The amino acid response to a mixed meal in patients with type 2 diabetes: effect of sitagliptin treatment

E. Muscelli1, S. Frascerra1, A. Casolaro1, S. Baldi1, A. Mari2, W. Galli3, J. Cobb3 & E. Ferrannini1

1 Department of Clinical & Experimental Medicine, University of Pisa, Pisa, Italy
2 CNR Institute of Biomedical Engineering, National Research Council, Padova, Italy
3 Department of Diabetes, Metabolon Inc., Durham, NC, USA

Aims: Amino acid (AA) metabolism is altered in type 2 diabetes (T2D), and fasting levels of α-hydroxybutyrate (α-HB), a biomarker for insulin resistance, have been suggested to track AA metabolism. We investigated the changes in AA and α-HB induced by a mixed-meal tolerance test (MTT) and the effects of sitagliptin treatment.

Material and Methods: Forty-seven T2D patients [56 ± 7 years, body mass index (BMI) 29.9 ± 4.2 kg/m²] were randomized to sitagliptin (100 mg/day, 6 weeks) or placebo. Seven age- and BMI-matched non-diabetic subjects served as control (CT).

Results: During a 5-h MTT, branched-chain AA (BCAA) peaked earlier in T2D than CT [75(25) vs. 62(3) mmol/l.h over 2 h, median (interquartile range), p = 0.05], and rose higher [5-h increment: 24(99) vs. 19(24) mmol/l.h, p = 0.05]. Fasting α-HB was higher [7.5(2.7) vs. 5.9(1.3) μg/ml, p = 0.04 T2D vs. CT], and its meal-induced increments were larger [24(99) vs. −41(86) μg/ml.h, p = 0.006]. Plasma non-esterified fatty acids (NEFA) declined during MTT, but their increments were greater in patients [53 ± 16 vs. 35 ± 10 mEq/l.h, p = 0.005]. Compared to placebo, both BCAA [−6.4(21.1) vs. 0.0(48.0) mmol/l.h, p = 0.01] and α-HB increments [−11.4(250) vs. 11.4(428) μg/ml.h, p = 0.002] decreased with sitagliptin, and meal-induced NEFA suppression was improved. Changes in BCAA and α-HB were reciprocally related to changes in insulin sensitivity (ρ = −0.37 and −0.43, p ≤ 0.01).

Conclusions: T2D is associated with a hyperaminoacidemic response to MTT, which circulating α-HB levels track. Sitagliptin-induced glycaemic improvement was associated with reductions in BCAA and α-HB excursions and better NEFA suppression, in parallel with improved insulin sensitivity, confirming that α-HB is a readout of metabolic overload.

Keywords: 1-linoleoyl-glycero phosphocholine, branched-chain aminoacids, sitagliptin, T2D, α-hydroxybutyrate

Date submitted 30 January 2014; date of first decision 18 March 2014; date of final acceptance 30 June 2014

Introduction

Almost a century ago, Luck and Morse [1] described insulin action to lower blood amino acid (AA) levels in normal subjects while avoiding hypoglycaemia. High plasma AA levels have since been reported in diabetic patients, with reduction after insulin therapy [2]. In obese non-diabetic subjects, branched-chain AA (BCAA; valine, leucine and isoleucine) were found to be 20% higher than in lean controls [3]. More recently, metabolomics platforms have made it possible to identify specific AA profiles in obese patients, including raised BCAA [4–6]. Some BCAA and the aromatic AA, tyrosine and phenylalanine, have been identified as biomarkers predictive of type 2 diabetes (T2D) in participants of both the Framingham Offspring Study and Malmö Diet and Cancer Study [7] and of insulin resistance in young healthy Finns [8], whereas higher glycine and some phosphatidyl-cholines have been associated with lower diabetes risk in large prospective studies [9]. In response to a glucose load, Shaham et al. [10] identified changes in the circulating levels of 21 metabolites, including reductions of leucine/isoleucine, valine, tyrosine, lysine, β-hydroxybutyrate and glycerol, and increased lactate. BCAA decreased more in insulin-sensitive than in insulin-resistant subjects with impaired glucose tolerance (IGT), again pointing to an interaction between insulin action and AA. A recent study of insulin-resistant subjects also identified differences in metabolite profile in pathways other than AA in response to an oral glucose tolerance test (OGTT) [11]. However, the response of plasma AA to glucose infusions with or without concomitant hyperinsulinaemia was found similar in obese, insulin-resistant subjects [12] and in obese diabetic women [13].

