

Uniqueness of Tryptophan in the Transport System in the Brain and Peripheral Tissues

Mutsumi Ogawa¹, Fumiko Shimizu¹, Yukie Ishii¹, Tetsuya Takao¹, Akikazu Takada²

¹Faculty of Food and Health Sciences, Showa Women's University, Tokyo, Japan

²International Projects on Food and Health (NPO), Tokyo, Japan

Email: takada-1a@kmd.biglobe.ne.jp

How to cite this paper: Ogawa, M., Shimizu, F., Ishii, Y., Takao, T. and Takada, A. (2023) Uniqueness of Tryptophan in the Transport System in the Brain and Peripheral Tissues. *Food and Nutrition Sciences*, 14, 401-414.

<https://doi.org/10.4236/fns.2023.145026>

Received: April 7, 2023

Accepted: May 16, 2023

Published: May 19, 2023

Copyright © 2023 by author(s) and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Amino acids are important substances that must be transported to tissues such as the brain and muscles. The process is considered insulin dependent. It is not known whether all the amino acids are almost equally dependent in their transportation to tissues. We want to know whether some important amino acids are transported differently from other amino acids. Especially tryptophan is important because it is converted to serotonin, melatonin or kynurenine. Results showed that Amino acids levels in the plasma were measured after the intakes of 50 grams of glucose or sucrose to young (18 - 22 years old) and old (≥ 50 years old) men. Total amino acids in the plasma decreased after the intakes of glucose. Total amino acids levels decreased more significantly in old men after the administration of sucrose. Total and non-essential amino acids in the plasma decreased significantly at 120 min after the intakes of glucose in young and old men, but only sucrose caused their decreases in both aged and young men. Both glucose and sucrose intakes decreased significantly the plasma levels of the total essential and branched amino acids in young and old men. Surprisingly, plasma levels of tryptophan did not decrease upon the administration of glucose but only slightly decreased upon the administration of sucrose in young men. In conclusion, not all the amino acids were transported well into tissues upon the administration of glucose or sucrose. Tryptophan seems to be relatively resistant for insulin to facilitate the transportation into tissues.

Keywords

Glucose, Sucrose, Insulin, Amino Acids, Transporter, mTORC1

1. Introduction

A progressive loss of physical independence is associated with aging, which sig-

nificantly changes the quality of life and increases morbidity and mortality. Sarcopenia is most likely a multifactorial disorder [1] [2], and recent evidence suggests that the anabolic actions of insulin are not effective in the protein synthesis in the elderly [3] [4] [5].

It has been hypothesized that insulin does not cause muscle protein synthesis in the elderly is resistant to the anabolic action, and that this effect of aging is independent of a normal glucose tolerance [6].

It has been strongly suggested that the primary reason for the reduced muscle anabolic response to feeding is this age-related insulin resistance. Indeed, it is indicated that the anabolic response of skeletal muscle proteins to mixed feeding is reduced with aging both in humans [3] and animals [7] [8].

As to roles of insulin in the transportation of amino acids and protein synthesis, it has been indicated [9] that insulin-stimulated vasodilation causes muscle protein synthesis by increasing nutritive flow and, consequently, induces mTORC1 signaling, whereas Akt/PKB signaling is either not directly involved or only facilitates the process. Local hyperinsulinemia at physiological postprandial levels caused a significant increase in leg blood flow [10] [11] and muscle perfusion [12], enhancing Akt and mTORC1 signaling and increasing skeletal muscle protein synthesis, resulting in an overall net anabolic effect.

Fernstrom, J.D. and Wurtman R.J. Indicated that when plasma levels of tryptophan were raised by taking tryptophan in foods or by injection of insulin, serotonin and tryptophan in the brain increased [13] [14]. They paid attention to tryptophan because tryptophan metabolite such as serotonin is a very important neuro-transmitter. They indicated that carbohydrate intakes increased the secretion of insulin which increased tryptophan levels in the plasma and lowered the concentrations of the competing amino acids such as branched neutral amino acids in rats [14]. Their results indicate that Carbohydrate intake was shown to decrease free amino acids levels in the plasma and glucose intake resulted in a decrease in large neutral amino acids such as methionine, phenylalanine, tyrosine, and tryptophan [15] [16]. These results suggest that intakes of foods that contain tryptophan are not good enough for increasing tryptophan, thus serotonin or melatonin in the brain. We must take carbohydrate together with such foods as meat. Increased levels of glucose or insulin in the plasma may increase the transport of some amino acids such as tryptophan and result in a decrease in the concentration of such amino acids

