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Higher Branched-chain Amino Acids and Lower Serine Exist in the Plasma of Nondiabetic Mice: A Comparison Between High- and Low-protein Diet Conditions

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Abstract. *Background/aim: The effects of dietary protein and carbohydrate content on the plasma amino acid profile of patients with diabetes are not fully understood. Therefore, we examined whether there are effects of diets with differing proportions of protein and carbohydrate on the plasma amino acid concentrations of control (CT) mice and mice with type 2 diabetes (db). Materials and Methods: We used db mice as an animal model of type 2 diabetes which are genetically deficient in leptin receptor. Diets with differing proportions of protein and carbohydrates (L diet: low protein/carbohydrate ratio, H diet: high protein/carbohydrate ratio) were supplied. db Mice were fed with a restriction on the basis of the consumption by CT-L mice, such that equivalent amounts of energy and fat were consumed. In CT mice fed the L or H diets, there was no significant difference in ad libitum food intake. Results: There were significant interactions between diet and genotype with respect to water intake, urine volume, urinary glucose concentration, and plasma isoleucine, leucine, valine, branched-chain amino acids, and serine concentrations. db-H mice showed significantly higher water intake, urine volume, and urinary glucose than db-L mice. db Mice fed the L or H diets had similar plasma amino acid profiles, except for valine. In contrast, CT-H mice showed significantly higher valine and branched-chain amino acids and lower serine concentrations than CT-L mice. Thus, the CT-H mice were more similar to db mice fed either of the diets. Conclusion: There were different effects of the dietary protein or carbohydrate content on the plasma amino acid profiles between nondiabetic and diabetic mice. In particular, the profiles in nondiabetic conditions were different between the low- and high-protein diet conditions.*

The increasing prevalence of type 2 diabetes worldwide can be explained by changes in lifestyle, including dietary habits (1). The principal change in dietary habits has been a higher intake of fat that is associated with insulin hypersecretion and resistance, which represents a prediabetic condition (2). In addition, it has been shown that higher energy intake in the form of protein, particularly in the form of animal protein, is related to the onset of type 2 diabetes in epidemiological studies, including studies conducted in Japan (3, 4). Moreover, a high plasma branched-chain amino acids (BCAAs) concentration prior to increase in blood glucose may predict the onset of type 2 diabetes, which suggests that blood BCAAs may promote the progression of prediabetes to type 2 diabetes (5-7).

The effects of dietary protein content on the complications of type 2 diabetes have also been shown in animals (8-11). We showed that mice with type 2 diabetes that consume a high-protein diet and are pair-fed according to the energy and fat intake of control mice demonstrate more rapid deteriorations in their diabetic and renal phenotypes than *db* mice fed a low-protein diet (9). In addition, control mice fed a high-protein diet have higher kidney mass, greater urinary albumin excretion, and higher renal expression of angiotensinogen and angiotensin-converting enzyme than mice fed a low-protein diet (9).

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The effects of the dietary protein and carbohydrate content on the plasma amino acid profile of patients with diabetes are not fully understood. In addition to evaluating the effects of diets with differing proportions of protein and carbohydrate on renal manifestations and insulin sensitivity, we examined whether there are different effects of the diets on the plasma amino acid concentrations of nondiabetic mice as control (CT) and diabetic mice (*db*) using restriction-feeding experiments.

