min⁻¹ was started and continued until the end of the study (i.e. 160 min). Intravenous glucose at variable rate was also infused to maintain euglycemia (plasma glucose at 90 mg/dl) throughout the study on one occasion (clamped euglycemic study), whereas on the other two occasions the rate of glucose infusion was decreased after 30 min to allow plasma glucose to fall and reach the target plasma glucose of 47 mg/dl at 120 min. This hypoglycemic plateau was maintained for the next 40 min, that is till the end of the study (clamped hypoglycemic studies).

On each occasion, at 30 min subjects ingested over a five minute period a 200 ml drink containing either a mixture of aminoacids (Lysine 3.1g, Histidine 1.7g, Arginine 3.1g, Aspartate 2.8g, Threonine 2.3g, Serine 2g, Glutamate 1g, Proline 3.3g, Glycine 2.7g, Alanine 1.7g, Cysteine 1.1g, Valine 3g, Methionine 0.7g, Isoleucine 2.7g, Leucine 4.6g, Tyrosine 4.1g, Phenylalanine 0.5g, Tryptophan 0.9g, Glutamine 2.1g,) or a seemingly identical placebo rendered palatable by flavouring it with artificial fruit flavour and adding sucrose (2g) and aspartame (1g). All drinks were prepared and administered by a research nurse not involved in the further execution of the study. The mixture of aminoacids was given both in the clamped euglycemia study (Eu-AA study), and in one of the two clamped hypoglycemia studies (Hypo-AA study), whereas in the other clamped hypoglycemia study subjects ingested placebo (Hypo-P study).

In all studies blood samples were drawn at 5-10 min intervals for bedside plasma glucose measurement and at 30-min intervals for measurement of plasma insulin, C-peptide, pancreatic polypeptide, amino-acids, counterregulatory hormone and non-glucose substrate concentration (see below).

A semiquantitative symptom questionnaire (25) was administered every 30 min. Subjects were asked to score from 0 (none) to 5 (severe) on each of the following symptoms: seven autonomic/neurogenic (adrenergic: heart pounding, tremor, anxiety and irritability; cholinergic: sweating, hunger, and tingling); five neuroglycopenic (difficulty in thinking, weakness, dizziness, blurred vision, drowsiness) and three non-specific (thirst, nausea and headache) (26). The sum of
each of these constituted the total symptom score.

In addition, at baseline, before inducing hypoglycemia and at the hypoglycemic plateau, (indicated as ‘time -30’, ‘time 0’, time 120’) and at similar times during the euglycemic studies, cognitive function was assessed by applying a battery of hypoglycemia-sensitive tests: Trail Making A and B tests (27), Verbal Fluency (28), Verbal Memory test (28), Digit Vigilance test (28), Forward and Backward Digit Span (29), Stroop word, color and color-word (interference) subtests (30), Paced Auditory Serial Addition Test (PASAT 2 and 3 sec) (31), with tests always being performed in this order.

Analytical methods

Plasma glucose was measured by means of a Beckman glucose analyzer (Glucose Analyzer II, Beckman Instruments, Fullerton, CA). Plasma insulin, C-peptide, pancreatic polypeptide, counterregulatory hormone, glucagon, adrenaline, noradrenaline, glycerol, β-OH-butyrate, lactate and alanine were measured by previously described assays (32). Plasma concentrations of amino acids were measured by high performance liquid chromatography with post-column o-phthalaldehyde derivatization (33). To remove antibody-bound insulin, plasma was mixed with an equal volume of 30% polyethylene glycol immediately after blood collection both in T1 DM and non-diabetic subjects (34). HbA1c was determined by a high performance liquid chromatography using a Hi-AUTO A1C, TM HA 8121 apparatus (DIC, Kyoto Daiichi, Kogaku Co., Ltd., Japan). Plasma free fatty acid (FFA) concentration was measured using a commercial kit (Wako NEFA C test kit, Wako Chemicals GmbH, Neuss, Germany).

Statistical analysis

All data were subjected to repeated measures analysis of variance (ANOVA) with Huynh-Feldt adjustment for nonsphericity (35). The ANOVA model included the sequence of studies as the between-subjects factor, whereas test condition (HYPO+P / HYPO+AA / EU+AA) and time were the within-subjects factors. Subjects were entered in the model as random factors. The factor group (nondiabetic / diabetic subjects) was entered in the model as between-subjects factor. If there were significant differences between baseline values, these were used as covariates. In this way, the data over the serial time points could be adjusted for any differences in baseline values (35). Post-hoc comparisons (Newman-Keuls test) were carried out to pinpoint specific differences on significant interaction terms.

The areas under the curve (AUC) of counterregulatory hormones and substrates at the clamped hypoglycemia period (120-160 min) were calculated according to the trapezoidal rule. The incremental area under the curve (iAUC) for glucagon response was calculated by subtracting plasma glucagon values before amino acids ingestion from the total AUC.

A modified Bonferroni procedure (36) for multiple cognitive test adjustments was used in order to maintain an overall type 1 error rate of 5% (alpha=0.05).

Data are given as means±SE, except where SD is specified. We considered differences to be statistically significant if the P value was 0.05 or less. We conducted the statistical analyses by using NCSS 2007 software (Kaysville, UT, USA) and Statistica software, version 6.0 (StatSoft, Tulsa, OK, USA).

RESULTS

Plasma glucose and insulin concentrations, and rates of glucose infusion (Figure 1)

In all study conditions, plasma glucose was maintained at the pre-selected plateaus, with no differences between groups and study conditions (p>0.2).

