Accepted Manuscript

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PII: S0003-9861(15)30029-1

DOI: 10.1016/j.abb.2015.08.003

Reference: YABBI 7037

To appear in: *Archives of Biochemistry and Biophysics*

Received Date: 26 April 2015

Revised Date: 15 July 2015

Accepted Date: 5 August 2015

Please cite this article as: D.O. Mook-Kanamori, R. de Mutsert, P.C.N. Rensen, C. Prehn, J. Adamski, M.d. Heijer, S. le Cessie, K. Suhre, F.R. Rosendaal, K. Willems van Dijk, Type 2 diabetes is associated with postprandial amino acid measures, *Archives of Biochemistry and Biophysics* (2015), doi: 10.1016/j.abb.2015.08.003.

Type 2 diabetes is associated with postprandial amino acid measures

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Word count (abstract): 200
Word count (main text): 3299
Keywords: Type 2 diabetes, metabolomics, amino acids, meal response, epidemiology

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Abstract

Most studies examining the association between type 2 diabetes (T2D) and amino acids have focused on fasting concentrations. We hypothesized that, besides fasting concentrations, amino acid responses to a standardized meal challenge are also associated with T2D. In a cross-sectional study of 525 participants (165 newly-diagnosed T2D, 186 newly-diagnosed impaired fasting glycaemia, and 174 normal fasting glucose), we examined postprandial amino acid concentrations and the responses (defined as the concentrations and responses 150 minutes after a standardized meal) of fourteen amino acids in relation to T2D. T2D was associated with lower postprandial concentration of seven amino acids compared to the normal fasting glucose group (lowest effect estimate for serine: -0.54 standard deviations (SD) (95% CI: -0.77, -0.32)), and higher concentrations of phenylalanine, tryptophan, tyrosine and (iso-)leucine (highest effect estimate for (iso-)leucine: 0.44 SD (95% CI: 0.20, 0.67)). Regarding the meal responses, T2D was associated with lower responses of seven amino acids (ranging from -0.55 SD ((95% CI): -0.78, -0.33) for serine to -0.25 SD ((95% CI: 0.-0.45, -0.02) for ornithine). We conclude that T2D is associated with postprandial concentrations of amino acids and a reduced amino acid meal response, indicating that these measures may also be potential markers of T2D.

Introduction

Research has focused on identifying pathophysiological pathways for the development of type 2 diabetes (1). Profiling an individual's global metabolism, or metabolomics, has shown to be a promising technique to investigate alterations in metabolism in type 2 diabetes, because it allows probing the endpoints of many disease relevant biological processes in a single experiment (2). Several studies have already been performed in the field of metabolomics and type 2 diabetes (3). Especially, concentrations of plasma amino acids have been associated with the risk of developing type 2 diabetes, both in cross-sectional and longitudinal studies (4-11). In almost all studies the branched-chain amino acids (valine, leucine and isoleucine) have been associated with type 2 diabetes risk. It has been hypothesized that these branched-chain amino acids interfere with mitochondrial metabolism of fatty acids (12). However, many other amino acids have also been shown to be involved, including phenylalanine (4-7, 9, 11), tyrosine (4, 6, 7, 9, 11), glutamine (6), glycine (8, 9, 11), arginine (5), histidine (8), ornithine (5), proline (5, 6, 10) and tryptophan (9).

So far most large scale studies were performed under fasting conditions, and did not examine the effect of nutritional interventions, such as glucose or meal challenges. A recent study showed that physiological challenges increased inter-individual variation even in phenotypically similar volunteers, revealing 'metabotypes' that were not observable in fasting metabolite profiles (13). A long fasting period leads to standardization of the metabolic state. However, the current-day human body is in a non-fasting state for the greater part of the day. Therefore, non-fasting readouts of metabolic processes are essential to assess the pathophysiology of metabolic disorders, because these represent the combined exposure to a perturbed metabolism. One population-based study on the effect of an oral glucose tolerance test (OGTT) observed that all amino acid concentrations decreased after the challenge (14). The authors also observed that for isoleucine the response to the OGTT was

less pronounced in individuals with insulin resistance (14). Conversely, a recent small interventional study showed that T2D was associated with hyperaminoacidaemia after a meal challenge (15). This metabolic inflexibility, expressed by an abnormal response to an OGTT or a meal, is assumed to be associated with an increased risk of disease. Therefore, studies focused on both fasting measurements and after a standardized meal challenge, will give a more complete and accurate picture of the metabolic pathways that are affected in diseases such as type 2 diabetes (16).

Here, we set out to investigate the behavior of amino acid metabolism in type 2 diabetes under nutritional challenge conditions. For this purpose, we examined the concentrations of fourteen amino acids in 174 individuals with newly diagnosed type 2 diabetes, 186 individuals with newly diagnosed impaired fasting glycaemia, and 165 persons with normal fasting glucose, before and after a standardized meal. We hypothesize that non-fasting amino acid concentration and the amino acid responses to a meal challenge will give more insight into the pathophysiological mechanisms involved in type 2 diabetes than fasting concentrations alone.

Research Design and Methods

Study Design

This study was embedded in the Netherlands Epidemiology of Obesity (NEO) study, a population-based, prospective cohort study designed to investigate pathways that lead to obesity-related diseases (17). The NEO study started recruitment in 2008 and includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the study. All participants gave their written informed consent.

Participants were invited to a baseline visit at NEO study center of the LUMC after an overnight fast. Prior to this study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information. At the baseline visit, participants underwent an extensive physical examination, including anthropometry, and fasting blood samples were drawn (17). Within 5 minutes after the fasting blood draw, a standardized liquid mixed meal (400mL, 600 kCal, with 16 En% protein, 50 En% carbohydrates, and 34 En% fat) was consumed and a subsequent blood sample was drawn after 30 and 150 minutes. For the present analyses we only measured amino acids concentrations in a fasting state and 150 minutes after the meal challenge. We selected participants with and without newly diagnosed impaired fasting glucose and type 2 diabetes on the basis of their baseline measurements as described below.

Population and diabetes classification

From the 6,671 participants of the NEO study, participants were selected without (1) a history of type 2 diabetes or impaired fasting glycaemia and (2) the use of any glucose- or lipid-lowering drugs. Information on diabetes status at baseline was verified via medical records of the general practitioners of the participants. A history of diabetes was defined as

the presence of a diagnosis coded in the medical records with International Classification of Primary Care (ICPC) codes T90 (diabetes mellitus, any type), T90.1 (type 1 diabetes mellitus) or T90.2 (type 2 diabetes mellitus) with a date of diagnosis before the date of the baseline study visit. A history of impaired fasting glycaemia was defined according to the presence of ICPC codes A91.05 or B85.01 (both impaired glucose tolerance) in absence of codes T90, T90.1 or T90.2, with a date of diagnosis before the baseline study visit.

The participants were then classified into three groups as described by the World Health Organization (18). Newly diagnosed type 2 diabetes was defined as having a fasting glucose ≥7.0 mmol/L at the baseline measurement without a previous history of type 2 diabetes. Newly diagnosed impaired fasting glycaemia was defined as having a fasting glucose concentration ≥6.1 mmol/L and <7.0 mmol/L at the baseline measurement without a previous history of impaired fasting glycaemia or type 2 diabetes. Participants with a fasting glucose concentration of ≤6.0 mmol/L were considered having a normal fasting glucose.

Metabolomics data (see below) were collected in a subgroup of 533 participants. All participants with newly diagnosed type 2 diabetes (n=175) were selected, which was complemented by random subset of the participants with newly diagnosed impaired fasting glycaemia (n=186) and normal fasting glucose (n=171). One participant (n=1) was excluded due to missing fasting glucose concentrations. As an additional quality control step, we compared fasting glucose concentrations with fasting hexose concentrations (which contains >90% glucose). Data were excluded from seven participants due to fasting glucose concentrations that deviated more than ±1.5 standard deviation from fasting hexose concentrations as measured as part of the metabolomics analyses, leaving 525 participants for the analyses.