Materials and Methods

Animals. Four-week-old male diabetic *db* mice [C57BLKS(BKS). Cg-+Lepr^{db}/+Lepr^{db}/J] that have a homozygous mutation in their leptin receptor gene and nondiabetic control (CT) mice (BKS. Cg-Dock7m +/Dock7m +/J) were purchased from Charles River Japan (Kanagawa, Japan) (12). This experiment was approved by the Animal Ethics Committee of Kagoshima University (approval number MD10051). The mice were housed individually in a humidity- and temperature-controlled (50±10%, 22±2˚C) facility under a 12-h light/dark cycle (07:00-19:00 h). The mice had *ad libitum* access to water. For acclimation to special foods and to avoid decreased food intake, six CT mice received 12% (low protein; L) or 24% (high protein; H) protein diets, which contained 50% animal and 50% plant protein (Table I) for three days. The amino acid amount of the diets was calculated using the information (13). At the initial time, the mice showed body weights of 10.6±0.3 g (CT-L, n=3), 10.6±0.6 g (CT-H, n=3), 17.7±0.3 g (*db*-L, n=5), and 18.3±0.6 g (*db*-H, n=5). There was no significant difference in body weight between mice fed the different diets of the respective genotype. Subsequently, they were housed individually in metabolic cages (3600 M021, Tecniplast Japan, Co., Ltd., Tokyo, Japan) for 15 days. We measured the amount of diet consumed by CT mice that were fed the L diet *ad libitum* (14) under metabolic cage conditions. As shown in Table II, the total amount of food consumed by the CT mice was a mean of 47.7 g over 15 days (the mean amount/day: 3.2 g), and CT mice that were fed the H diet *ad libitum* consumed a similar amount of food. In the restriction-feeding experiment for *db* mice, we supplied 3.2 g of each diet per day to the *db* mice for three days as the acclimation phase except the first day (2.0 g due to the arrival time). Subsequently, they were housed individually in metabolic cages for 15 days, and the respective diet was supplied at 3.2 g per day. Water intake was measured once per week. Urine was collected, the volume was measured between 07:00 and 07:00 h on days 13-14 and stored at −80˚C for later analysis. On the final day (day 15), the mice were anesthetized using pentobarbital (100 mg/kg) after 6 h of fasting (07:00-13:00 h), blood was collected by cardiac puncture, mixed with EDTA (final concentration 4 mM) and centrifuged, and the supernatant was stored at −80˚C until analysis. Finally, the mice were euthanized with a neck dislocation procedure, and their organs were carefully dissected and stored at −80˚C until analysis.

Biochemical measurements. The blood and urinary glucose concentrations were measured using a commercial kit (glucose CII-test Wako; WAKO, Tokyo, Japan) according to the manufacturer's instructions. The plasma insulin and urinary C-peptide concentrations were measured using ELISA kits (Morinaga Institute of Biological Science Inc., Kanagawa, Japan and Yanaihara Institute Inc., Shizuoka, Japan, respectively). Amino acid and urea concentrations were measured using an amino acid analyzer and the ninhydrin reaction (JLC-500/V, JEOL Ltd., Tokyo, Japan).

Table I. *Composition of the diets fed to the mice*.

A

Dietary component	Diets (% energy)	
	L	H
Protein	12	24
Carbohydrates	71	59
Fat	17	17
Total	100	100
kcal/g (kJ/g)	4.3 (18.0)	4.3 (18.0)
P/C ratio	0.17	0.41

B

	L	H
Essential amino acids (nmol/g)		
Histidine	206.2	412.4
Isoleucine	429.4	858.8
Leucine	746.5	1,493.0
Lysine	551.6	1,103.2
Methionine	158.7	317.4
Threonine	381.5	763.0
Tryptophan	72.1	144.2
Phenylalanine	348.7	697.4
Valine	546.3	1,092.6
Non-essential amino acids (nmol/g)		
Alanine	452.6	905.2
Arginine	367.4	734.8
Aspartic acid	783.8	1,576.6
Cystine	40.7	81.4
Glutamic acid	1,566.0	3,132.0
Glycine	443.3	886.6
Proline	817.2	1,634.4
Serine	560.3	1,120.6
Tyrosine	293.2	586.4
Branched-chain amino acids (nmol/g)	1,722.2	3,444.4
Aromatic amino acids (nmol/g)	641.9	1,283.8

P/C ratio means protein/carbohydrates. The amount of amino acids of the diets was calculated (see Materials and Methods).

Statistical analyses. Data are shown as the means±standard errors (SE). Statistical analyses were performed using two-way analysis of variance (ANOVA) and a *post hoc* Tukey's HSD test or the Mann-Whitney *U*-test, as appropriate. $p<0.05$ was accepted as indicating statistical significance (SPSS, Medical model version 24., IBM Japan).