Now we wanted to know if there are differences in uptakes of amino acids from the blood to tissues (mainly muscles) after the administration of glucose or sucrose in young and old men

2. Methods

Men older than 50 years old and male college students were recruited in the experiments. Their health states were checked carefully and recruited them if there were no health problems such as diabetes, hypertension nor serious diseases ex-

perienced in the past. They did not smoke in the past. We also excluded people who took drugs for dyslipidemia, hyperglycemia, or hypertension. After fasting overnight, participants were randomly assigned to groups. Depending on their group, each participant received a 550-mL solution containing 50 g of glucose or sucrose (or 500 mL water as a control). Either 50 g of glucose or sucrose was added and dissolved in each bottle containing 500 mL of water. Between 9:00 AM and 10:00 AM, blood was sampled using a syringe, and participants were given either glucose or sucrose solution or water as a control. We measured blood glucose using a finger stick (TERUMO kit) before and 120 min after the administration of glucose or sucrose. Furthermore, other plasma factors were measured after plasma was separated from blood. Ethylenediamine tetra acetic acid (EDTA) was used as an anticoagulant. Plasma was obtained by centrifuging the blood samples, and the amino acids and insulin levels were measured for backgrounds of these participants. The samples were analyzed by SRL, Inc. (Tokyo Japan) using the UF-Amino Station[®], which is a liquid chromatography-mass spectrometry system with an automated pre-column derivatization for simultaneous determination of amino acids (Shimadzu Corporation, Kyoto Japan). The original concept of this system was developed by Ajinomoto Co., Inc. (Tokyo Japan) as an automated method of analyzing major free amino acids in human plasma in the field of clinical chemistry. The human plasma samples were cryopreserved with EDTANa₂ before the analysis. The thawed samples were deproteinized with acetonitrile followed by the amino acid analysis. Pre-column derivatization in the UF-Amino Station was automatically performed using an automated sample injector with the reagent APDSTAG[®] (Wako Pure Chemical Industries, Ltd., Osaka Japan). Target-free amino acids as derivatized compounds were separated under a reversed-phase ultrahigh performance liquid chromatography condition and determined by the liquid chromatograph mass spectrometer. Insulin was measured by the CLEIA (chemiluminescent immunoassay) method.

2.1. Ethics

This work was approved by the ethical committees of Showa Women's University and the NPO "International projects on food and health" and was conducted A. Takada *et al.* DOI: 10.4236/fns.2019.101005 54 Food and Nutrition Sciences in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments.

2.2. Statistics

The results are presented as means \pm SD. Statistical significance of the differences between groups was calculated according by one-way ANOVA. When ANOVA indicated a significant difference ($p < 0.05$), the mean values of the treatment were compared using Tukey's least significant difference test at $p < 0.05$.

3. Results

1) Measurements of plasma levels of amino acids in old and young men.

a) Background of participants.

Table 1 shows the background of participants. There was no difference on energy intake between young and old men. Young men take more lipids and old men take more carbohydrate.

b) Changes of blood glucose or insulin levels after the administration of glucose or sucrose in young or old men.

Figure 1 shows plasma levels of insulin after the administration of glucose or sucrose in young men. Blood insulin levels increased at the same rate after the administration of glucose or sucrose up to 30 min. then blood glucose levels declined more rapidly after the administration of sucrose compared with that of glucose. The levels were equal after 120 min.

Insulin levels were higher at 30 min after the administration of sucrose than that of glucose in young men.

Table 1. Background of participants.