Metabolomics

Metabolomic measurements were performed in both the fasting and postprandial blood samples at t=150 minutes after meal at the Genome Analysis Center at the Helmholtz Zentrum München, Germany using the Biocrates Absolute*IDQ*^{TM} p150 kit (BIOCRATES Life Science AG, Innsbruck, Austria) and ESI-FIA-MS/MS measurements (19). The p150 assay is a lipidomics assay, covering a wide range of acyl-carnitines, sphingolipids, and glycerophosphocholines, complemented by a set of amino acid measures and hexose concentrations. The method of Absolute*IDQ*^{TM} p150 kit has been proven to be in agreement with FDA-Guidelines. The assay was applied following the manufacturer's instructions and has been described in detail before (19, 20). For this study, we examined fourteen amino acids (thirteen proteinogenic amino acids and ornithine) both in a fasting state and after the meal challenge. In addition xLeucine (xLeu) was determined, which actually is the sum of leucine and isoleucine concentrations. Five amino acids could not be determined by the high throughput assay (p150) used in this study (alanine, asparagine, aspartic acid, glutamic acid, and lysine). The measurement of other blood parameters (glucose, HbA1c, and lipid profile) has been described previously (17).

Statistical analyses

First, we examined the response of the fourteen amino acid concentrations to the meal challenge using paired t-tests in each group. Then, using linear regression we examined the relation of diabetes state and each amino acid concentration by calculating effect estimates with 95% confidence intervals, adjusting for sex and age (model 1), or sex, age and BMI (model 2). We performed the linear regression for three distinct comparisons: (1) normal fasting glucose (reference) versus type 2 diabetes, (2) normal fasting glucose (reference) versus impaired fasting glycaemia, and (3) impaired fasting glycaemia (reference) versus type 2 diabetes. We examined both the effect of diabetes state on fasting and postprandial

amino acids concentrations, in addition to the response of the meal challenge [$aminoacid_{t=150}$] – [$aminoacid_{t=0}$]). Subsequently, we examined whether the association with postprandial amino acid concentrations and responses to the meal challenge differed between men and women by repeating the linear regression stratified by sex. Finally, to examine whether the association between type 2 diabetes and amino acid response is explained by fasting insulin concentrations, postprandial insulin concentrations or insulin resistance, we repeated the linear regression of amino acid response on diabetes, additionally adjusting for fasting insulin and insulin resistance (using the homeostatic model assessment of insulin resistance, HOMA-IR ([glucose x insulin]/22.5)) (21).

All statistical analyses were performed in Stata 12.1 (StataCorp, Texas, USA). For all regression analyses, amino acid concentrations were Z-score normalized (i.e. standardized to a mean of zero with a standard deviation of 1) to make effect estimates comparable. As a result, the effect estimates are expressed in standard deviations of amino acid concentration. We report both nominal significance levels ($p<0.05$) and after Bonferroni correction ($p < 1.19 \times 10^{-3}$ (=0.05/(14 x 3 comparisons))).

Results

The key characteristics of the study participants are shown in Table 1. Age and body mass index were higher in the impaired fasting glycaemia and type 2 diabetes groups than in the normal fasting glucose group. In Table 2 and Figures 1a-b the fasting and postprandial amino acid concentration are shown, stratified by diabetes state. In all three groups (normal fasting glucose, impaired fasting glycaemia and type 2 diabetes), arginine, methionine, ornithine, phenylalanine, proline, tyrosine, tryptophan, valine and (iso-)leucine concentrations increased after the meal challenge, while glycine concentrations decreased in all groups. For histidine, serine and threonine, there was a postprandial increase in the participants with normal fasting glucose and a decrease in participants with type 2 diabetes.

Table 3 shows the results from the linear regression (type 2 diabetes versus normal fasting glucose) for amino acid concentrations in a fasting state, postprandial, and for the amino acid responses to the meal challenge. After adjusting for age, sex and BMI, type 2 diabetes was associated with lower fasting glycine and valine concentrations (lowest effect estimate for glycine: -0.40 standard deviations (SD) (95% CI: -0.62, -0.18)), and higher fasting phenylalanine, tryptophan, tyrosine and (iso-)leucine concentrations associated with type 2 diabetes (highest effect estimate for (iso-)leucine: 0.62 SD (95% CI: 0.42, 0.81)). In a postprandial state, type 2 diabetes was associated with lower concentration of seven amino acids (lowest effect estimate for serine: -0.54 SD (95% CI: -0.77, -0.32)), and higher concentrations of phenylalanine, tryptophan, tyrosine and (iso-)leucine (highest effect estimate for (iso-)leucine: 0.44 SD (95% CI: 0.20, 0.67). Regarding the meal response, lower responses were observed in type 2 diabetes for seven amino acids. Effect estimates ranged from -0.55 SD ((95% CI): -0.78, -0.33) for serine to -0.25 SD ((95% CI: -0.48, -0.02) for ornithine. For thirteen of the fourteen amino acids (exception Tryptophan) the effect estimate was negative. Regarding the sex-stratified analyses, type 2 diabetes was associated with a

more negative response in men than in women (Table S1), with this sex-interaction being significant in seven of the fourteen amino acids.

Table S2 show the results from the linear regression analyses for the impaired fasting glycaemia versus normal fasting glucose. Higher fasting concentrations of four amino acids (methionine, phenylalanine, tyrosine and (iso-)leucine) were found in impaired fasting glycaemia, while valine concentrations was lower in the impaired fasting glycaemia group. Postprandially, impaired fasting glycaemia was associated with higher concentrations of phenylalanine, tryptophan, tyrosine and (iso-)leucine. For the meal responses, impaired fasting glycaemia was only associated with a lower serine response (in contrast to seven amino acids associated with type 2 diabetes (Table 3)). Regarding the comparison between the type 2 diabetes and impaired fasting glycaemia group, several associations were observed in fasting state (six associations), postprandial state (nine associations) and for the meal response (seven associations) (Table S3). However, the effect estimates were generally smaller than those observed in the comparison between the type 2 diabetes and normal fasting glucose groups. For nearly all associations shown in Table 3, Table S2 and Table S3, the effect estimates attenuated after adjustment for BMI. Finally, we examined the potentially confounding effect of insulin concentrations (both fasting and postprandial) and insulin resistance (defined by HOMA-IR) in the association between amino acid response and type 2 diabetes by adding these variables into the model (Table S4). Compared with the effect estimates for the responses shown in Table 3, the point estimates marginally shifted towards to the null in all amino acids, but for all seven associated amino acids from Table 3 the effect estimate remained below one.

Discussion

To examine how amino acid metabolism (both fasting and non-fasting) is affected in type 2 diabetes we studied the response of fourteen amino acids to a standardized meal challenge in relation to impaired fasting glycaemia and type 2 diabetes. The mixed meal of the meal challenge contained 16 En% proteins. We expected that, through protein degradation in the gut and subsequent uptake of amino acids and oligopeptides via enterocytes, the amino acid concentrations would in general increase after the meal challenge (13). In the current study most amino acids increased, but glycine decreased after the meal challenge, irrespective of type 2 diabetes. Glycine is essential for the conversion of primary bile acids to conjugated bile acids in the liver. Therefore, our findings most likely reflect increased hepatic clearance of postprandial glycine to replenish the conjugated bile acid pool in the gall bladder (22).