Results

Effects of the diets with differing proportions of protein and carbohydrates on diabetic conditions. As shown in Table

Table II. *Characteristics of control and diabetic mice fed diets composed of differing proportions of proteins and carbohydrates.*

	CT		*db*		*p*-Value		
Parameter	L	H	L	H	Diet	Genotype	Diet×Genotype
n	3	3	5	5			
Food intake (g)	47.7±1.8	49.1±1.1^{NS}	48.0	48.0			NA
Body mass (g)	19.0±0.8	20.4±0.4	23.2±0.3	23.5±0.4	0.128	**0.000**	0.265
Water intake (g/week)	18.2±1.4^{a}	17.5±1.7^{a}	30.1±1.8^{b}	40.3±1.1^{c}			**0.005**
Urine volume (g/day)	1.04±0.13^{a}	0.85±0.14^{a}	3.07±0.28^{b}	4.65±0.24^{c}			**0.005**
Urinary glucose (mg/day)	0.20±0.02^{a}	0.93±0.36^{a}	369.52±29.55^{b}	545.45±18.23^{c}			**0.003**
Urinary C-peptide (ng/day)	0.8±0.3	1.5±0.2	2.6±0.9	6.5±2.1	0.148	**0.045**	0.312
Fasting blood glucose (mg/dl)	157±3	132±24	135±20	79±15	0.053	0.073	0.418
Plasma insulin (μg/l)	0.7±0.1	0.5±0.4	5.0±1.4	5.1±0.8	0.954	**0.002**	0.892

Data are means±SE and were analyzed using two-way ANOVA except food intake. Food intake was analyzed using Mann-Whitney *U*-test. Values sharing identical superscripts ($^{a, b, c}$) are not significantly different. Significant differences by two-way ANOVA are shown in bold. NA: Not applicable; NS: not significant *vs*. CT (control)-L.

II, there were significant interactions between diet and genotype with respect to water intake, urine volume, and urinary glucose concentration among the four groups. *db*-H mice had significantly higher values for all these parameters than the *db*-L, CT-L, and CT-H mice. Regarding the parameters that showed no significant interaction, *db* mice had significantly higher body mass, urinary C-peptide concentration, and plasma insulin concentration than CT mice.

Effects of the diets with differing proportions of carbohydrates and protein on plasma amino acid profile. As shown in Table III, there were significant interactions between diet and genotype with respect to plasma isoleucine, leucine, valine, serine, and BCAA concentrations among the four groups. CT-H mice had significantly higher valine and lower serine concentrations than CT-L mice. In addition, *db*-H and *db*-L mice had significantly higher isoleucine and leucine concentrations than CT-L and CT-H mice. Furthermore, CT-H mice had significantly higher BCAAs concentrations than CT-L mice, and *db*-H and *db*-L mice had significantly higher concentrations than CT-L and CT-H mice. With respect to genotype, *db* mice had significantly higher plasma phenylalanine, citrulline, and urea concentrations but lower lysine, methionine, threonine, alanine, asparagine, cystine, glycine, proline, and tyrosine concentrations than CT mice. With respect to diet, mice fed the H diet had significantly lower threonine, tryptophan, and citrulline concentrations than mice fed the L diet. Mice fed the H-diet containing higher amino acid amounts showed lower values in plasma than mice fed the L-diet, suggesting that plasma amino acid levels are modulated by metabolic conditions.

Discussion

In the present study, we investigated the influence of dietary protein content on plasma amino acid concentrations in nondiabetic and leptin receptor-deficient diabetic mice fed a similar amount of food. There were significant interactions between diet and genotype with respect to the plasma isoleucine, leucine, valine, BCAAs, and serine concentrations. Nondiabetic mice fed a high-protein diet had significantly higher valine and BCAAs and lower serine concentrations than nondiabetic mice fed a low-protein diet. In contrast, diabetic mice had similar amino acid profiles while consuming each diet, with the exception of lower valine.

Herein, we used the two diets (12% and 24% of energy from the protein, and the same 17% of energy from fat), which are based on the previous mouse studies (8, 9), as a low- and high-protein diet, leading to the relatively high content of carbohydrate. We set the range between 12 and 24% protein content as the energy base, which is the range used in the regular diet of humans (15, 16). Namely, a 12-24% protein energy containing diet corresponds to the consumption of 0.9-1.8 g protein/kg of body weight (BW)/day, in the case of 30 kcal energy expenditure/kg of BW/day.