Individual AA or AA mixtures are physiological stimuli for insulin secretion both in normal and diabetic patients [14–16]. The insulin response to a meal containing AA (or protein) and glucose is higher than the response to the same amount of glucose [17]. Protein ingestion, such as glucose, stimulates glucagon-like peptide-1 (GLP-1) and GIP (gastric inhibitory polypeptide) secretion, hormones with strong incretin effect, with divergent effects on glucagon secretion – stimulated by GIP and inhibited by GLP-1 [18]. The incretin effect is typically blunted in diabetic patients [19–21]. Sitagliptin, a DPP-IV (dipeptidyl peptidase 4) inhibitor, raises intact GLP-1 levels and improves diabetes control [22] through improved β-cell function, decreased glucagon release and endogenous glucose production [23].
α-Hydroxybutyrate (α-HB), an organic acid product of AA degradation, has been described as a marker of insulin resistance [24]. In a study including two large cohorts of non-diabetic subjects, fasting α-HB was directly related to clamp-derived insulin resistance, while a lipid metabolite, linoleoyl-glycerophosphocholine (L-GPC) was a negative correlate of insulin resistance. Furthermore, both metabolites were predictive of incident dysglycaemia or diabetes, significantly adding to the predictability of either outcome [25].

The aim of this study was to investigate in patients with T2D whether raising incretin hormones with sitagliptin impacts on the AA response to a meal and on markers of insulin resistance.

Methods

Study Population and Protocol

Fifty patients with T2D [either gender, age between 30 and 70 years, and body mass index (BMI) between 20 and 40 kg/m²] diagnosed within the past 5 years were recruited. At screening, antihyperglycaemic therapy-naive patients had to have HbA1c between 7 and 10%, whereas patients on treatment (monotherapy or low-dose oral combination therapy) had to have HbA1c between 6.5 and 10%. After 4 weeks of washout and prior to randomization, fasting plasma glucose (FPG) was required to range between 7.2 and 14.4 mmol/l. Exclusion criteria were: unstable body weight; history of malignancy in the last 5 years; significant cardiovascular disorder within the last 6 months; treatment with >12.5 mg daily of thiazide, β-blocker therapy; monotherapy or combination with PPARδ agonists in the last 12 weeks; pregnant women or women expecting to conceive within the study duration; plasma creatinine ≥1.5 mg/dl; ALT (alanine aminotransferase) and AST (aspartate aminotransferase) >2.0 × ULN (upper limit of normal); abnormal thyroid-stimulating hormone (TSH) levels; triacylglycerols >3.39 mmol/l; creatinine clearance <60 ml/min; blood pressure ≥160/95 mmHg.

T2D patients were randomized to sitagliptin (100 mg/day) or placebo for 6 weeks in a double-blind design, with an equal fraction of drug-naive and treated patients in each group. Seven subjects with FPG <5.6 mmol/l participated as controls in all baseline experiments. The results of glucose metabolism have been reported previously [23].

Each subject underwent a meal tolerance test (MTT; 53% carbohydrate, 30% fat, 17% protein, 560 kcal) before and after treatment. The meal, consisting of 1 egg, 50 g of Parmesan cheese and 75 g of glucose in water, was consumed in <10 min.

Measurements

Fat-free mass (FFM) was measured by electrical bioimpedance using a Body Composition Analyser Model TB-300 (TANITA, Tokyo, Japan); fat mass was then obtained as the difference between body weight and FFM. Plasma glucose was measured by the glucose oxidase technique (Beckman Glucose Analysers; Beckman, Fullerton, CA, USA), plasma insulin and C-peptide by electrochemiluminescence (on a COBAS e411 instrument, Roche, IN, USA). Plasma triacylglycerols and serum high-density lipoprotein (HDL) cholesterol and non-esterified fatty acids (NEFA) were assayed in duplicate by standard spectrophotometric methods on a Synchron Clinical System CX4 (Beckman Instruments, Fullerton, CA, USA). Plasma AA (valine, isoleucine, leucine, glycine, alanine, arginine and proline) were measured at the time-points −30, 0, 15, 30, 60, 90, 120, 150, 180, 240 and 300 min using a reverse-phase, high-performance liquid chromatographic system, HPLC, as previously described [26]. This method has a within-assay relative standard deviation of <5%.