We were able to confirm several already known associations between fasting amino acid concentrations and type 2 diabetes (e.g. phenylalanine) (4-11). An important exception in our study was valine, of which concentrations were lower in both the fasting and especially in the postprandial state amongst participants with type 2 diabetes. One possible explanation for this finding is that none of the participants were taking any glucose- or lipid lowering drugs, thus warranting further investigation into the role of valine in type 2 diabetes under physiological conditions. Interestingly, we observed more associations amongst the postprandial amino acid concentrations than in the fasting state. Furthermore, almost all the amino acid responses to the meal challenge were lower in participants with type 2 diabetes. Prior to the study, it was difficult to predict whether the amino acid response would mirror the response of glucose and lipids metabolism (higher), or whether amino acids response would be lower in participants with type 2 diabetes. Indeed, for almost all amino acids a lower response was observed in type 2 diabetes compared to the normal fasting glucose group. For example, histidine, serine and threonine concentrations increased after the meal

challenge in participants with a normal fasting glucose, but decreased in participants with type 2 diabetes. Adjusting for insulin concentrations or insulin resistance only slightly changed the effect estimates for the association between amino acid responses and type 2 diabetes, indicating that the lower response is only partly explained by insulin resistance or beta-cell function. Since insulin resistance lowers the cellular uptake of postprandial glucose as well as fatty acids from the circulation in participants with impaired fasting glycaemia and type 2 diabetes, it could be hypothesized that this would also be true for circulating amino acids. However, our data indicate that the cellular uptake of amino acids is not influenced by insulin resistance. Interestingly, we observed that in the impaired fasting glycaemia group the amino acid concentrations (fasting and postprandial) and the responses were generally intermediate between the normal fasting glucose and the type 2 diabetes groups, indicating that such physiological changes have already partly occurred in participants with impaired fasting glycaemia.

The associations between type 2 diabetes and the amino acid response attenuated after additionally adjustment for BMI, indicating that body mass partly explains the relation between amino acids and type 2 diabetes. This finding is in line with most metabolomics studies that adjust for BMI as a measure of obesity. There has been evidence for sex dimorphism regarding fasting amino acid concentrations (23). A stronger relationship between several amino acids and type 2 diabetes (and insulin resistance) amongst men than women has been described previously (7, 24). In the current study, we also found evidence for a stronger association in men between type 2 diabetes and amino acid response than in women. Insulin resistance of protein metabolism has found to be most pronounced in men (25). This sex effect may be due to differences in the metabolic regulation through sex hormones (7) and differences in fat distribution between the sexes could also play a role (7, 25).

Some methodological aspects should be considered. A major strength of the current study was that all participants were naïve to drug treatment (6). In most cross-sectional metabolomics studies on type 2 diabetes the participants were using glucose- or lipid-lowering drugs, which could influence the results (5, 10, 26). We did not perform any matching in our study and instead adjusted for covariates, while some metabolomics studies have been performed in matched case-control designs on age, sex and BMI (4, 8). Studies have shown that matching gives similar results as after adjustment for covariates, but diminishes statistical power (27). Many metabolomics studies on type 2 diabetes confirmed the diagnosis based on questionnaire or by a physician. Since we were assessing newly diagnosed patients, we used fasting glucose to define the diabetes state. Unfortunately, we only had a single measurement and no oral glucose tolerance test as is recommended by the World Health Organization (18). Therefore, there may be some misclassification in the diabetes classification. This misclassification may be assumed to be random and would therefore attenuate the size of the effect estimates toward the null. Also, the associations observed in this study with amino acid response were based on only one postprandial measurement (150 minutes), leaving it unclear how type 2 diabetes may be associated with amino acid fluctuations over a longer time course after a meal. Additionally, we examined fourteen amino acids that are highly correlated to each other, due their interlinked biochemical pathways (Table S5). Many metabolomics studies use a Bonferroni correction for multiple testing. However, due to these high correlations between the amino acids, this type of correction would be overly conservative. We therefore decided to show whether the p-values were significant at both a nominal and a Bonferroni-corrected level. Finally, studies have yet to show whether amino acids truly play a causative role in the pathophysiology of type 2 diabetes, or whether they are merely a marker. To examine whether the observed associations could be explained by insulin resistance we adjusted for fasting insulin and

HOMA-IR. The implementation of a glucose clamp test, which is the golden standard for insulin sensitivity, was unfeasible in such a large population-based cohort study. Also, many recent studies were performed in a follow-up setting (4, 9, 11, 28), which is suitable for disease prediction of potential biomarkers. But neither from these prospective follow-up studies nor from cross-sectional studies, such as the current, can one determine whether the lower amino acid response observed in participants with newly diagnosed type 2 diabetes is part of the pathophysiological pathway, or whether the disease is causing this lower meal response.

In conclusion, studies on non-fasting amino acid concentrations, and especially amino acid responses to meal challenges, can give a more complete picture of the pathophysiological mechanisms involved in type 2 diabetes. However, it remains unclear whether these lower responses are causative for disease. Furthermore, longitudinal studies will need to be performed to examine whether a lower amino acid response can be used as a potential screening tool for early detection of type 2 diabetes.

Acknowledgements

The authors thank all individuals who participated in the Netherlands Epidemiology in Obesity study, and all participating general practitioners for inviting eligible participants. The authors also thank all research nurses for collection of the data, Anne de Boer, Margot de Waal, Henk de Jong, and Ralph Rippe for collection and merging of data from the medical records of the general practitioners, and Ingeborg de Jonge for all data management of the NEO study. We thank Julia Scarpa, Dr. Werner Römisch-Margl and Katharina Sckell for support by metabolomics measurements performed at the Helmholtz Centrum München, Genome Analysis Center, Metabolomics Core Facility.

Funding

The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area Vascular and Regenerative Medicine. In this study the metabolomics measurements and the work of Dennis Mook-Kanamori were supported by Dutch Science Organization (ZonMW-VENI Grant 916.14.023). Patrick C.N. Rensen is Established Investigator of the Dutch Heart Foundation (2009T038). This study was supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center Diabetes Research (DZD e.V.). Karsten Suhre was supported by 'Biomedical Research Program' funds at Weill Cornell Medical College in Qatar, a program funded by the Qatar Foundation.

Duality of Interest

No authors declare a conflict of interest.

Author Contributions

Study Design: RdM, MdH, SlC, FRR, KWvD

Data Analyses: DOMK, RdM, SlC, KS, KWvD

Supervision Metabolomic Measurements: CP, JA

Biological Discussion: DOMK, PCNR, CP, JA, KS, KWvD

Manuscript Writing: DOMK, RdM, FRR, KWvD

Manuscript Revision: All authors

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Table 1: Characteristics of the study population, stratified by disease state.