Plasma insulin concentrations achieved during the study did not differ either between nondiabetic and diabetic subjects, or in relation to treatment conditions. On average, baseline plasma insulin concentrations were lower in nondiabetic as compared to diabetic (p<0.001) in all study conditions.

The rates of glucose infusion were lower during hypoglycemia plateau in the HYPO-AA study as compared to HYPO-P and EU-AA studies in both nondiabetic subjects (2.6±0.4 vs 5.5±0.6 and 8.8±0.9 mg·kg⁻¹·min⁻¹).
Plasma glucagon, C-peptide and pancreatic polypeptide concentrations (Figure 2, Table I)

Plasma glucagon concentrations were similar at baseline in nondiabetic and diabetic subjects in all study conditions. In the HYPO-P study, plasma glucagon concentrations after an initial decrease at 60 min, increased (p=0.001) in nondiabetic subjects, whereas it did not increase in diabetic subjects (p=0.867). The ingestion of amino acids in the HYPO-AA study increased significantly the response of glucagon to hypoglycemia in nondiabetic and, to a lower extent, in diabetic subjects as compared to the HYPO-P study (Table I). Amino acids stimulated glucagon response also in the EU+AA study, which was maximal between 75-120 min and then tended to decrease to baseline values at 120-160 min. In diabetic subjects, the response of glucagon to hypoglycemia stimulated by amino acids was lower than that of nondiabetics. However, it was superimposable on that of nondiabetic subjects in the HYPO+P study (p=0.581, Table I). Interestingly, although the iAUC of glucagon response in the HYPO+AA study was 4.7 times greater in nondiabetic as compared to diabetic subjects (7468±835 and 1599±142 ng/l, respectively, p<0.001), the estimated contribution of amino acids per se in directly stimulating glucagon secretion, as derived from the EU+AA study, was not statistically different both in nondiabetic and diabetic subjects (19±4 and 35±7 %, respectively, p=0.061).

Plasma C-peptide concentrations in non-diabetic subjects decreased at 30 min in all studies; then they remained suppressed in the HYPO-P study, whereas they increased following the ingestion of amino acids more in the EU-AA than in HYPO-AA study (p<0.05, figure 2). By the end of study, however, C-peptide concentrations returned to basal values and were suppressed in the EU-AA and HYPO-AA study, respectively. Plasma C-peptide concentrations were undetectable in diabetic subjects in all studies.

Plasma pancreatic polypeptide (PP) concentration increased during HYPO+P and HYPO+AA both in nondiabetic and diabetic subjects, although the peak response was higher in HYPO+AA in nondiabetics but not in diabetics (Table I). PP levels did not change in EU+AA studies.

Plasma adrenaline, noradrenaline, cortisol and GH concentrations (Figure 3, Table I)

Plasma adrenaline levels increased in HYPO-P and HYPO-AA both in nondiabetic and diabetic subjects. However, responses were lower in diabetic as compared to nondiabetic subjects (Table I, p<0.05). In EU-AA study adrenaline levels did not change as compared to basal values in both groups (Table I).

Plasma noradrenaline concentrations were not different in all study conditions both in nondiabetic and diabetic subjects (Table I, p>0.2).

Responses of plasma cortisol and GH increased similarly in response to HYPO+P and HYPO+AA as compared to EU+AA both in nondiabetic and diabetic subjects (Fig. 3, Table I).

Plasma non-glucose substrate (Figure 4).

Plasma FFA levels decreased similarly in all studies in both nondiabetic and diabetic subjects. However, FFA at 120-160 min were higher in HYPO+P and HYPO+AA as compared to EU+AA (p=0.018) in nondiabetic subjects. They were not different in diabetic subjects (p=0.712).

Plasma glycerol concentrations decreased from basal values in all studies both in nondiabetic and diabetic subjects. However, similarly to FFA, glycerol levels were higher in HYPO+P and HYPO+AA as compared to EU+AA (p=0.018) only in nondiabetic subjects.

Plasma β-OH-butyrate concentrations, after an initial decrease to nadir values at 90 and 60 min in nondiabetic and diabetic subjects, respectively, increased in the HYPO+AA and EU+AA but not in the HYPO+P studies in both nondiabetic (p<0.001) and diabetic subjects (p<0.001).
Plasma lactate concentrations increased in all studies in both nondiabetic and diabetic subjects. In nondiabetic subjects plasma lactate increased more in HYPO+P as compared to both HYPO+AA (p=0.025) and EU+AA (p=0.008). In diabetic subjects lactate was similar in HYPO+P, HYPO+AA and EU+AA (p=0.2). Overall, plasma lactate response in nondiabetic was greater than that of diabetic subjects (nondiabetics vs diabetics: HYPO+P p<0.001, HYPO+AA p=0.005 and EU+AA p=0.046).

**Plasma branched and nonbranched chain amino acid concentrations (Figure 5)**

Branched and non-branched chain aminoacids (BCAA and N-BCAA, respectively) concentrations were similar at baseline in non-diabetic and diabetic subjects in all studies (Fig.5). After the ingestion of amino acids both BCAA and N-BCAA levels increased similarly in HYPO+AA and EU+AA studies. In the HYPO+P studies both BCCA and N-BCCA concentrations tended to decrease from basal values by the end of study with no difference between the two groups (Fig. 5).

**Symptoms (Figure 6)**

The score of autonomic and neuroglycopenic symptoms increased in HYPO+P and HYPO+AA with no difference (p=0.2) between studies both in nondiabetic and diabetic subjects. Whereas it did not change in the EU+AA studies in nondiabetic and diabetic subjects (Fig. 6).