Plasma samples for targeted metabolite analysis (α-HB and linoleoylglycerophosphocholine (L-GPC)) were obtained at 0, 60 and 120 min of the MTT. Metabolites were analysed by isotope dilution ultra high-performance liquid chromatography–tandem mass spectrometer (UHPLC-MS-MS) [24]. In brief, 50 μl of ethylenediaminetetraacetic (EDTA) plasma samples were spiked with internal standards, and subsequently subjected to protein precipitation by mixing 200 μl of 1% formic acid in methanol. Following centrifugation, 100-μl aliquots of clear supernatant were diluted with 30 μl 1% formic acid and injected onto an UHPLC-MS/MS system, consisting of a Thermo TSQ Quantum Ultra Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Waters Acquity UHPLC system (Waters Corporation, Milford, MA, USA) equipped with a column manager module in 2.5 min assay. α-HB and L-GPC were eluted with a gradient on Waters Acquity single RPC-18 column (Waters Corporation) (2.1 × 50 mm, 1.7 mm particle size) at a mobile phase flow rate of 0.4 ml/min at 40 °C. Ionization was achieved by HESI source. Quantification was performed based on the area ratios of analyte and internal standard peaks using a weighted linear least-squares regression analysis generated from fortified calibration standards in water, prepared immediately prior to each run. Stable deuterium-labelled compounds (α-HB-D3 and L-GPC-D9) were used as internal standards.

Calculations

AA results were grouped as BCAA (sum of valine, isoleucine and leucine) and glycogenic amino acids, GGA (sum of glycine, alanine, arginine and proline concentrations). Calculation of metabolite concentrations based on internal standards was performed using the MetIQ™ package, which is an integral part of the kit. Additionally, the data were corrected for batch effects. The AbsolutIDQ™ methods were proven to be in conformance with the Food and Drug Administration (FDA) Guideline (Guidance for Industry – Bioanalytical Method Validation, May 2001) requiring proof of reproducibility within an given error range [24].

β-Cell function was assessed with the use of a previously described mathematical model [27]. One of the model blocks describes the dependence of insulin (or C-peptide) secretion on glucose concentration; the mean slope of this dose-response function (i.e. the relationship between insulin release and plasma glucose concentrations) was previously reported and represents β-cell glucose sensitivity (βCGS). Insulin sensitivity was estimated from the plasma glucose and insulin responses to MTT between 0 and 120 min by the Oral Glucose Insulin Sensitivity (OGIS) index, which has been validated against the euglycaemic insulin clamp, and expressed in units of ml/min/m² of body surface area [28]. Areas under
time–concentration curves (AUC) were calculated by the trapezium rule.

**Statistical Analysis**

Data are given as mean ± SD or median (interquartile range) for variables with a normal or skewed distribution, respectively. Differences between control and T2D subjects were analysed by Mann–Whitney test, intragroup comparison (post vs. pretreatment) by Wilcoxon signed rank test. Treatment responses were analysed by an analysis of variance (ANCOVA) model with change from baseline as the outcome variable and baseline value and treatment (sitagliptin vs. placebo) as covariates. Univariate associations were tested by Spearman regression (ρ value). A p-value of ≤0.05 was considered statistically significant.

**Results**

**T2D Patients Versus Controls**

Forty-seven T2D patients completed all studies and had plasma AA measurements; metabolites were determined in 19 and 20 patients from the placebo and sitagliptin group, respectively. Age and BMI were similar between controls and T2D patients (60 ± 6 and 56 ± 7 years and 28.8 ± 2.3 vs. 29.9 ± 4.2 kg/m², respectively, both p = ns). Baseline HbA1c (39 ± 2 vs. 57 ± 6 mmol/mol, p < 0.0001), FPG (5.56 ± 0.33 vs. 9.28 ± 1.89 mmol/l, p < 0.0001) and fasting plasma insulin concentrations [41 (22) and 67 (53) pmol/l, p = 0.03] were higher in the patients, whereas insulin sensitivity (389 ± 27 vs. 271 ± 42 ml/min/m², p < 0.0001) and β-cell glucose sensitivity [98 (115) vs. 32 (30) pmol/min/m²/mM, p < 0.001] were markedly reduced, as previously reported [23]. All baseline characteristics were similar between patients randomized to sitagliptin or placebo (data not shown).