	Normal fasting glucose (n=174)	Impaired fasting glycaemia (n=186)	Type 2 diabetes (n=165)
Demographic/Anthropometric			
Age (years)	55.5 (46.0 – 65.0)	58.0 (46.0 – 65.0)	58.0 (47.0 – 64.0)
Males (%)	84 (48.3)	118 (63.4)	90 (54.6)
BMI (kg/m^2)	28.1 (20.7 – 36.7)	29.9 (25.1 – 38.5)	31.5 (26.2 – 42.9)
Fasting blood measures			
Glucose (mmol/L)	5.3 (4.5 – 6.0)	6.3 (6.1 – 6.8)	7.5 (7.0 – 12.1)
Insulin (mU/L)	9.1 (3.6 – 28.1)	13.5 (6.1 – 29.3)	17.1 (6.4 – 46.6)
HOMA-IR	2.2 (0.7 – 7.3)	3.9 (1.7 – 8.6)	6.0 (2.2 – 18.1)
HbA1c (%)	5.3 (4.9 – 5.7)	5.5 (5.1 – 6.0)	6.0 (5.3 – 8.6)
HbA1c (mmol/mol)	34.4 (30.0 – 38.4)	36.6 (32.5 – 42.1)	42.1 (34.4 – 70.5)
Total cholesterol (mmol/L)	5.8 (4.3 – 7.8)	5.9 (4.4 – 7.9)	5.9 (4.3 – 7.7)
HDL-cholesterol (mmol/L)	1.5 (0.9 – 2.3)	1.3 (0.8 – 2.1)	1.2 (0.8 – 1.8)
LDL-cholesterol (mmol/L)	3.8 (2.2 – 5.5)	3.7 (2.3 – 5.4)	3.7 (2.4 – 5.4)
Triglycerides(mmol/L)	1.0 (0.4 – 2.7)	1.4 (0.6 – 4.1)	1.7 (0.8 – 3.6)
Serum creatinine (µmol/L)	78 (57 – 103)	77 (59 – 97)	74 (55 – 99)
eGFR-MDRD (mL/min/1.73 m2)	81 (63 – 108)	86 (66 – 108)	87 (64 – 115)
ASAT (U/L)	23 (16 – 35)	24 (17 – 44)	25 (16 – 55)
ALAT (U/L)	22 (12 – 53)	28 (16 – 59)	33 (14 – 83)

Values represent medians (90% range).
Normal fasting glucose: Fasting glucose ≤ 6.0 mmol/L
Impaired fasting glycaemia: Fasting glucose ≥ 6.1 mmol/L and <7.0 mmol/L
Type 2 Diabetes: Fasting glucose ≥ 7.0 mmol/L

Table 2: Differences between postprandial and fasting amino acid concentrations stratified by diabetes state.

	Normal fasting glucose (n=174)		Impaired fasting glycaemia (n=186)		Type 2 diabetes (n=165)	
	Fasting	Postprandial	Fasting	Postprandial	Fasting	Postprandial
Amino acid						
Arginine	78.1 (55.4 – 104.3)	82.3 (52.9 – 109.4)**	80.8 (58.2 – 106.9)	83.7 (57.0 – 113.0)**	76.2 (58.8 – 100.1)	78.7 (54.9 – 100.6)
Glutamine	524.3 (412.5 – 619.4)	526.7 (411.2 – 639.0)	522.3 (429.8 – 638.0)	527.6 (416.8 – 637.8)	504.8 (414.4 – 607.3)	491.1 (385.7 – 608.3)*
Glycine	221.3 (150.7 – 384.4)	196.0 (133.0 – 356.7)**	205.2 (151.0 – 340.6)	179.2 (120.9 – 284.2)**	191.5 (137.3 – 290.5)	158.4 (116.0 – 255.4)**
Histidine	63.0 (51.7 – 82.0)	65.8 (50.7 – 82.0)**	63.4 (48.7 – 77.7)	63.8 (47.7 – 79.3)	62.6 (50.1 – 73.3)	59.7 (45.2 – 74.2)**
Methionine	32.6 (24.2 – 41.6)	39.4 (28.0 – 52.6)**	34.1 (25.4 – 43.8)	40.2 (28.5 – 52.7)**	33.1 (26.7 – 43.9)	37.0 (25.0 – 49.9)**
Ornithine	62.7 (39.9 – 85.6)	74.3 (48.0 – 103.0)**	65.4 (48.2 – 86.2)	74.6 (51.4 – 103.5)**	63.6 (41.7 – 90.5)	70.4 (49.0 – 102.3)**
Phenylalanine	50.2 (38.8 – 60.2)	58.1 (44.5 – 73.7)**	53.2 (43.2 – 68.7)	62.6 (47.9 – 77.3)**	54.4 (45.2 – 71.1)	61.4 (48.4 – 76.8)**
Proline	197.6 (118.5 – 309.7)	286.5 (196.3 – 447.5)**	210.7 (142.8 – 325.5)	307.9 (211.1 – 446.5)**	222.3 (150.4 – 335.9)	298.4 (198.1 – 427.8)**
Serine	99.5 (66.8 – 132.9)	100.1 (70.4 – 146.2)*	97.7 (71.0 – 134.2)	95.0 (64.7 – 133.8)*	93.0 (68.2 – 124.5)	85.1 (59.4 – 120.9)**
Threonine	112.9 (86.2 – 148.0)	117.2 (88.0 – 164.2)**	114.4 (84.8 – 162.5)	117.6 (77.6 – 160.3)	108.7 (83.5 – 160.0)	105.5 (72.3 – 164.0)
Tryptophan	83.0 (67.0 – 99.5)	84.5 (67.4 – 97.3)	85.4 (70.2 – 98.0)	87.0 (70.9 – 101.3)*	85.2 (72.1 – 103.2)	86.5 (70.8 – 103.2)
Tyrosine	71.2 (48.0 – 100.0)	90.9 (57.9 – 123.5)**	81.2 (58.7 – 108.0)	100.2 (68.5 – 133.1)**	81.3 (61.0 – 108.6)	99.1 (71.6 – 133.1)**
Valine	174.7 (127.6 – 226.4)	216.0 (161.5 – 269.2)**	171.8 (131.6 – 221.6)	217.6 (164.5 – 274.1)**	171.4 (126.6 – 233.0)	201.2 (139.8 – 259.5)**
xLeucine	184.4 (133.5 – 244.6)	242.0 (163.0 – 318.9)**	206.3 (143.2 – 270.0)	257.1 (179.8 – 337.0)**	216.6 (157.3 – 295.0)	263.6 (170.7 – 375.7)**

Values represent medians (90% range) concentrations of amino acids (µmol/L).

P-values are calculated by paired t-test.

xLeucine represents the sum of Leucine and Isoleucine.

*: $p < 0.05$ for difference between fasting and postprandial state.

**: $p < 1.19 \times 10^{-3}$ (=0.05/42 for Bonferroni correction) for difference between fasting and postprandial state.

Normal fasting glucose: Fasting glucose ≤ 6.0 mmol/L

Impaired fasting glycaemia: Fasting glucose ≥ 6.1 mmol/L and <7.0 mmol/L

Type 2 Diabetes: Fasting glucose ≥ 7.0 mmol/L

Table 3: Association between type 2 diabetes and amino acid concentrations compared with participants with normal fasting glucose.