**Cognitive function (Table II)**

With the exception of Trail-Making A, Digit Vigilance and Stroop Words and Colors tests in nondiabetic subjects, and of Trail-Making A, Digit Vigilance tests in diabetic subjects, all cognitive tests deteriorated significantly during hypoglycemia, as compared to EU+AA, both in HYPO+ P and HYPO+AA (Table II). However, the degree of deterioration was lower in HYPO+AA than in HYPO+P in the following tests: Trail-making B, PASAT (2 sec), Digit span forward, Stroop colored words and verbal memory tests in nondiabetic subjects, and Trail-Making B, Digit span backwards and Stroop color tests in diabetic subjects.

**DISCUSSION**

The present study was undertaken to examine the effects of the ingestion of a mixture of amino acids on the counterregulatory, symptomatic and cognitive responses to hypoglycemia. Both nondiabetic and type 1 diabetic subjects were studied. First, the results indicate that oral amino acids enhance glucagon response to hypoglycemia in both nondiabetic and diabetic subjects, although to a lesser extent in the latter. Second, oral amino acids affect the responses of β-cells of pancreatic islets, as shown by the lower suppression of C-peptide. Third, amino acids preserve several aspects of cognitive responses to hypoglycemia which, to the best of our knowledge, is a novel finding.

In type 1 diabetic subjects the glucagon response to hypoglycemia with amino acid administration was nearly similar to the glucagon response to hypoglycemia alone (without amino acid administration) in the nondiabetic subjects. Such result is in line with recent evidence indicating that the sensitivity of pancreatic α-cells to amino acids is markedly reduced in diabetics as compared to nondiabetics, and that a near normal glucagon response is seen when the amino acid alanine is infused in diabetic subjects in hypoglycemia. (13).

Interestingly, the near normal response of glucagon to hypoglycemia after oral amino acids observed in the present study in diabetics is also similar to that described after ingestion of a mixed meal (15). Indeed, it is currently believed that the protein component of the meal is responsible for such effect. As reported previously, the mechanisms of amino acids-induced amplification of the glucagon response to hypoglycemia likely involve both direct and indirect effects (13). In this study, a direct stimulatory effect of oral amino acids on glucagon response was evident at 60-90 min in both the euglycemic clamp and the hypoglycemic clamp before hypoglycemia was established. However, the stimulatory effect of oral amino acids on glucagon release was modulated by plasma glucose concentration. It
was amplified by the induction of hypoglycemia both in nondiabetic and diabetic subjects, but suppressed by euglycemia in both groups of subjects. This indicates that amino acids directly stimulate glucagon secretion from the α-cell (37) and that hypoglycemia amplifies the direct stimulatory effect of amino acids on glucagon release. Furthermore, oral amino acids may also act through different indirect mechanisms which involve gastroenteric peptides such as GLP-1 (38) and a decrease of tonic intraislet α-cell inhibition by insulin and hypoglycemia (39). With regard to the latter, it is possible that the initially greater stimulation of beta cell function by amino acids was followed by a greater decrement in intraislet insulin concentration during hypoglycemia resulting in a greater decrease in tonic intraislet α-cell inhibition by insulin and signaling for increased glucagon secretion during hypoglycemia in normal non-diabetic subjects (39). Most likely, the decrement in intraislet insulin may be an important factor for the increase in glucagon secretion during hypoglycemia (40).

The ingestion of amino acids induced a greater peak response of pancreatic polypeptide compared with placebo in the hypoglycemic clamp studies in nondiabetic but not in diabetic subjects. It is well known that pancreatic polypeptide secretion after ingestion of a mixed meal is mostly mediated by the vagus nerve or by the extravagal cholinergic system (41). In addition, hypoglycemia per se represents a strong stimulus to pancreatic polypeptide secretion (41). Furthermore, autonomic vagal neuropathy blunts pancreatic polypeptide response to hypoglycemia (41). Although the diabetic subjects we studied had no evident signs of autonomic neuropathy, we can not exclude the possibility that the lower response of pancreatic polypeptide concentrations to hypoglycemia in these subjects as compared to nondiabetics might be related to subclinical autonomic neuropathy or to other diabetes-related causes including diabetes duration (41).

Elevation of plasma amino acids concentrations, following ingestion of amino acids, did not affect counterregulatory hormones, with the exception of glucagon, and symptomatic responses to hypoglycemia, whereas they clearly resulted in preservation of some aspects of cognitive function during hypoglycemia. This is a new finding, which points towards a net effect of amino acids in supporting cognition while having no relevant effects on hormonal and symptomatic responses to hypoglycemia. It should be noted that previous studies indicate only a partial or no role of amino acids in sustaining cognition during hypoglycemia. In fact, Evans et al. (18) have shown that alanine infusion can sustain performance of Stroop word and color tests during hypoglycemia, although a contribution of elevated lactate levels to performance in those tests can not be completely excluded (18). In contrast, M’Bemba et al. found no effect of infusion of a dipeptide made of alanine and glutamine on four-choice reaction time test (12). One possible explanation for a specific effect of amino acids on cognitive function is that elevated plasma amino acids do not affect metabolism and neurotransmission of brain centers which physiologically direct counterregulation and symptoms, whereas they may sustain brain areas capable of controlling hypoglycemic-induced deterioration of cognitive function. Therefore, in contrast to the role of other non-glucose substrates such as lactate (19) and ketones (20) amino acids limit their effect only to cognitive function. That would seem advantageous since the reduced counterregulatory hormone release and lower symptoms associated with lactate (19) or ketones (20) use by the brain might weaken defensive responses to hypoglycemia and generate hypoglycemia unawareness, a well known risk factor for severe hypoglycemia (42), even in the face of preserved cognitive function.