The high BCAAs and low serine concentrations in nondiabetic mice fed a high-protein diet are interesting. High concentrations of BCAAs have been shown to induce insulin resistance in several organs, including skeletal muscle (17-19). Moreover, high BCAAs concentrations may influence neurotransmitter concentrations in the brain (20). At the blood-brain barrier, BCAAs compete with large neutral amino acids, such as tryptophan and glutamate, for uptake. Tryptophan is converted to serotonin, a key neurotransmitter in the brain (21), and in the presence of high BCAAs

Table III. *Plasma amino acid and urea concentrations in control and diabetic mice fed diets composed of differing proportions of protein and carbohydrate.*

	CT		*db*		*p*-Value		
Diet	L	H	L	H	Diet	Genotype	Diet×Genotype
n	3	3	5	5			
Essential amino acids (nmol/ml)							
Histidine	52.5±1.3	45.9±8.8	45.6±1.6	39.6±3.1	0.139	0.127	0.944
Isoleucine	50.0±4.4[a]	64.2±4.0[a]	93.1±2.4[b]	89.8±3.4[b]			**0.029**
Leucine	78.4±6.6[a]	101.3±9.0[a]	132.3±2.6[b]	129.0±5.8[b]			**0.046**
Lysine	271.2±3.4	210.0±40.9	158.2±4.8	163.3±18.5	0.180	**0.002**	0.118
Methionine	43.1±0.2	34.3±7.4	23.3±1.0	22.6±2.0	0.152	**0.000**	0.216
Threonine	125.1±4.6	98.8±16.0	71.7±3.2	59.7±1.4	**0.012**	**0.000**	0.292
Tryptophan	62.8±2.4	49.7±2.0	68.1±6.0	56.8±3.6	**0.026**	0.222	0.860
Phenylalanine	45.5±1.2	57.0±8.2	67.1±2.7	67.7±4.7	0.222	**0.005**	0.267
Valine	131.8±5.4[a]	159.5±2.3[b]	200.7±4.7[c]	181.3±5.4[b]			**0.001**
Non-essential amino acids (nmol/ml)							
Alanine	249.5±8.6	254.1±106.0	124.9±15.2	132.2±14.5	0.887	**0.011**	0.975
Arginine	74.7±6.4	38.6±18.3	36.7±5.5	40.2±7.9	0.114	0.081	0.061
Aspartic acid	7.8±0.5	7.9±1.7	7.9±1.2	6.6±0.6	0.598	0.606	0.561
Asparagine	33.2±0.5	30.0±7.9	20.4±1.2	17.4±2.4	0.330	**0.002**	0.976
Cystine	7.7±0.3	4.6±2.1	1.3±0.2	1.3±0.2	0.079	**0.000**	0.074
Glutamic acid	37.3±2.6	32.5±11.5	33.1±4.0	28.3±3.9	0.413	0.474	0.997
Glutamine	623.0±21.5	563.2±64.7	518.5±35.3	506.4±40.3	0.425	0.089	0.594
Glycine	223.3±15.1	199.8±19.4	146.3±9.0	156.3±8.2	0.589	**0.000**	0.193
Proline	77.0±5.7	56.5±15.6	41.1±2.9	32.2±8.8	0.115	**0.004**	0.516
Serine	114.1±3.3[a]	86.8±13.2[b]	56.9±2.4[c]	56.9±3.4[c]			**0.033**
Tyrosine	68.1±2.7	64.7±18.7	45.6±4.6	48.0±5.1	0.949	**0.036**	0.731
Citrulline	55.2±5.0	43.3±4.9	63.2±2.0	56.7±2.6	**0.019**	**0.008**	0.436
Ornithine	46.2±3.1	56.1±4.1	66.0±3.8	56.9±6.0	0.941	0.066	0.086
Miscellaneous (nmol/ml)							
Branched-chain amino acids	260.3±16.3[a]	325.0±12.0[b]	426.0±8.2[c]	400.1±11.1[c]			**0.002**
Urea	4,631.9±329.2	6,495.7±376.8	9,582.1±1025.8	9,318.4±686.8	0.361	**0.000**	0.231

Data are means±SE and were analyzed using two-way ANOVA. Values sharing identical superscripts ([a, b, c]) are not significantly different. Significant differences by two-way ANOVA are shown in bold. CT means control.

concentrations, the uptake of tryptophan may decrease, resulting in a lower concentration of serotonin. A low serotonin concentration may influence mental and physical health by affecting depression (21). For example, consuming a high-protein diet that includes a higher ratio of BCAAs/non-BCAAs, including tryptophan, might cause depression due to lower serotonin in the brain (22), which may affect the development of diabetes. Patients with diabetes also have lower plasma serine concentrations (6, 7), and dietary supplementation with serine ameliorates diabetes (23). Thus, a low serine concentration may be significant in both diabetes and prediabetes. Further study of these potential mechanisms is required.