	Fasting		Postprandial		Response	
	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Amino acid						
Arginine	-0.10 (-0.30, 0.11)	0.05 (-0.18, 0.27)	-0.30 (-0.51, -0.10)*	-0.15 (-0.37, 0.08)	-0.29, -0.50, -0.08)*	-0.25 (-0.48, -0.02)*
Glutamine	-0.21 (-0.43, 0.00)	-0.14 (-0.38, 0.09)	-0.43 (-0.64, -0.22)**	-0.30 (-0.53, -0.07)*	-0.29 (-0.49, -0.08)*	-0.21 (-0.43, 0.02)
Glycine	-0.52 (-0.72, -0.32)**	-0.40 (-0.62, -0.18)**	-0.66 (-0.86, -0.46)**	-0.53 (-0.75, -0.31)**	-0.16 (-0.36, 0.04)	-0.16 (-0.38, 0.06)
Histidine	-0.22 (-0.42, -0.01)*	-0.18 (-0.40, 0.04)	-0.59 (-0.80, -0.38)**	-0.48 (-0.70, -0.25)**	-0.49 (-0.69, -0.28)**	-0.39 (-0.61, -0.16)**
Methionine	0.22 (0.02, 0.42)*	0.15 (-0.07, 0.36)	-0.34 (-0.55, -0.12)*	-0.25 (-0.48, -0.02)*	-0.53 (-0.74, -0.32)**	-0.38 (-0.61, -0.15)**
Ornithine	0.10 (-0.12, 0.32)	0.00 (-0.25, 0.24)	-0.19 (-0.40, 0.03)	-0.20 (-0.43, 0.04)	-0.35 (-0.56, -0.14)*	-0.25 (-0.48, -0.02)*
Phenylalanine	0.64 (0.44, 0.83)**	0.53 (0.32, 0.75)**	0.32 (0.12, 0.53)*	0.31 (0.08, 0.53)*	-0.22 (-0.42, -0.01)*	-0.14 (-0.37, 0.08)
Proline	0.19 (-0.05, 0.42)	0.12 (0.35, 0.38)	0.05 (-0.19, 0.29)	0.04 (-0.23, 0.30)	-0.23 (-0.44, -0.01)*	-0.14 (-0.37, 0.10)
Serine	-0.31 (-0.52, -0.10)*	-0.21 (-0.44, 0.02)	-0.67 (-0.88, -0.46)**	-0.54 (-0.77, -0.32)**	-0.62 (-0.83, -0.42)**	-0.55 (-0.78, -0.33)**
Threonine	-0.12 (-0.32, 0.08)	-0.12 (-0.34, 0.10)	-0.42 (-0.63, -0.21)**	-0.34 (-0.57, -0.12)*	-0.45 (-0.67, -0.24)**	-0.35 (-0.58, -0.12)*
Tryptophan	0.38 (0.17, 0.59)**	0.33 (0.10, 0.56)*	0.40 (0.19, 0.61)**	0.36 (0.13, 0.59)*	0.02 (-0.19, 0.23)	0.03 (-0.20, 0.26)
Tyrosine	0.70 (0.50, 0.90)**	0.52 (0.30, 0.74)**	0.47 (0.26, 0.68)**	0.38 (0.15, 0.62)*	-0.09 (-0.30, 0.12)	-0.02 (-0.25, 0.20)
Valine	-0.14 (-0.35, 0.07)	-0.26 (-0.49, -0.04)*	-0.46 (-0.66, -0.25)**	-0.44 (-0.67, -0.22)**	-0.42 (-0.63, -0.21)**	-0.29 (-0.52, -0.06)*
xLeucine	0.74 (0.56, 0.92)**	0.62 (0.42, 0.81)**	0.46 (0.25, 0.68)**	0.44 (0.20, 0.67)**	-0.13 (-0.34, 0.08)	-0.06 (-0.29, 0.18)

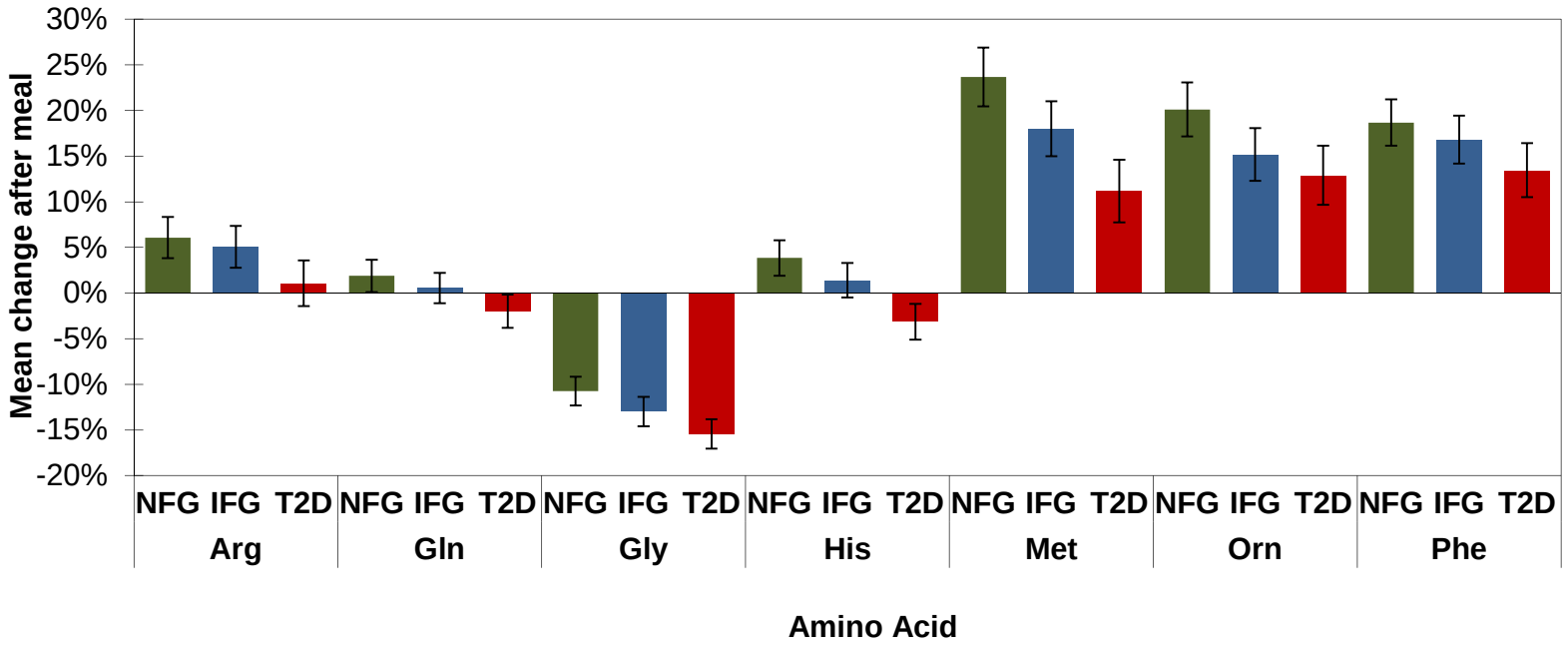
Results are based on analyses of the study population (n=174 with normal fasting glucose and n=165 with type 2 diabetes).
xLeucine represents the sum of Leucine and Isoleucine.
Amino acid concentrations have been Z-score standardized.
Values represent regression coefficients (standard deviations) from the linear regression analyses and their 95% confidence intervals (C.I.) for the risk of type 2 diabetes.
Model 1: adjusted for sex and age.
Model 2: adjusted for sex, age and BMI.
*: $p < 0.05$
**: $p < 1.19 \times 10^{-3}$ (=0.05/42 for Bonferroni correction)
Normal fasting glucose: Fasting glucose ≤ 6.0 mmol/L
Type 2 Diabetes: Fasting glucose ≥ 7.0 mmol/L