During the MTT, both BCAA and GGA increased by approximately 50%, with BCAA concentrations peaking earlier in T2D compared to control subjects [75 (25) vs. 62 (3) mmol/l/h over the first 2 h, p = 0.05]. Over the entire 5 h of meal absorption, BCAA levels tended to be higher in T2D than control subjects and the meal-induced increment was larger [31 (23) vs. 19 (24) mmol/l/h, p = 0.05]. In contrast, the GGA response to the meal was similar in the two groups (Table 1, Figure 1).

Fasting plasma α-HB was higher in T2D than control subjects (Table 2). Following meal ingestion, plasma α-HB decreased in controls, while it increased at 60 min and then declined in the T2D group; over 2 h postmeal, the α-HB AUC was higher in patients than controls (Figure 2). In contrast, fasting plasma L-GPC concentrations were not significantly different between patients and controls, and rose during the meal similarly in the two groups. Fasting NEFA decreased sharply following meal ingestion, plateaued between 60 and 180 min, and then started to rise again; the NEFA AUC over 3 h was greater in patients than controls (53 ± 16 vs. 35 ± 10 mEq/l/h, p = 0.005, Figure 2).

**Response to Sitagliptin Treatment**

BMI did not change after either placebo or sitagliptin treatment. Diabetes control improved after sitagliptin as compared to placebo, as did insulin sensitivity [placebo: 271 (78)–258 (87) vs. sitagliptin: 268 (59)–295 (50) ml/min/m², p = 0.01] and β-cell glucose sensitivity [33 (30)–35 (33) vs. 31 (36)–53 (52) pmol/min/m²/mM, p = 0.01], as previously reported [23]. The serum lipid profile was similar in the two groups, at baseline and following treatment (data not shown).

Sitagliptin treatment was associated with significantly lower concentrations of BCAA and α-HB, but not GGA or L-GPC, compared to placebo (Figures 1 and 2, Tables 1 and 2). Sitagliptin treatment also reduced fasting NEFA and improved their suppression during MTT (α fasting = −0.09 ± 0.11 vs. 0.01 ± 0.15 mEq/l, p = 0.02 for the placebo-adjusted difference; α AUC = −6.2 ± 31.5 vs. 21.6 ± 47.6 mEq/l/h, p = 0.05).

In the complete set of baseline data, FPG and glucose AUC were reciprocally associated with insulin sensitivity and βCGS (all p ≤ 0.001), as expected. The BCAA response to the meal was inversely related to insulin sensitivity (ρ = −0.55, p = 0.0002), while α-HB AUC was positively related to glucose (ρ = 0.38) and to the ratio BCAA/total AA (ρ = 0.42), and reciprocally to insulin sensitivity (ρ = −0.47) (all p ≤ 0.05) and glycine AUC (ρ = −0.58, p = 0.002). At follow up, the changes in BCAA/total AA and glycine were significantly related to the changes in glucose AUC in opposite directions, while BCAA and α-HB changes were reciprocally related to the changes in insulin sensitivity. Only α-HB changes were associated with the changes in β-cell function (Table S1, Supporting Information and Figure 3).

**Discussion**

The main findings of this study were: (i) at baseline, the plasma BCAA response to the mixed meal was higher in T2D patients than in age- and BMI-matched non-diabetic controls, especially during the first 2 h of the 5-h test; the same was not generally true for the measured GAA; (ii) coherently with this, α-HB – a product of amino acid catabolism – increased in response to the meal in T2D, while it decreased in controls and (iii) compared to placebo, sitagliptin treatment was associated with a selective reduction in both BCAA and α-HB response to the meal. These findings deserve further specification.