The mechanism underlying the high BCAAs concentration in nondiabetic mice is not understood. Under high protein conditions, the increased BCAAs in plasma may correspond to a higher intake of dietary BCAAs. In addition, *db* mice showed different BCAAs metabolism, such as suppressed BCAAs oxidation in liver and lipid tissues, compared to healthy mice (24). Therefore, the current results showing the different responses to the different protein content diets might be related to the influence on BCAAs metabolism. In particular, the enzymes related to BCAAs oxidation in liver and lipid tissues should be further examined. The low serine concentration in nondiabetic mice is likely the result of an induction of gluconeogenesis (25), which may be explained by greater excretion of glucagon and suppressed excretion of insulin (26), leading to more rapid glycogen turnover and gluconeogenesis. However, in Table II, the levels of plasma insulin and urinary C-peptide were not significantly increased in the H-diet condition. Furthermore, a high-protein diet induces a higher rate of urea synthesis (27). As shown in Table III, nondiabetic

mice consuming a high-protein diet had higher plasma urea concentrations than mice consuming a low-protein diet. The higher rate of urea synthesis induced by the higher nitrogen load may be related to the greater gluconeogenesis by neuronal and hormonal mechanisms (27). However, further study of these physiological implications is required.

As a result, the concentrations of threonine, tryptophan, and citrulline were lower in mice fed the H-diet than in mice fed the L-diet. There is a possibility of modulating metabolism in the liver. With high protein feeding conditions, tryptophan and threonine might be converted by enzymes that are enhanced in expression (28, 29). On citrulline, the explanation is not clear. The consumption of diets differing in their proportions of protein and carbohydrate was associated with differences in the urinary volume, urinary glucose, and water intake in *db* mice, which is consistent with previous findings (9, 10). This may be explained by an induction of gluconeogenesis by the high-protein diet.

This study had some limitations. The mice were restricted in sex, age, and numbers. Hence, further studies should be conducted using female mice, mice of more adult age, and increased numbers of mice. The difference in plasma amino acid profiles in nondiabetic mice between diets composed of differing proportions of protein and carbohydrate is an interesting finding, and more mechanistic studies are needed to explain the mechanism underlying this change.

Conclusion

The differences in dietary protein and carbohydrate content did not affect the plasma amino acid profile of diabetic mice, regardless of their urine volume or urinary glucose concentration. On the other hand, the nondiabetic mice showed a difference in plasma amino acid profile under diets composed of differing proportions of protein and carbohydrate. Although the physiological significance of this difference in the amino acid profile in nondiabetic mice has not been fully explored, the consumption of a high-protein diet causes the amino acid profile to be more similar to that of diabetic mice. High BCAAs in plasma are related to diabetic conditions, cancer, and heart failure, leading to reduced lifespan (22, 24). Based on the results of high BCAAs in plasma in nondiabetic mice fed a high-protein diet, we suggest avoidance of a high-protein diet for subjects with health conditions.

Conflicts of Interest

The Authors declare no conflicts of interest with regard to the study.

Authors' Contributions

E.A. and M.H. designed and performed experiments, analyzed data and wrote paper; M.U. gave technical support and conceptual advice, and undertook data curation.

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References

1 Zimmet P, Alberti KG and Shaw J: Global and societal implications of the diabetes epidemic. Nature *414(6865)*: 782-787, 2001. PMID: 11742409. DOI: 10.1038/414782a

2 López S, Bermúdez B, Pacheco YM, Villar J, Abia R and Muriana FJ: Distinctive postprandial modulation of beta cell function and insulin sensitivity by dietary fats: Monounsaturated compared with saturated fatty acids. Am J Clin Nutr *88(3)*: 638-644, 2008. PMID: 18779278. DOI: 10.1093/ajcn/88.3.638

3 Shang X, Scott D, Hodge AM, English DR, Giles GG, Ebeling PR and Sanders KM: Dietary protein intake and risk of type 2 diabetes: Results from the melbourne collaborative cohort study and a meta-analysis of prospective studies. Am J Clin Nutr *104(5)*: 1352-1365, 2016. PMID: 27629053. DOI: 10.3945/ajcn.116.140954

4 Zhao LG, Zhang QL, Liu XL, Wu H, Zheng JL and Xiang YB: Dietary protein intake and risk of type 2 diabetes: A dose-response meta-analysis of prospective studies. Eur J Nutr *58(4)*: 1351-1367, 2019. PMID: 29858629. DOI: 10.1007/s00394-018-1737-7