FIGURE LEGENDS

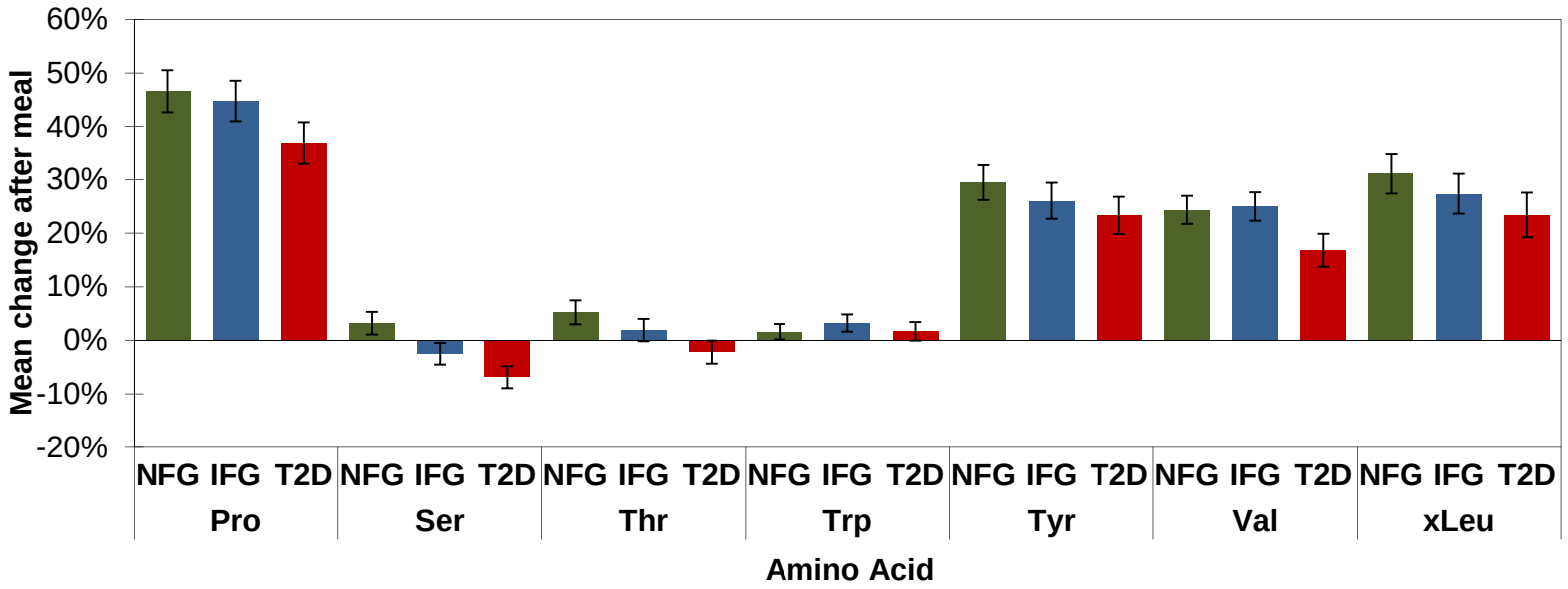
Figure 1a-b: Mean change after meal challenge in amino acid concentrations stratified by diabetes state.

Bars represent mean changes (standard deviations) in response to the meal challenge.
Arg: Arginine; Gln: Glutamine; Gly: Glycine; His: Histidine; Met: Methionine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine; xLeu: Leucine + Isoleucine.
NFG: Normal fasting glucose; IFG: Impaired fasting glycaemia; T2D: Type 2 Diabetes.

1A)


30%
25%
20%
15%
10%
5%
0%
-5%
-10%
-15%
-20%
Mean change after meal
NFG IFG T2D NFG IFG T2D NFG IFG T2D NFG IFG T2D NFG IFG T2D NFG IFG T2D NFG IFG T2D
Arg
Gln
Gly
His
Met
Orn
Phe
Amino Acid

1B)


60%
50%
40%
30%
20%
10%
0%
-10%
-20%
Mean change after meal
NFG IFG T2D
Pro
Ser
Thr
Trp
Tyr
Val
xLeu
Amino Acid

We examined postprandial amino acid concentrations in relation to type 2 diabetes

Type 2 diabetes is associated with postprandial amino acid concentrations

Physiological changes also present in patients with impaired fasting glycaemia

Associations appear not be me mediated by insulin resistance

ONLINE SUPPLEMENTAL TABLES

Table S1: Association between type 2 diabetes and postprandial amino acid concentration and the response to the meal challenge compared with participants with normal fasting glucose, stratified by sex.

	Postprandial		Response		Males versus Females	
	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	p-value interaction	p-value interaction
	Males	Females	Males	Females	Postprandial	Response
Amino acid						
Arginine	-0.21 (-0.52, 0.09)	-0.07 (-0.39, 0.26)	-0.40 (-0.72, -0.09)*	-0.07 (-0.42, 0.27)	0.32	0.39
Glutamine	-0.35 (-0.65, -0.05)*	-0.25 (-0.60, 0.11)	-0.27 (-0.58, 0.04)	-0.13 (-0.47, 0.22)	0.31	0.76
Glycine	-0.45 (-0.66, -0.23)**	-0.62 (-1.02, -0.22)*	-0.26 (-0.51, 0.00)*	-0.05 (-0.42, 0.32)	3.2×10^{-4}	1.6×10^{-4}
Histidine	-0.57 (-0.89, -0.26)**	-0.37 (-0.68, -0.05)*	-0.49 (-0.80, -0.18)*	-0.26 (-0.59, 0.07)	0.55	0.71
Methionine	-0.36 (-0.68, -0.05)*	-0.11 (-0.47, 0.24)	-0.55 (-0.83, -0.26)**	-0.18 (-0.53, 0.18)	0.29	1.3×10^{-3}
Ornithine	-0.17 (-0.46, 0.13)	-0.22 (-0.59, 0.16)	-0.28 (-0.55, 0.00)*	-0.21 (-0.59, 0.18)	0.86	0.85
Phenylalanine	0.16 (-0.15, 0.46)	0.48 (0.15, 0.81)*	-0.33 (-0.63, -0.03)*	0.07 (-0.27, 0.42)	0.57	3.6×10^{-5}
Proline	-0.21 (-0.59, 0.17)	0.34 (-0.01, 0.69)	-0.34 (-0.64, -0.05)*	0.12 (-0.25, 0.48)	0.57	2.4×10^{-5}
Serine	-0.41 (-0.69, -0.12)*	-0.69 (-1.05, -0.34)**	-0.60 (-0.89, -0.31)**	-0.49 (-0.84, -0.14)*	0.16	0.78
Threonine	-0.36 (-0.67, -0.04)*	-0.33 (-0.66, 0.02)	-0.45 (-0.74, -0.15)*	-0.24 (-0.60, 0.12)	0.85	0.93
Tryptophan	0.22 (-0.08, 0.53)	0.51 (0.17, 0.85)*	-0.16 (-0.47, 0.15)	0.25 (-0.09, 0.60)	0.01	0.03
Tyrosine	0.25 (-0.04, 0.53)	0.53 (0.16, 0.89)	-0.15 (-0.44, 0.13)	0.13 (-0.23, 0.49)	0.45	1.0×10^{-4}
Valine	-0.55 (-0.85, -0.24)**	-0.32 (-0.66, 0.02)	-0.37 (-0.68, -0.06)*	-0.19 (-0.53, 0.15)	3.8×10^{-5}	0.24
xLeucine	0.34 (0.02, 0.67)*	0.55 (0.21, 0.90)*	-0.23 (-0.54, 0.09)	0.16 (-0.19, 0.50)	3.0×10^{-4}	8.4×10^{-4}

Results are based on analyses of the study population (n=174 males (84 normal fasting glucose/90 type 2 diabetes) and n=165 females (90 normal fasting glucose/75 type 2 diabetes).
xLeucine represents the sum of Leucine and Isoleucine.
Amino acid concentrations have been Z-score standardized.
Values represent regression coefficients (standard deviations) from the linear regression analyses and their 95% confidence intervals (C.I.) for the risk of type 2 diabetes.
Model: adjusted for age and BMI.
*: $p < 0.05$
**: $p < 8.93 \times 10^{-4}$ (=0.05/56 for Bonferroni correction)
Normal fasting glucose: Fasting glucose ≤ 6.0 mmol/L
Type 2 Diabetes: Fasting glucose ≥ 7.0 mmol/L

Table S2: Association between impaired fasting glycaemia and amino acid concentrations compared with participants with normal fasting glucose.