Earlier evidence indicates that amino acids can serve as energy sources in the brain during insulin-induced hypoglycemia (43). Therefore, under such circumstances, they can be used by neurons and glia cells as an alternative substrate to glucose in order to limit the detrimental effect of glucopenia on cerebral metabolism and function. However, it has been recently shown that brain amino acids (alanine and leucine) uptake is not sufficient to offset energy deficit due to reduced glucose uptake during hypoglycemia (44) and that increased
availability of amino acids does not result in net brain uptake of amino acids during hypoglycemia (45). Additionally, several amino acids such as tryptophan, tyrosine, phenylalanine, histidine, and arginine are used by the brain for the synthesis of various neurotransmitters and neuromodulators (46) which play a variety of functions in the brain, including a role in the regulation of mood state, fatigue, attention and memory (47). Therefore, on theoretical grounds, our study result of preservation of some aspects of cognitive function during hypoglycemia supplemented with oral amino acids might be due either to a direct use of amino acids by neuronal cells to derive energy and maintain cognitive function or to their conversion to neurotransmitters and/or neuromodulators able to affect cognition. However, whichever is the mechanism, levels of different classes of amino acids in the brain depend largely on their availability in blood, and when individuals are exposed to stressful conditions, such as hypoglycemia, brain requirements for specific amino acids may be particularly critical. In the present study, supplementation of oral amino acids, not only prevented the insulin-induced fall in plasma amino acids observed in the hypoglycemia and placebo studies, it actually increased the overall concentrations of amino acids. Likely, these increased concentrations favored amino acids use by the brain.

The battery of tests we used assessed a broad range of cognitive aspects. Overall, amino acids ingestion sustained cognitive domains pertaining to memory, attention, psychomotor efficiency, information processing and immediate memory in nondiabetics and all of these but immediate memory in diabetics. This inter-groups difference can be most likely attributed to either diabetes or to a general verbal ability and/or general intelligence condition. Notably, these results have been obtained with plasma amino acids concentrations in the physiological range of the postprandial condition in humans (~2-3 mM/l) (15).

Plasma β-OH-butyrate concentrations were suppressed in all studies. However, they were less suppressed following oral amino acids ingestion both in eu- and hypoglycemia. Their use by the brain as an alternative substrate to sustain brain metabolism and function during hypoglycemia can not be completely excluded. However, this is unlikely because first, the levels of β-OH-butyrate in our study were much lower than those that have been shown to be effective in maintaining cognitive function during hypoglycemia in previous studies (20), and second, β-OH-butyrate diminished counterregulatory and symptomatic responses to hypoglycemia in those studies (20), but not in our present study.

In conclusion, the present study indicates that oral amino acids improve some aspects of cognitive function in response to hypoglycemia, and potentiate (in non-diabetic subjects) and recover (in subjects with T1DM) responses of glucagon to hypoglycemia. Additional studies are required to prove that these beneficial effects of oral amino acids during hypoglycemia translate into clinical advantages for people with T1DM and to possibly demonstrate that they contribute to reduction of the risk of severe hypoglycemia in T1DM.

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REFERENCES
1. Gerich J, Langlois M, Noacco C, Karam J, and Forsham P: Lack of glucagon response to hypoglycemia in diabetes: Evidence for an intrinsic pancreatic alpha-cell defect. Science 182:171, 1973
2. Bolli G, de Feo P, Compagnucci P, Cartechini MG, Angeletti G, Santeusanio F, Brunetti P, Gerich JE: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus. Interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. Diabetes 32:134-141, 1983
3. Bolli GB, Fanelli CG: Physiology of glucose counterregulation to hypoglycemia. In: Hypoglycemic Disorders, Service FJ (Ed.), Endocrinology and Metabolism Clinics of North America, W.B. Saunders, Philadelphia, 28: 467-493, 1999
4. Cryer PE: Hypoglycemia: the limiting factor in the management of IDDM. Diabetes 43:1378-1389, 1994
5. White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV: Identification of type 1 diabetic patients at increased risk for hypoglycemia during intensive therapy. N Engl J Med 308:485–491, 1983
6. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977–986, 1993
7. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Di Vincenzo A, Modarelli F, Ciofetta M, Lepore M, Annibale B, Torlone E, Perriello G, De Feo P, Santeusanio F, Brunetti P, Bolli GB: Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycaemia, following institution of rational, intensive insulin therapy in IDDM. Diabetologia 37:1265-1276, 1994
8. Bolli GB: How to ameliorate the problem of hypoglycemia in intensive as well as non-intensive treatment of Type 1 diabetes mellitus. Diabetes Care 22 (Suppl.2):B43-B52, 1999
9. Rossetti P, Porcellati F, Bolli GB, Fanelli CG: Prevention of hypoglycemia while achieving good glycemic control in type 1 diabetes mellitus: the role of insulin analogs. Diabetes Care 31 Suppl 2:S113-120, 2008
10. Kuhara T, Ikeda S, Ohneda A, and Sasaki Y: Effects of intravenous infusion of 17 amino acids on the secretion of Gh, glucagon and insulin in sheep. Am J Physiol 260:E21-E26, 1991
11. Caprio S, Tamborlane WV, Zych K, Gerow K, and Sherwin RS: Loss of potentiating effect of hypoglycemia on the glucagon response to hyperaminoacidemia in IDDM. Diabetes 42:550-555, 1993
12. M'bemba J, Cynober L, de Bandt P, Taverna M, Chevalier A, Bardin C, Slama G, Selam JL: Effects of dipeptide administration on hypoglycaemic counterregulation in type 1 diabetes. Diabetes Metab 29:412-417, 2003
13. Porcellati F, Pampanelli S, Rossetti P, Busciantella Ricci N, Marzotti S, Lucidi P, Santeusanio F, Bolli GB, Fanelli CG: Effect of the amino acid alanine on glucagon secretion in non-diabetic and type 1 diabetic subjects during hyperinsulinaemic euglycaemia, hypoglycaemia and post-hypoglycaemic hyperglycaemia. Diabetologia 50:422-430, 2007
14. Fanelli CG, Pampanelli S, Porcellati F, Bartocci L, Scionti L, Rossetti P, Bolli GB: Rate of fall of blood glucose and physiological responses of counterregulatory hormones, clinical symptoms and cognitive function to hypoglycaemia in Type I diabetes mellitus in the postprandial state. *Diabetologia* 46: 53–64, 2003.