5 Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB and Gerszten RE: Metabolite profiles and the risk of developing diabetes. Nat Med *17(4)*: 448-453, 2011. PMID: 21423183. DOI: 10.1038/nm.2307

6 Yamakado M, Nagao K, Imaizumi A, Tani M, Toda A, Tanaka T, Jinzu H, Miyano H, Yamamoto H, Daimon T, Horimoto K and Ishizaka Y: plasma free amino acid profiles predict four-year risk of developing diabetes, metabolic syndrome, dyslipidemia, and hypertension in Japanese population. Sci Rep *5*: 11918, 2015. PMID: 26156880. DOI: 10.1038/srep11918

7 Yamaguchi N, Mahbub MH, Takahashi H, Hase R, Ishimaru Y, Sunagawa H, Amano H, Kobayashi-Miura M, Kanda H, Fujita Y, Yamamoto H, Yamamoto M, Kikuchi S, Ikeda A, Takasu M, Kageyama N, Nakamura M and Tanabe T: Plasma free amino acid profiles evaluate risk of metabolic syndrome, diabetes, dyslipidemia, and hypertension in a large Asian population. Environ Health Prev Med *22(1)*: 35, 2017. PMID: 29165132. DOI: 10.1186/s12199-017-0642-7

8 Teixeira SR, Tappenden KA and Erdman JW Jr: Altering dietary protein type and quantity reduces urinary albumin excretion without affecting plasma glucose concentrations in BKS.cg-m +Lepr db/+Lepr db (db/db) mice. J Nutr *133(3)*: 673-678, 2003. PMID: 12612136. DOI: 10.1093/jn/133.3.673

9 Arimura E, Horiuchi M, Kawaguchi H, Miyoshi N, Aoyama K and Takeuchi T: Low-protein diet improves blood and urinary glucose levels and renal manifestations of diabetes in C57BLKS-db/db mice. Eur J Nutr *52(2)*: 813-824, 2013. PMID: 22692501. DOI: 10.1007/s00394-012-0387-4

10 Arimura E, Pulong WP, Marchianti ACN, Nakakuma M, Abe M, Ushikai M and Horiuchi M: Deteriorated glucose metabolism with a high-protein, low-carbohydrate diet in db mice, an animal model of type 2 diabetes, might be caused by insufficient insulin secretion. Eur J Nutr *56(1)*: 237-246, 2017. PMID: 26497335. DOI: 10.1007/s00394-015-1075-y

11 Arimura E, Okatani H, Araki T, Ushikai M, Nakakuma M, Abe M, Kawaguchi H, Izumi H and Horiuchi M: Effects of diets with different proportions of protein/carbohydrate on retinal manifestations in *db* mice. In Vivo *32(2)*: 265-272, 2018. PMID: 29475908. DOI: 10.21873/invivo.11233

12 Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI and Morgenstern JP: Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in db/db mice. Cell *84(3)*: 491-495, 1996. PMID: 8608603. DOI: 10.1016/s0092-8674(00)81294-5

13 Standard tables of food composition in Japan 2015 (Seventh Revised Version): Amino acids. Tokyo, Ministry of Education, Culture, Sports, Science and Technology, Japan. Availableat: https://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/sdetail01/1388553.htm [Last accessed on 26th February 2021]

14 Kamimura N, Nishimaki K, Ohsawa I and Ohta S: Molecular hydrogen improves obesity and diabetes by inducing hepatic FGF21 and stimulating energy metabolism in db/db mice. Obesity (Silver Spring) *19(7)*: 1396-1403, 2011. PMID: 21293445. DOI: 10.1038/oby.2011.6

15 Layman DK, Clifton P, Gannon MC, Krauss RM and Nuttall FQ: Protein in optimal health: Heart disease and type 2 diabetes. Am J Clin Nutr *87(5)*: 1571S-1575S, 2008. PMID: 18469290. DOI: 10.1093/ajcn/87.5.1571S

16 Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, Ilkayeva OR, Wenner BR, Bain JR, Lee JJ, Lim SC, Khoo CM, Shah SH and Newgard CB: Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia *53(4)*: 757-767, 2010. PMID: 20076942. DOI: 10.1007/s00125-009-1637-8