	Fasting		Postprandial		Response	
	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Amino acid						
Arginine	0.16 (-0.07, 0.38)	0.22 (-0.01, 0.45)	0.11 (-0.11, 0.33)	0.22 (-0.01, 0.44)	-0.04 (-0.24, 0.17)	0.03 (-0.19, 0.24)
Glutamine	0.05 (-0.17, 0.27)	0.10 (-0.13, 0.32)	-0.03 (-0.24, 0.18)	0.07 (-0.14, 0.28)	-0.09 (-0.30, 0.12)	-0.02 (-0.23, 0.20)
Glycine	-0.13 (-0.34, 0.08)	0.00 (-0.21, 0.21)	-0.22 (-0.43, -0.01)*	-0.08 (-0.30, 0.13)	-0.17 (-0.39, 0.05)	-0.18 (-0.41, 0.05)
Histidine	0.00 (-0.22, 0.22)	0.02 (-0.21, 0.25)	-0.16 (-0.37, 0.05)	-0.07 (-0.29, 0.14)	-0.20 (-0.41, 0.01)	-0.11 (-0.33, 0.10)
Methionine	0.29 (0.08, 0.49)*	0.26 (0.05, 0.48)*	0.03 (-0.18, 0.23)	0.12 (-0.10, 0.33)	-0.19 (-0.39, 0.01)	-0.08 (-0.28, 0.13)
Ornithine	0.22 (0.02, 0.42)*	0.17 (-0.04, 0.38)	0.03 (-0.18, 0.24)	0.04 (-0.17, 0.26)	-0.20 (-0.41, 0.01)	-0.13 (-0.35, 0.09)
Phenylalanine	0.46 (0.26, 0.65)**	0.40 (0.20, 0.60)**	0.37 (0.16, 0.58)**	0.41 (0.20, 0.63)**	-0.01 (-0.21, 0.19)	0.08 (-0.13, 0.29)
Proline	0.01 (-0.20, 0.22)	0.01 (-0.21, 0.23)	0.03 (-0.18, 0.24)	0.07 (-0.15, 0.29)	0.04 (-0.16, 0.24)	0.13 (-0.08, 0.33)
Serine	0.02 (-0.19, 0.23)	0.11 (-0.11, 0.32)	-0.23 (-0.43, -0.03)*	-0.10 (-0.31, 0.10)	-0.38 (-0.59, -0.17)**	-0.30 (-0.52, -0.08)*
Threonine	0.15 (-0.06, 0.36)	0.13 (-0.09, 0.34)	-0.03 (-0.23, 0.17)	0.02 (-0.19, 0.22)	-0.24 (-0.45, -0.02)*	-0.15 (-0.36, 0.07)
Tryptophan	0.21 (0.01, 0.41)*	0.18 (-0.03, 0.40)	0.36 (0.16, 0.57)**	0.37 (0.16, 0.58)**	0.16 (-0.05, 0.36)	0.19 (-0.02, 0.40)
Tyrosine	0.59 (0.39, 0.80)**	0.47 (0.27, 0.68)**	0.47 (0.27, 0.67)**	0.43 (0.22, 0.64)**	0.02 (-0.19, 0.22)	0.08 (-0.13, 0.28)
Valine	-0.19 (-0.38, 0.00)*	-0.29 (-0.49, -0.10)*	-0.09 (-0.28, 0.10)	-0.08 (-0.28, 0.12)	0.08 (-0.12, 0.28)	0.19 (-0.02, 0.39)
xLeucine	0.32 (0.16, 0.48)**	0.23 (0.06, 0.40)*	0.22 (0.04, 0.41)*	0.21 (0.02, 0.40)*	-0.03 (-0.23, 0.16)	0.04 (-0.17, 0.24)

Results are based on analyses of the study population (n=174 normal fasting glucose and n=186 impaired fasting glycaemia).
xLeucine represents the sum of Leucine and Isoleucine.
Amino acid concentrations have been Z-score standardized.
Values represent regression coefficients (standard deviations) from the linear regression analyses and their 95% confidence intervals (C.I.) for the risk of impaired fasting glycaemia.
Model 1: adjusted for sex and age.
Model 2: adjusted for sex, age and BMI.
*: $p < 0.05$
**: $p < 1.19 \times 10^{-3}$ (=0.05/42 for Bonferroni correction)
Normal fasting glucose: Fasting glucose ≤ 6.0 mmol/L
Impaired fasting glycaemia: Fasting glucose ≥ 6.1 mmol/L and <7.0 mmol/L

Table S3: Association between type 2 diabetes and amino acid concentrations compared with participants with impaired fasting glycaemia.

	Fasting		Postprandial		Response	
	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Amino acid						
Arginine	-0.24 (-0.44, -0.03)*	-0.21 (-0.41, 0.00)*	-0.42 (-0.62, -0.21)**	-0.35 (-0.56, -0.14)**	-0.28 (-0.50, -0.07)*	-0.23 (-0.44, -0.01)*
Glutamine	-0.27 (-0.47, -0.06)*	-0.26 (-0.47, -0.05)*	-0.42 (-0.63, -0.21)**	-0.36 (-0.57, -0.15)**	-0.22 (-0.43, -0.00)*	-0.16 (-0.37, 0.06)
Glycine	-0.38 (-0.56, -0.20)**	-0.37 (-0.55, -0.18)**	-0.44 (-0.62, -0.26)**	-0.41 (-0.60, -0.23)**	-0.01 (-0.21, 0.19)	0.00 (-0.20, 0.20)
Histidine	-0.21 (-0.42, 0.00)	-0.20 (-0.41, 0.02)	-0.45 (-0.65, -0.25)**	-0.39 (-0.60, -0.19)**	-0.33 (-0.53, -0.12)*	-0.27 (-0.48, -0.06)*
Methionine	-0.05 (-0.25, 0.16)	-0.08 (-0.29, 0.13)	-0.38 (-0.59, -0.17)**	-0.32 (-0.53, -0.11)*	-0.37 (-0.58, -0.16)**	-0.29 (-0.49, -0.08)*
Ornithine	-0.11 (-0.33, 0.10)	-0.18 (-0.39, 0.04)	-0.22 (-0.43, 0.00)*	-0.24 (-0.46, -0.02)*	-0.16 (-0.38, 0.05)	-0.12 (-0.33, 0.10)
Phenylalanine	0.20 (-0.01, 0.41)	0.14 (-0.08, 0.35)	-0.07 (-0.28, 0.14)	-0.06 (-0.27, 0.16)	-0.24 (-0.46, -0.03)*	-0.18 (-0.40, 0.04)
Proline	0.14 (-0.04, 0.32)	0.09 (-0.09, 0.27)	-0.04 (-0.22, 0.15)	-0.05 (-0.24, 0.14)	-0.31 (-0.52, -0.11)*	-0.26 (-0.46, -0.05)*
Serine	-0.30 (-0.51, -0.10)*	-0.31 (-0.52, -0.10)*	-0.43 (-0.63, -0.24)**	-0.41 (-0.61, -0.21)**	-0.26 (-0.47, -0.06)*	-0.22 (-0.42, -0.01)*
Threonine	-0.28 (-0.49, -0.06)*	-0.30 (-0.53, -0.08)*	-0.41 (-0.62, -0.20)**	-0.39 (-0.61, -0.18)**	-0.24 (-0.44, -0.03)*	-0.18 (-0.39, 0.03)
Tryptophan	0.18 (-0.02, 0.38)	0.14 (-0.06, 0.35)	0.02 (-0.20, 0.23)	0.01 (-0.21, 0.22)	-0.17 (-0.39, 0.05)	-0.14 (-0.37, 0.08)
Tyrosine	0.10 (-0.10, 0.30)	0.01 (-0.19, 0.21)	-0.02 (-0.23, 0.19)	-0.05 (-0.26, 0.16)	-0.13 (-0.34, 0.09)	-0.07 (-0.29, 0.14)
Valine	0.04 (-0.16, 0.23)	-0.03 (-0.22, 0.17)	-0.38 (-0.59, -0.17)**	-0.37 (-0.58, -0.16)**	-0.50 (-0.71, -0.29)**	-0.43 (-0.64, -0.21)**
xLeucine	0.42 (0.23, 0.90)**	0.36 (0.18, 0.54)**	0.21 (0.00, 0.43)	0.23 (0.01, 0.45)*	-0.13 (-0.35, 0.09)	-0.06 (-0.28, 0.16)