15. Porcellati F, Pampanelli S, Rossetti P, Cordoni C, Marzotti S, Scionti L, Bolli GB, Fanelli CG: Counterregulatory hormone and symptom responses to insulin-induced hypoglycemia in the postprandial state in humans. *Diabetes* 52:2774-2783, 2003.

16. Wiethop BV and Cryer PE: Alanine and terbutaline in treatment of hypoglycemia in IDDM. *Diabetes Care* 16:1131-1136, 1993.

17. Wiethop BV and Cryer PE: Glycemic actions of alanine and terbutaline in IDDM. *Diabetes Care* 16:1124-1130, 1993.

18. Evans ML, Hopkins D, Macdonald IA, Amiel SA: Alanine infusion during hypoglycaemia partly supports cognitive performance in healthy human subjects. *Diabet Med* 21:440-446, 2004.

19. Maran A, Cranston I, Lomas J, Macdonald I, Amiel SA: Protection by lactate of cerebral function during hypoglycaemia. *Lancet* 343:16-20, 1994.

20. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J: Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans. *Diabetes* 43:1311-1317, 1994.

21. Ewing DJ, Clarke BF: Autonomic neuropathy: its diagnosis and prognosis. *Clin Endocrinol Metab* 15:855-888, 1986.

22. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683-1689, 1993.

23. McGuire E, Helderman J, Tobin J, Andres R, Berman M: Effects of arterial venous sampling on analysis of glucose kinetics in man. *J Appl Physiol* 41:565-573, 1976.

24. De Feo P, Perriello G, Ventura MM, Calcinaro F, Basta G, Lolli C, Cruciani C, Dell'Olio A, Santeusanio F, Brunetti P, Bolli GB: Studies on overnight insulin requirements and metabolic clearance rate of insulin in normal and diabetic man: relevance to the pathogenesis of the dawn phenomenon. *Diabetologia* 29:475-480, 1986.

25. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991.

26. Cryer PE: Symptoms of hypoglycemia, thresholds for their occurrence, and hypoglycemia unawareness. *Endocrinol Metab Clin North Am* 28:495-500, 1999.

27. Boll T. and Barth J: Neuropsychology of Brain Damage. In: *Handbook of Clinical Neuropsychology*, edited by S. Filskov and T. Boll. New York: Wiley, 1981, p418-452.
28. Lezak MD, Howieson DB, Loring DW, Hannay HJ, Fischer JS: *Neuropsychological Assessment*. Oxford University Press, USA; 4th ed., pp. 429-550, 2004
29. Wechsler D: *Manual of the Wechsler Adult Intelligence Scale-Revised*. The Psychological Corporation Limited, New York, 1981
30. Golden C: *Stroop Color and Word Test*. Chicago: Stoelting, 1978.
31. Gronwall DMA: Paced auditory serial-addition task: a measure of recovery from concussion. *Percept Mot Skills* 44:367–373, 1977
32. Fanelli CG, De Feo P, Porcellati F, Perriello G, Torlone E, Santeusanio F, Brunetti P, Bolli GB: Adreneric mechanisms contribute to the late phase of hypoglycemic glucose counterregulation in humans by stimulating lipolysis. *J Clin Invest* 89:2005-2013, 1992
33. Nair KS, Welle SL, Tito J: Effect of plasma amino acid replacement on glucagon and substrate responses to insulin-induced hypoglycemia in humans. *Diabetes* 39:376-82, 1990
34. Kuzuya H, Blix PN, Horwitz DL, Steiner DF, Rubenstein AH: Determination of free and total insulin and C-peptide in insulin treated diabetics. *Diabetes* 26:22-29, 1977
35. Winer BJ, Brown DR, Michels KM: *Statistical Principles in Experimental Design*. 3rd ed. New York: McGraw Hill 497-582, 1991
36. Holland BS, Copenhaver M: Improved Bonferroni type multiple testing procedures. *Psychol Bull* 104:145–148, 1988
37. Iversen J: Secretion of glucagon from the isolated, perfused canine pancreas. *J Clin Invest* 50:2123–2136, 1971
38. Dunning BE, Foley JE, Ahrén B: Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* 48:1700–1713, 2005
39. Raju B, Cryer PE: Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin deficient diabetes. *Diabetes* 54:757–764, 2005
40. Banarer S, McGregor VP, Cryer PE: Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. *Diabetes* 51:958–965, 2002
41. Krarup T, Schwartz TW, Hilsted J, Madsbad S, Overlaege O, Sestoft L: Impaired response of pancreatic polypeptide to hypoglycaemia: an early sign of autonomic neuropathy in diabetics. *Br Med J* 2:1544-6, 1976
42. Gold AE, MacLeod KM, Frier BM: Frequency of severe hypoglycemia in patients with type 1 diabetes with impaired awareness of hypoglycemia. *Diabetes Care* 17:697–703, 1994
43. Wong KL, Tyce GM: Glucose and amino acid metabolism in rat brain during sustained hypoglycaemia. *Neurochem Res* 8:401-415, 1983
44. Lubow JM, Piñón IG, Avogaro A, Cobelli C, Treeson DM, Mandeville KA, Toffolo G, Boyle PJ: Brain oxygen utilization is unchanged by hypoglycemia in normal humans: lactate, alanine, and leucine uptake are not sufficient to offset energy deficit. *Am J Physiol Endocrinol Metab* 290:E149-E153, 2006
45. Wahren J, Ekberg K, Fernqvist-Forbes E, Nair S: Brain substrate utilisation during acute hypoglycaemia. *Diabetologia* 42:812-818, 1999
46. Betz AL, Goldstein GW, Katzman R: Blood-brain-cerebrospinal fluid barriers. in *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*, 5th ed., G.J. Siegel, ed. New York: Raven Press, Pp. 681-698, 1994