Subjects	Young (n = 35)	Old (n = 44)	ss
Age (years)	20.7 ± 1.5	62.4 ± 9.6	**
Height (m)	1.72 ± 0.06	1.68 ± 0.07	*
BMI (kg/m ²)	22.2 ± 3.3	24.3 ± 3.3	**
Energy intake (kcal/day)	1962 ± 578	2115 ± 460	
Protein intake (g/day)	67.5 ± 23	66.6 ± 28.8	
Lipid intake (g/day)	58.3 ± 21.7	49.1 ± 22.6	*
Carbohydrate intake (g/day)	271.1 ± 92.6	198.6 ± 89.4	**
Insulin (μIU/mL)	6.97 ± 4.21	6.19 ± 3.79	

ss: *p < 0.05, **p < 0.01.

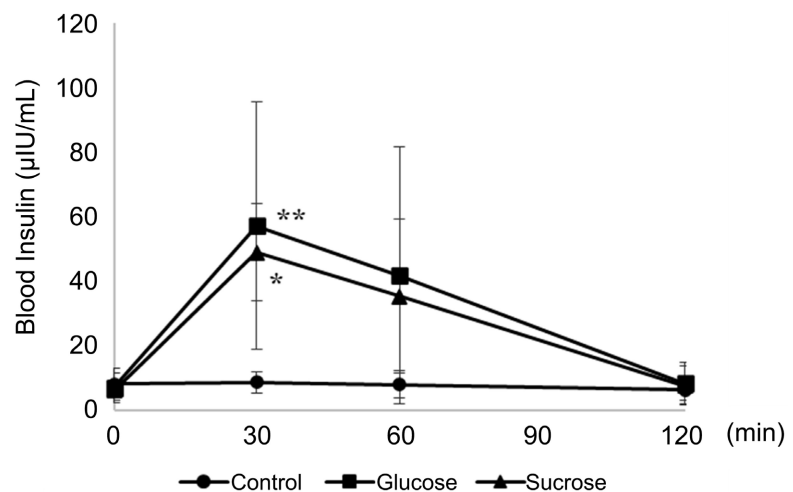


Figure 1. Changes in blood insulin levels after the administration of sucrose or glucose to young men. *: p < 0.05, **: p < 0.01, control vs. glucose, sucrose.

Figure 2 shows changes in blood insulin levels after the administration of glucose or sucrose to old men.

It is shown that the rates of increase in insulin levels were higher after intakes of glucose than sucrose in old men.

c) Plasma levels of amino acids.

Plasma levels of amino acids in young and old men were examined. Although there were no differences in plasma levels of total amino acids and total essential and branched amino acids between young and old men, levels of nonessential amino acids in the plasma were higher in old men. Levels of phenylalanine, tyrosine, alanine, A-aminobutyric acid, citrulline, cystine, glutamic acid, ornithine, and taurine were higher in old men, and serine was higher in young men.

2) The administration of glucose or sucrose in young and old men.

Blood was taken before the experiments from old men and amino acids levels were measured at 0 min and 120 min after the intakes of glucose (15 men) or sucrose (16 men) or water as a control (13 men) and amino acids levels in the plasma were measured. Essential amino acids (EAA) such as histidine, lysine, methionine, phenylalanine, threonine, tryptophan, leucine, isoleucine, and valine and branched amino acids (BAA) such as leucine, isoleucine, and valine decreased significantly 120 min after the administration

Figure 3 and **Figure 4** shows changes of total, nonessential, essential, branched amino acids and tryptophan levels in plasma after the administration of glucose or sucrose in young and old men.

Figure 3 and **Figure 4** indicates that intakes of both glucose and sucrose resulted in decrease in plasma levels of amino acids, but amino acids levels decreased more significantly after the administration of glucose than that of sucrose. In young men, plasma levels of tryptophan were not significantly higher in comparison between the administration of glucose or sucrose.