With regard to AA metabolism, early studies showed that hyperaminoacidaemia is frequently found in T2D patients – especially under conditions of poor glycaemic control [29] – as well as in non-diabetic obese individuals [4]. More recently, this abnormality has been further characterized as involving predominantly, although not exclusively, BCAA. In euglycaemic hyperinsulinaemic clamp studies, the ability of insulin to lower circulating AA concentrations by restraining proteolysis and accelerating AA catabolism has been shown to be impaired in individuals with insulin resistance of glucose metabolism [30]. Moreover, raised BCAA have been associated with insulin resistance and incident diabetes in large observational studies [7]. This study extends previous findings by showing that in patients with typical T2D an earlier and larger excursion in plasma AA also occurs after a mixed meal (the majority of previous studies have used oral glucose loads), and that this enhanced aminoacidaemic response comprises mostly BCAA. For want of AA turnover measurements, the probable explanation for the hyperaminoacidaemia of our T2D
Table 1. Plasma amino acids during the MTT in controls and diabetic patients.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>T2D</th>
<th>p1</th>
<th>Placebo pre</th>
<th>Placebo post</th>
<th>Sita pre</th>
<th>Sita post</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branched-chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>𝜕 AUC (mmol/lh)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0 [48]</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>𝜕 increment AUC (mmol/lh)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3 [24]</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glycogenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (μmol/l)</td>
<td>921 [160]</td>
<td>778 [369]</td>
<td>0.60</td>
<td>968 [403]</td>
<td>791 [301]*</td>
<td>763 [197]</td>
<td>732 [240]</td>
<td></td>
</tr>
<tr>
<td>Increment AUC (mmol/lh)</td>
<td>30 [32]</td>
<td>33 [38]</td>
<td>0.71</td>
<td>25 [40]</td>
<td>39 [36]</td>
<td>36 [34]</td>
<td>26 [20]</td>
<td></td>
</tr>
<tr>
<td>𝜕 AUC (mmol/lh)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—22 [49]</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>𝜕 increment AUC (mmol/lh)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—10 [35]</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

p1, for the comparison of controls versus all diabetic patients by Mann–Whitney test; p2, for the placebo-adjusted difference between baseline and sitagliptin treatment. HB, hydroxybutyrate; MTT, meal tolerance test; T2D, type 2 diabetes; AUC, areas under time–concentration curves.

*p < 0.05 for the intragroup comparison (post vs. pre) by Wilcoxon signed rank test.

Figure 1. Plasma concentrations of branched-chain amino acids (AA) (sum of leucine, isoleucine and valine) and glycogenic amino acids (sum of glycine, alanine, arginine and proline) in the fasting state and following a mixed meal in type 2 diabetic patients randomized to placebo or sitagliptin treatment for 6 weeks. Plots are mean ± standard mean error (SEM); complete lines are pretreatment values, dashed lines are post-treatment data. The shaded area encompasses mean ± SEM of the non-diabetic control group.

patients is a combination of reduced inhibition of proteolysis and reduced uptake/degradation because of insulin resistance. The observed correlation between changes in BCAA and changes in insulin sensitivity (Figure 3) supports this interpretation. Whether glycaemia itself may exert an independent effect on AA response remains undetermined.

Targeted metabolomics have identified small circulating molecules that track with both hyperaminoacidaemia and insulin resistance. Because insulin resistance is strongly associated with both prevalent and incident diabetes [31], metabolic markers of AA degradation – such as α-HB – also track with both hyperaminoacidaemia and insulin resistance and predict
incident diabetes. In a recent study, \( \alpha \)-ketobutyrate – the direct precursor of \( \alpha \)-HB – and 2-oxo-branched-chain AA – the first products of BCAA catabolism – are both substrate for a shared enzyme, branched-chain \( \alpha \)-ketoacid dehydrogenase [32]. The current results show that \( \alpha \)-HB follows BCAA both in direction of change and in time-course. Even within the limits of a small patient sample, the observed pattern of correlations confirms the operation of a metabolic network, in which impaired glucose metabolism clusters with higher plasma AA and NEFA concentrations as a joint result of insulin resistance of glucose turnover, proteolysis and lipolysis. The higher \( \alpha \)HB levels are the read-out of the resulting overload of mitochondrial metabolism [24]. Genetic determinants may also play a part in the altered metabolic cluster in T2D; in fact, the expression of genes involved in BCAA metabolism and mitochondrial \( \beta \)-oxidation of fatty acids is downregulated in adipose tissue proportionally to the degree of insulin resistance [33].