47. Lieberman HR: Nutrition, brain function and cognitive performance. *Appetite* 40:245-54, 2003
LEGENDS TO TABLES AND FIGURES

Table I. Basal plasma levels, maximal concentrations and AUC of glucagon, adrenaline, noradrenaline, cortisol, growth hormone and pancreatic polypeptide in ten nondiabetic and ten type 1 diabetic subjects studied during clamped hypoglycemia (120-160 min) both with amino acids and placebo ingestion, and during clamped euglycemia (120-160 min) with amino acids ingestion.

Table II. Cognitive tests scores in ten nondiabetic and ten type 1 diabetic subjects studied during clamped hypoglycemia both with amino acids and placebo ingestion, and during clamped euglycemia with amino acids ingestion.

Figure 1. Plasma glucose and free insulin concentrations, and rates of glucose infusion during clamped euglycemia with amino acids (closed circles) ingestion, and clamped hypoglycemia both with placebo (open circles) and amino acids (closed triangles) ingestion in ten nondiabetic (left panel) and ten type 1 diabetic (right panel) subjects.

Figure 2. Plasma glucagon, C-peptide and pancreatic polypeptide concentrations during clamped euglycemia with amino acids (closed circles) ingestion, and clamped hypoglycemia both with placebo (open circles) and amino acids (closed triangles) ingestion in ten nondiabetic (left panel) and ten type 1 diabetic (right panel) subjects.

Figure 3. Plasma adrenaline, noradrenaline, cortisol and growth hormone concentrations during clamped euglycemia with amino acids (closed circles) ingestion, and clamped hypoglycemia both with placebo (open circles) and amino acids (closed triangles) ingestion in ten nondiabetic (left panel) and ten type 1 diabetic (right panel) subjects.

Figure 4. Plasma concentrations of free fatty acids, glycerol, β-hydroxybutyrate, lactate and alanine during clamped euglycemia with amino acids (closed circles) ingestion, and clamped hypoglycemia both with placebo (open circles) and amino acids (closed triangles) ingestion in ten nondiabetic (left panel) and ten type 1 diabetic (right panel) subjects.

Figure 5. Plasma concentrations of branched chain aminoacids (BCAA) and non-branched chain aminoacids (N-BCAA) during clamped euglycemia with amino acids (closed circles) ingestion, and clamped hypoglycemia both with placebo (open circles) and amino acids (closed triangles) ingestion in ten nondiabetic (left panel) and ten type 1 diabetic (right panel) subjects.

Figure 6. Autonomic and neuroglycopenic symptom scores during clamped euglycemia with amino acids (closed circles) ingestion, and clamped hypoglycemia both with placebo (open circles) and amino acids (closed triangles) ingestion in ten nondiabetic (left panel) and ten type 1 diabetic (right panel) subjects.
Table 1