Results are based on analyses of the study population (n=186 impaired fasting glycaemia and n=165 type 2 diabetes).
xLeucine represents the sum of Leucine and Isoleucine.
Amino acid concentrations have been Z-score standardized.
Values represent regression coefficients (standard deviations) from the linear regression analyses and their 95% confidence intervals (C.I.) for the risk of type 2 diabetes.
Model 1: adjusted for sex and age.
Model 2: adjusted for sex, age and BMI.
*: $p < 0.05$
**: $p < 1.19 \times 10^{-3}$ (=0.05/42 for Bonferroni correction)
Impaired fasting glycaemia: Fasting glucose ≥ 6.1 mmol/L and <7.0 mmol/L
Type 2 Diabetes: Fasting glucose ≥ 7.0 mmol/L

Table S4: Association between type 2 diabetes and postprandial amino acid concentration and the response to the meal challenge compared with participants with normal fasting glucose, adjusted for fasting insulin and insulin resistance (HOMA-IR).

	Fasting insulin		Postprandial insulin		Insulin resistance (HOMA-IR)	
	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)	Beta (95% C.I.)
	Postprandial	Response	Postprandial	Response	Postprandial	Response
Amino acid						
Arginine	-0.11 (-0.33, 0.12)	-0.24 (-0.48, 0.00)*	-0.10 (-0.33, 0.13)	-0.26 (-0.50, -0.02)*	-0.08 (-0.31, 0.15)	-0.23 (-0.47, 0.02)
Glutamine	-0.28 (-0.51, -0.04)*	-0.21 (-0.44, 0.02)	-0.26 (-0.50, -0.03)*	-0.17 (-0.40, 0.07)	-0.26 (-0.50, -0.02)*	-0.22 (-0.46, 0.02)
Glycine	-0.49 (-0.71, -0.26)**	-0.17 (-0.39, 0.06)	-0.43 (-0.65, -0.20)**	-0.14 (-0.37, 0.08)	-0.46 (-0.69, -0.23)**	-0.17 (-0.40, 0.06)
Histidine	-0.45 (-0.68, -0.22)**	-0.39 (-0.62, -0.16)*	-0.46 (-0.69, -0.23)**	-0.36 (-0.59, -0.13)*	-0.42 (-0.65, -0.19)**	-0.39 (-0.63, -0.16)*
Methionine	-0.21 (-0.45, 0.03)	-0.33 (-0.56, -0.10)*	-0.31 (-0.54, -0.07)*	-0.38 (-0.61, -0.14)*	-0.19 (-0.43, 0.06)	-0.30 (-0.54, -0.07)*
Ornithine	-0.15 (-0.40, 0.09)	-0.24 (-0.47, 0.00)*	-0.16 (-0.40, 0.09)	-0.21 (-0.45, 0.02)	-0.12 (-0.37, 0.13)	-0.23 (-0.47, 0.01)
Phenylalanine	0.30 (0.07, 0.53)*	-0.12 (-0.35, 0.11)	0.22 (-0.01, 0.45)	-0.16 (-0.40, 0.07)	0.31 (0.07, 0.54)*	-0.10 (-0.33, 0.14)
Proline	0.06 (-0.21, 0.32)	-0.10 (-0.33, 0.14)	0.03 (-0.24, 0.30)	-0.16 (-0.40, 0.08)	0.07 (-0.21, 0.34)	-0.07 (-0.31, 0.17)
Serine	-0.53 (-0.76, -0.30)**	-0.54 (-0.77, -0.31)**	-0.54 (-0.77, -0.30)**	-0.53 (-0.76, -0.30)**	-0.53 (-0.76, -0.29)**	-0.54 (-0.77, -0.30)**
Threonine	-0.31 (-0.54, -0.08)*	-0.33 (-0.57, -0.10)*	-0.36 (-0.59, -0.12)*	-0.34 (-0.57, -0.10)*	-0.28 (-0.52, -0.04)*	-0.32 (-0.56, -0.08)*
Tryptophan	0.36 (0.13, 0.60)*	0.05 (-0.18, 0.29)	0.32 (0.08, 0.56)*	0.05 (-0.19, 0.29)	0.36 (0.12, 0.60)*	0.07 (-0.17, 0.31)
Tyrosine	0.38 (0.14, 0.62)*	0.02 (-0.21, 0.24)	0.29 (0.05, 0.53)*	-0.03 (-0.26, 0.21)	0.39 (0.14, 0.63)*	0.04 (-0.20, 0.27)
Valine	-0.42 (-0.65, -0.19)**	-0.27 (-0.51, -0.04)*	-0.42 (-0.65, -0.19)**	-0.21 (-0.44, 0.02)	-0.36 (-0.60, -0.13)*	-0.24 (-0.48, 0.00)*
xleucine	0.46 (0.21, 0.70)**	-0.03 (-0.27, 0.20)	0.37 (0.13, 0.62)*	-0.09 (-0.33, 0.15)	0.46 (0.21, 0.71)**	-0.02 (-0.26, 0.22)

Results are based on analyses of the study population (n=174 normal fasting glucose and n=165 type 2 diabetes).
xLeucine represents the sum of Leucine and Isoleucine.
Amino acid concentrations have been Z-score standardized.
Values represent regression coefficients (standard deviations) from the linear regression analyses and their 95% confidence intervals (C.I.) for the risk of type 2 diabetes.
Model for fasting insulin: adjusted for sex, age, fasting insulin and BMI.
Model for postprandial insulin: adjusted for sex, age, fasting insulin and BMI.
Model for HOMA-IR: adjusted for sex, age, HOMA-IR and BMI.
HOMA-IR: (fasting glucose * fasting insulin) / 22.5.
*: $p < 0.05$
**: $p < 6.0 \times 10^{-4}$ (=0.05/84 for Bonferroni correction)
Normal fasting glucose: Fasting glucose ≤ 6.0 mmol/L
Type 2 Diabetes: Fasting glucose ≥ 7.0 mmol/L

Table S5: Pearson correlation coefficient between the fourteen amino acids.

	Gln	Gly	His	Met	Orn	Phe	Pro	Ser	Thr	Trp	Tyr	Val	xLeu
Arg	.51*	.25*	.42*	.48**	.19*	.36*	.17*	.28*	.42*	.42*	.30*	.16*	.25*
Gln		.35*	.56*	.50**	.43*	.37*	.21*	.44*	.47*	.52*	.30*	.22*	.24*
Gly			.16*	.13**	.24*	-.05	.10*	.58*	.27*	.07	-.04	-.12*	-.17*
His				.54**	.25*	.42*	.14*	.35*	.52*	.45*	.33*	.25*	.26*
Met					.33*	.71*	.31*	.31*	.58*	.67*	.69*	.42*	.58*
Orn						.31*	.24*	.29*	.37*	.35*	.33*	.30*	.30*
Phe							.22*	.12*	.31*	.67*	.68*	.46*	.65*
Pro								.05	.24*	.25*	.24*	.22*	.27*
Ser									.47*	.19*	.13*	.01	.03
Thr										.39*	.37*	.22*	.21*
Trp											.63*	.48*	.61*
Tyr												.36*	.55*
Val													.76*

Values represent Pearson correlation coefficients.
*: $p<0.05$