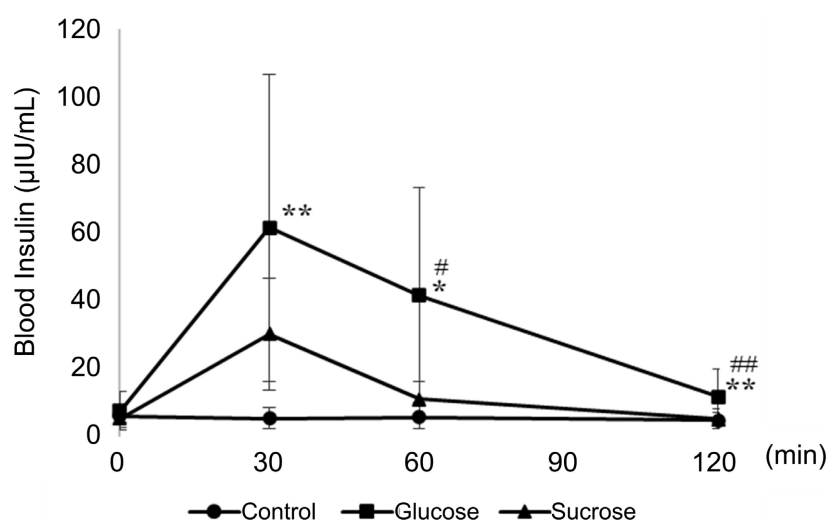


Figure 2. Changes in blood insulin levels after the administration of sucrose or glucose to old men. *: $p < 0.05$, **: $p < 0.01$, control vs. glucose or sucrose. #: $p < 0.01$, ##: $p < 0.01$, glucose vs. sucrose. #: $p < 0.05$, ###: $p < 0.01$, glucose vs sucrose.