|                      | Nondiabetic subjects          | Type 1 diabetic subjects          |
|----------------------|-------------------------------|----------------------------------|
|                      | HYPO +                        | HYPO +                           | p     | HYPO +                        | HYPO +                           | p     |
|                      | P                             | AA                              | EU+AA| P                             | AA                              | EU+AA| p     |
| GLUCAGON             |                               |                                 |      |                               |                                 |      |       |
| Basal levels (ng/l)  | 67±4                          | 69±5                            | 77±4 | 0.373                         | 49±7                            | 56±4 | 51±3 | 0.843 |
| Cmax (ng/l)          | 142±20                        | 394±36§                         | 137±18| 0.001                         | 49±9                            | 124±25§| 64±9| 0.016 |
| AUC (ng·l⁻¹·min⁻¹)  | 116±16                        | 318±30§                         | 111±13| 0.001                         | 44±7                            | 101±18§| 57±7| 0.028 |
| ADRENALINE          |                               |                                 |      |                               |                                 |      |       |
| Basal levels (nmol/l)| 0.2±0.1                       | 0.1±0.1†                        | 0.4±0.1§ | 0.034                        | 0.5±0.3                         | 0.3±0.2 | 0.3±0.2| 0.756 |
| Cmax (nmol/l)        | 2.8±0.4                       | 2.7±0.5                         | 0.7±0.1§ | 0.001                         | 1.6±0.4                         | 1.3±0.1 | 0.8±0.3| 0.165 |
| AUC (nmol·l⁻¹·min⁻¹) | 2.1±0.4                       | 1.6±0.3                         | 0.4±0.1§ | 0.003                         | 1.0±0.2                         | 0.8±0.1 | 0.5±0.1§| 0.048 |
| NORADRENALINE       |                               |                                 |      |                               |                                 |      |       |
| Basal levels (nmol/l)| 0.8±0.2                       | 0.6±0.2                         | 1.1±0.2 | 0.124                         | 1.3±0.3                         | 1.0±0.2 | 1.3±0.2| 0.411 |
| Cmax (nmol/l)        | 1.5±0.2                       | 1.3±0.2                         | 1.9±0.2 | 0.132                         | 2.0±0.5                         | 1.7±0.2 | 1.9±0.3| 0.796 |
| AUC (nmol·l⁻¹·min⁻¹) | 1.2±0.2                       | 0.9±0.1                         | 1.2±0.1 | 0.060                         | 1.3±0.3                         | 1.3±0.2 | 1.2±0.1| 0.963 |
| CORTISOL            |                               |                                 |      |                               |                                 |      |       |
| Basal levels (μg/l)  | 9.1±1.0                       | 9.8±0.9                         | 10±1.2 | 0.794                         | 11.3±1.3                        | 12±1.7 | 11.8±1.1| 0.846 |
| Cmax (μg/l)          | 22±4.5                        | 21±4.5                          | 11±1.2§ | 0.013                         | 20±2.1§                         | 17±1.2 | 14±0.4| 0.014 |
| AUC (μg·dl⁻¹·min⁻¹)  | 15±3.4                        | 13±2.8                          | 6.6±0.6§ | 0.011                         | 16±2.1                         | 15±1.2 | 9.0±0.5§| 0.004 |
| GROWTH HORMONE      |                               |                                 |      |                               |                                 |      |       |
| Basal levels (μg/l)  | 0.6±0.2                       | 0.9±0.3                         | 0.9±0.3 | 0.451                         | 2.0±1.6                         | 2.0±1.9 | 1.6±1.2| 0.772 |
| Cmax (μg/l)          | 17±3.7                        | 16±2.0                          | 3.2±1.7§ | 0.002                         | 19±3.1                         | 20±3.9 | 3.1±0.9§| 0.001 |
| AUC (μg·l⁻¹·min⁻¹)  | 9.5±2.3                       | 7.5±1.0                         | 0.5±0.2§ | 0.001                         | 8.7±1.9                        | 8.4±1.8 | 0.9±0.6§| 0.003 |
| PANCREATIC POLYPEPTIDE |                              |                                 |      |                               |                                 |      |       |
| Basal levels (pmol/l)| 22±1                         | 23±1.3                          | 24±1.2 | 0.799                         | 18±1.1                         | 21±1.1 | 20±1.2| 0.684 |
| Cmax (pmol/l)        | 168±19§                       | 194±11§                         | 30±4.0 | 0.001                         | 127±40                         | 141±34 | 23±3.0§| 0.012 |
| AUC (pmol·l⁻¹·min⁻¹) | 131±21                       | 143±11                          | 26±2.0§ | 0.001                         | 70±23†                         | 80±21§ | 20±3.0| 0.047 |

Data are mean±SE. Basal levels were the average of -30 and 0 minutes values. P values calculated from repeated measures ANOVA. The symbols § and †indicate significant within group differences.
### Table II