Information on the effect of treatment on the AA response is limited; it includes the acute injection of insulin in experimental animals [1] and in humans with ketotic diabetes [2], the use of thiazolidinediones [33] and short-term weight loss intervention [34]. The present results show that placebo-controlled sitagliptin treatment showed that, in addition to improving insulin sensitivity and \( \beta \)-cell function [23], sitagliptin also reduces the enhanced AA and \( \alpha \)-HB response to the meal at the same time as it strengthens meal-induced suppression of NEFA levels. These treatment-induced changes varied consensually with one another and with insulin sensitivity (Figure 3). Thus, the metabolic cluster linking insulin action with glucose, AA and NEFA handling improved as a whole with the glucose-lowering therapy. This study does not allow separation of any inherent sitagliptin effects from those mediated by the treatment-induced improvement in insulin sensitivity and abatement of glucose toxicity. Matching of glycaemia and insulin sensitivity by an active comparator would be necessary to test the potential role of different targets of DPP-IV inhibition.

In observational studies of incident diabetes [25], L-GPC behaved as a consistent, independent negative predictor of progression to dysglycaemia or T2D. In the present studies, however, circulating L-GPC levels did not differ between T2D patients and controls, and did not change significantly with sitagliptin treatment vs. placebo (Table 2). The reason could be the small number of study participants. However, \( \alpha \)-HB was the only parameter that changed consensually with the treatment-induced changes in \( \beta \)-cell function (Table S1), confirming the negative association of this molecule with \( \beta \)-cell glucose sensitivity found in large cohorts [25].

Among the glycogenic AA, reduced glycine levels have been reported in individuals developing diabetes [9]. Because glycine is a component of glutathione, it has been hypothesized that low glycine may result from increased oxidative stress and glutathione consumption [25]. In this dataset, glycine was reciprocally associated with \( \alpha \)-HB in the baseline set, and its changes with treatment were inversely related to changes in \( \alpha \)-HB and the BCCA/totalAA ratio. Thus, there is support for the notion that, among glycogenic AA a low glycine concentration is a marker for a higher cellular redox state and metabolic overload.

Several AA are established insulin secretagogues [35,36]. Thus, it is conceivable that in T2D hyperaminoacidemia supplements hyperglycaemia in promoting insulin release by dysfunctional \( \beta \)-cells. Following sitagliptin treatment, both basal and postmeal insulin secretion rates were unchanged despite a significant fall in plasma glucose [23] and AA levels (Table 1). This occurred primarily because the \( \beta \)-cell sensitivity to glucose was improved by treatment, thereby offsetting the reduction in nutrient stimulation.

In conclusion, T2D is associated with a hyperaminoacidemia response to a mixed meal, which circulating \( \alpha \)-HB levels track. Sitagliptin-induced glycaemic improvement was associated with consensus reductions in BCAA and \( \alpha \)-HB excursions, and better NEFA suppression, in parallel with
Improved insulin sensitivity, confirming that α-HB is readout of metabolic overload.

Acknowledgements

The authors thank Elisabetta Barsotti and Brenno D’Astiaraga (Department of Clinical & Experimental Medicine, University of Pisa School of Medicine, Pisa, Italy) for their excellent technical assistance. We also express gratitude to the patients who generously agreed to the complex protocol of the study. The work was aided in part by a grant from Merck Sharp & Dohme and by funds from the Italian Ministry of University and Research (MIUR 2007BRR57M-001).
Figure 3. Association between baseline (left panels) and treatment-induced changes (right panels) in insulin sensitivity, $\alpha$-HB and the ratio of branched-chain to total amino acids (BCAA/AA) in patients with type 2 diabetes. Values are areas-under-curve (AUC) for the pooled placebo and sitagliptin data.

Conflict of interest
S. F. and S. B. were principally responsible for the laboratory determinations; W. G. and J. C. for the metabolomics; E. M. and A. C. for the study conduct; E. M., A. M. and E. F. for the study design, data analysis and interpretation. E. M. and E. F. wrote the draft text; all authors read, commented on and approved the final text. W. G. and J. C. are employees of Metabolon Inc., none of the authors has any conflicting interest with regard to the material presented.

Supporting Information
Additional Supporting Information may be found in the online version of this article:


Table S1. Pattern of relationships among sitagliptin-induced changes of metabolic variables, amino acids and metabolites in type 2 diabetes patients (T2D) patients.*

References