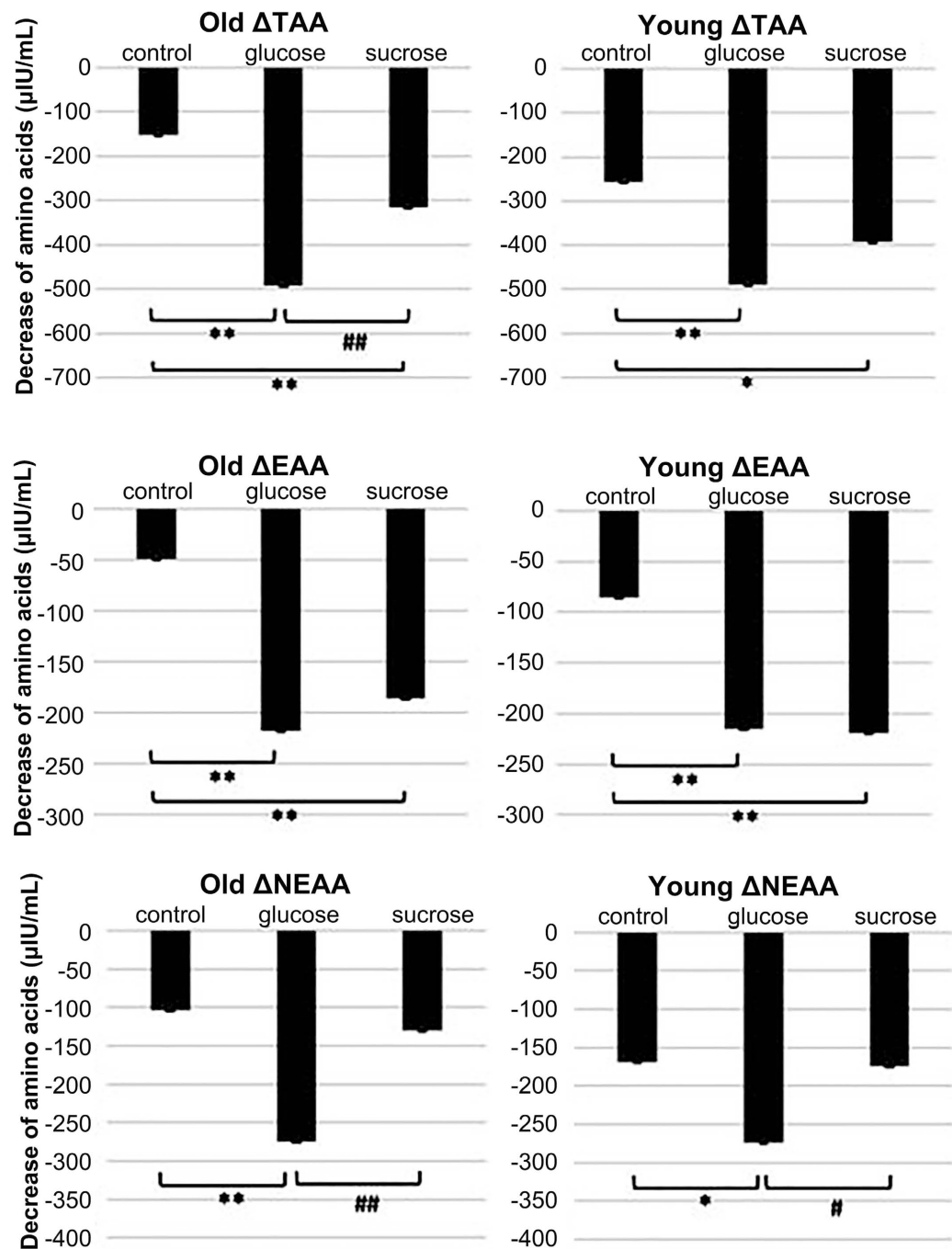


Figure 3. Changes of plasma levels of total (TAA), essential (EAA), non-essential (NEAA) amino acids after the administration of glucose or sucrose in young or old men. *: $p < 0.05$, **: $p < 0.01$, control vs. glucose, sucrose. #: $p < 0.05$, ##: $p < 0.01$, glucose vs. sucrose.

Figure 5 indicates that in all the cases the rate of decrease of these amino acids were higher after the administration of glucose than sucrose.

Figure 6 indicates that glucose administration resulted in higher decrease rate of branched amino acids than that of sucrose in young and old men. But no change of the rate of decrease in plasma levels of tryptophan after the administration of glucose or sucrose in old men, but in young men the rate of decrease

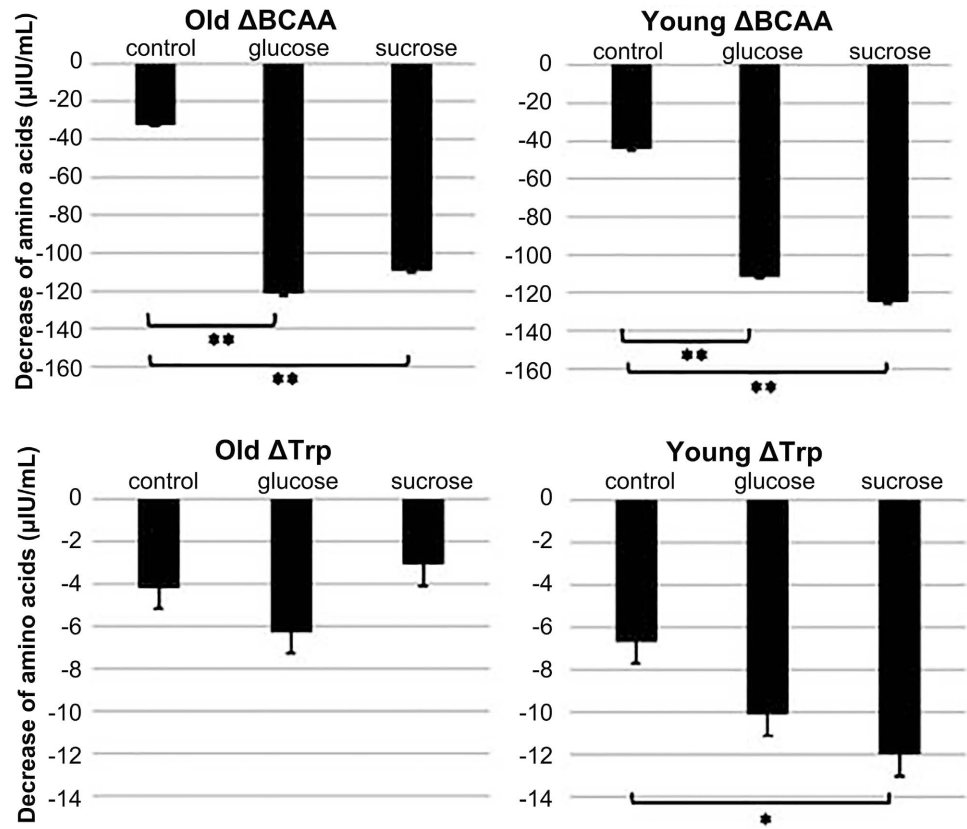
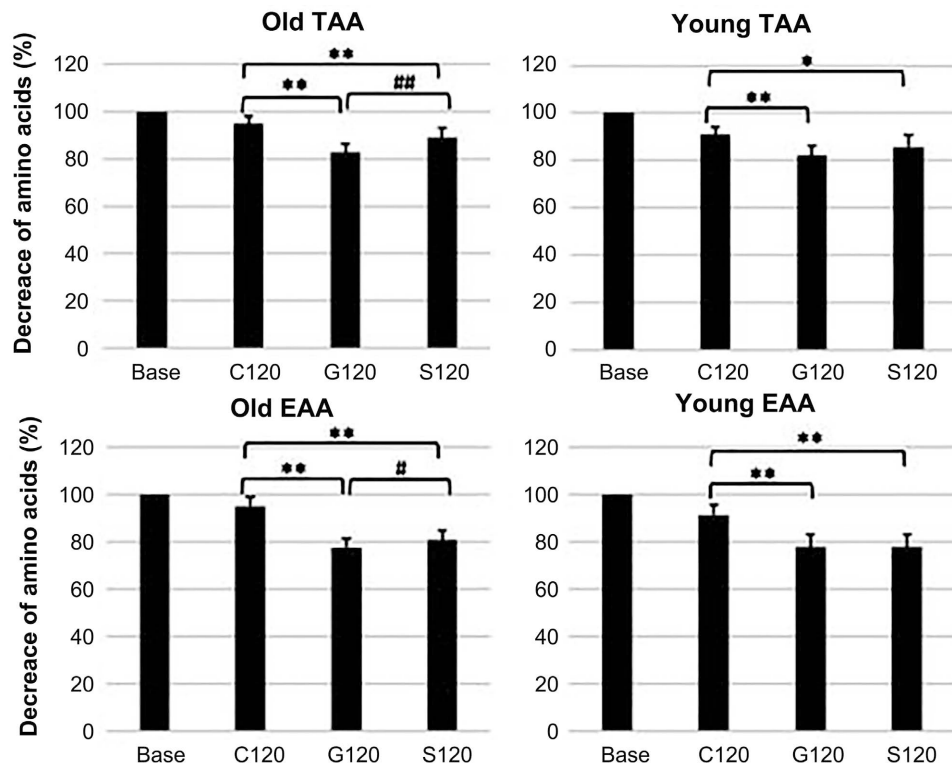