| Nominal PG(Eu/Hypo) | Minutes | Type 1 diabetic subjects |
|---------------------|---------|--------------------------|
|                     | 90/90   | 90/90 90/47 mg/dl        | 90/90 90/90 90/47 mg/dl |
| Trail-Making A (a)  |         |                          |                        |
| Hypo+P              | 39±2.6  | 34±5.0 70±13 0.234       | 42±5.2 60±9 109±27 0.072 |
| Hypo+AA             | 36±4.6  | 36±5.7 65±8.0 32±4.9     | 68±7.2 63±9.1          |
| EU+AA               | 30±3.8  | 67±4.7 61±3.6 39±6.2     | 65±9.6 78±5.4          |
| Trail-Making B (b)  |         |                          |                        |
| Hypo+P              | 53±4.9  | 71±9.4 89±10 0.002       | 49±2.2 64±9.3 115±26 0.014 |
| Hypo+AA             | 54±4.1  | 56±7.6 74±6.6*†          | 52±5.3 63±7.8 69±9.9*  |
| EU+AA               | 62±6.4  | 57±6.4 50±2.5*           | 64±5.6 62±3.5 64±6.7*  |
| PASAT (3 sec) (b)   |         |                          |                        |
| Hypo+P              | 59±0.6  | 58±0.6 41±7.5*†          | 55±0.5 55±0.2 51±1.7*‡ 0.031 |
| Hypo+AA             | 58±0.5  | 58±0.7 49±3.2†           | 59±1.2 55±0.4 54±1.0‡  |
| EU+AA               | 52±2.3  | 54±1.9 57±0.8            | 53±1.7 54±1.3 58±1.0   |
| PASAT (2 sec) (b)   |         |                          |                        |
| Hypo+P              | 47±2.8  | 52±2.5 33±5.9 0.042      | 36±1.2 38±3.5 35±3.2‡ 0.038 |
| Hypo+AA             | 50±2.3  | 51±3.0 46±2.4*           | 40±2.8 47±1.7 36±1.6‡  |
| EU+AA               | 40±3.5  | 48±3.8 45±2.4*           | 41±1.5 48±1.2 45±0.8   |
| Digit Span Forw. (c)|         |                          |                        |
| Hypo+P              | 4.0±0.3 | 4.6±0.3 3.3±0.5 0.034    | 4.2±0.2 5.0±0.3 3.3±0.3† 0.028 |
| Hypo+AA             | 4.3±0.1 | 4.5±0.2 4.4±0.2*         | 4.0±0.3 3.8±0.3 3.6±0.2* |
| EU+AA               | 4.3±0.1 | 4.5±0.1 4.3±0.2*         | 4.1±0.1 4.2±0.1 4.0±0.1  |
| Digit Span Back. (c)|         |                          |                        |
| Hypo+P              | 5.0±0.4 | 4.6±0.4 3.8±0.6†         | 4.8±0.3 4.7±0.1 4.0±0.3 0.011 |
| Hypo+AA             | 5.1±0.4 | 4.4±0.4 4.3±0.5†         | 4.9±0.3 4.5±0.3 4.8±0.2* |
| EU+AA               | 4.3±0.6 | 4.5±0.4 4.8±0.3          | 4.8±0.2 4.8±0.3 4.5±0.3* |
| Digit Vigilance (d) |         |                          |                        |
| Hypo+P              | 33±2.4  | 33±2.1 28±3.6 0.149      | 31±1.2 36±1.2 33±1.1 0.065 |
| Hypo+AA             | 33±1.4  | 33±1.9 30±2.5            | 33±1.4 38±1.4 32±1.0   |
| EU+AA               | 32±2.1  | 31±2.7 33±1.5            | 36±1.4 32±1.3 37±1.5   |
| Verbal Fluency (e)  |         |                          |                        |
| Hypo+P              | 9.5±0.5 | 11.3±0.9 8.2±1.3‡ 0.001  | 13.5±0.1 12.4±1.0 10.8±0.9‡ 0.002 |
| Hypo+AA             | 13±0.9  | 11.4±1.0 9.8±1.1‡         | 12.5±0.3 10.8±0.7 9.0±0.6‡  |
| EU+AA               | 12.3±0.3| 11.8±0.6 13.8±0.7        | 11.0±1.0 12.0±1.4 14.0±1.0 |
| Stroop Word (f)     |         |                          |                        |
| Hypo+P              | 94±5.8  | 104±8.0 80±15.8 0.458    | 104±4.0 98±3.8 96±1.9‡ 0.014 |
| Hypo+AA             | 107±7.8 | 104±7.3 94±8.0 103±1.4   | 108±1.7 102±1.3‡ 114±2.8  |
| EU+AA               | 98±4.3  | 103±5.0 104±5.5          | 106±3.3 109±4.1 114±2.8  |
| Stroop Color (f)    |         |                          |                        |
| Hypo+P              | 73±3.2  | 82±3.4 55±10 0.066       | 78±2.5 79±2.9 59±0.8 0.002 |
| Hypo+AA             | 78±4.1  | 79±2.7 69±3.8            | 76±9.9 77±1.7 75±2.3*‡  |
| EU+AA               | 74±1.2  | 79±3.1 79±1.1            | 81±2.5 83±2.5 87±1.9   |
| Stroop Col -Words (f)|         |                          |                        |
| Hypo+P              | 51±2.3  | 55±1.3 38±6.8 0.025      | 48±1.7 52±0.9 50±0.4‡ 0.042 |
| Hypo+AA             | 62±3.2  | 59±2.0 51±2.0*‡          | 49±0.5 50±1.7 49±1.6‡  |
| EU+AA               | 56±2.6  | 55±2.2 60±3.2            | 46±2.1 53±1.5 60±1.7   |
| Verbal Memory Test (g)|         |                          |                        |
| Hypo+P              | 4.8±0.1 | 3.6±0.6 2.2±0.7 0.042    | 5.0±0.0 5.0±0.0 3.5±0.4‡ 0.015 |
| Hypo+AA             | 4.3±0.2 | 4.2±0.3 3.4±0.5*         | 5.0±0.0 4.8±0.1 4.0±0.3‡  |
|        | Data are mean±SE. *P < 0.05 vs HYPO+P; †P < 0.05 vs EU+AA; (a) time (sec) required to complete the task; (b) number of correct responses; (c) number of digit sequences correctly repeated; (d) number of correct targets crossed out in 90 sec; (e) number of words named in 60 sec; (f) number of correct responses in 45 sec; (g) number of words recalled. |
Figure 1

NON-DIABETIC SUBJECTS

Plasma Glucose

DIABETIC SUBJECTS

Plasma Insulin

Glucose Infusion Rate

Time (min)

-30 -15 0 15 30 45 60 75 90 105 120 140 160

Time (min)

-30 -15 0 15 30 45 60 75 90 105 120 140 160
Figure 2

Non-diabetic Subjects

Diabetic Subjects

Plasma Glucagon

Plasma C-Peptide

Plasma Pancreatic Polypeptide

Nominal PG (mg/dl)

Time (min)

ng/dl

mmol/l

pg/ml
Figure 4

**Plasma Free Fatty Acids**

**Plasma Glycerol**

**Plasma Beta Hydroxybutyrate**

**Plasma Lactate**

**NON-DIABETIC SUBJECTS**

**DIABETIC SUBJECTS**

Nominal PG (mg/dl)

- Eu-AA
- Hypo-P
- Hypo-AA

Time (min)
Figure 5

**Plasma Branched Amino Acids**
- Non-Diabetic Subjects
- Diabetic Subjects

**Plasma Non-branched Amino Acids**
- Non-Diabetic Subjects
- Diabetic Subjects