17 Krebs M, Krssak M, Bernroider E, Anderwald C, Brehm A, Meyerspeer M, Nowotny P, Roth E, Waldhäusl W and Roden M: Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes *51(3)*: 599-605, 2002. PMID: 11872656. DOI: 10.2337/diabetes.51.3.599

18 Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD and Svetkey LP: A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab *9(4)*: 311-326, 2009. PMID: 19356713. DOI: 10.1016/j.cmet.2009.02.002

19 Jang C, Oh SF, Wada S, Rowe GC, Liu L, Chan MC, Rhee J, Hoshino A, Kim B, Ibrahim A, Baca LG, Kim E, Ghosh CC, Parikh SM, Jiang A, Chu Q, Forman DE, Lecker SH, Krishnaiah S, Rabinowitz JD, Weljie AM, Baur JA, Kasper DL and Arany Z: A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. Nat Med *22(4)*: 421-426, 2016. PMID: 26950361. DOI: 10.1038/nm.4057

20 Fernstrom JD: Branched-chain amino acids and brain function. J Nutr *135(6 Suppl)*: 1539S-1546S, 2005. PMID: 15930466. DOI: 10.1093/jn/135.6.1539S

21 Shabbir F, Patel A, Mattison C, Bose S, Krishnamohan R, Sweeney E, Sandhu S, Nel W, Rais A, Sandhu R, Ngu N and Sharma S: Effect of diet on serotonergic neurotransmission in depression. Neurochem Int *62(3)*: 324-329, 2013. PMID: 23306210. DOI: 10.1016/j.neuint.2012.12.014

22 Solon-Biet SM, Cogger VC, Pulpitel T, Wahl D, Clark X, Bagley E, Gregoriou GC, Senior AM, Wang QP, Brandon AE, Perks R, O'Sullivan J, Koay YC, Bell-Anderson K, Kebede M, Yau B, Atkinson C, Svineng G, Dodgson T, Wali JA, Piper MDW, Juricic P, Partridge L, Rose AJ, Raubenheimer D, Cooney GJ, Le Couteur DG and Simpson SJ: Branched chain amino acids impact health and lifespan indirectly *via* amino acid balance and appetite control. Nat Metab *1(5)*: 532-545, 2019. PMID: 31656947. DOI: 10.1038/s42255-019-0059-2

23 Holm LJ and Buschard K: L-serine: A neglected amino acid with a potential therapeutic role in diabetes. APMIS *127(10)*: 655-659, 2019. PMID: 31344283. DOI: 10.1111/apm.12987

24 Neinast MD, Jang C, Hui S, Murashige DS, Chu Q, Morscher RJ, Li X, Zhan L, White E, Anthony TG, Rabinowitz JD and Arany Z: Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. Cell Metab *29(2)*: 417-429.e4, 2019. PMID: 30449684. DOI: 10.1016/j.cmet.2018.10.013

25 Hetenyi G Jr., Anderson PJ, Raman M and Ferrarotto C: Gluconeogenesis from glycine and serine in fasted normal and diabetic rats. Biochem J *253(1)*: 27-32, 1988. PMID: 3138983. DOI: 10.1042/bj2530027

26 Linn T, Santosa B, Grönemeyer D, Aygen S, Scholz N, Busch M and Bretzel RG: Effect of long-term dietary protein intake on glucose metabolism in humans. Diabetologia *43(10)*: 1257-1265, 2000. PMID: 11079744. DOI: 10.1007/s001250051521

27 Noguchi Y, Shikata N, Furuhata Y, Kimura T and Takahashi M: Characterization of dietary protein-dependent amino acid metabolism by linking free amino acids with transcriptional profiles through analysis of correlation. Physiol Genomics *34(3)*: 315-326, 2008. PMID: 18559966. DOI: 10.1152/physiolgenomics.00007.2008

28 Davis AJ and Austic RE: Dietary protein and amino acid levels alter threonine dehydrogenase activity in hepatic mitochondria of Gallus domesticus. J Nutr *127(5)*: 738-744, 1997. PMID: 9164995. DOI: 10.1093/jn/127.5.738

29 Badawy AA, Lake SL and Dougherty DM: Mechanisms of the pellagragenic effect of leucine: stimulation of hepatic tryptophan oxidation by administration of branched-chain amino acids to healthy human volunteers and the role of plasma free tryptophan and total kynurenines. Int J Tryptophan Res *7*: 23-32, 2014. PMID: 25520560. DOI: 10.4137/IJTR.S18231

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