Figure 4. Changes of plasma levels of branched amino acids (BCAA), and tryptophan (Trp) after the administration of glucose or sucrose in young or old men. *: $p < 0.05$, **: $p < 0.01$, control vs. sucrose. #: $p < 0.05$, ##: $p < 0.01$, glucose vs. sucrose.



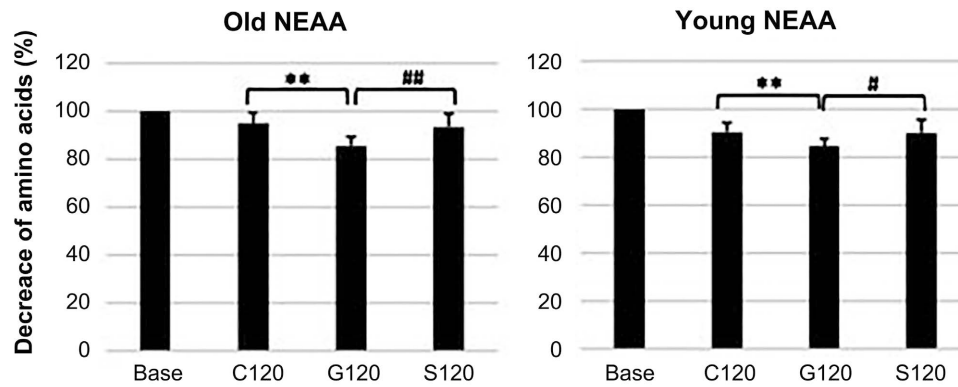


Figure 5. The rate of changes in plasma levels of total (TAA), essential (EAA), non-essential (NEAA) amino acids after the administration of glucose or sucrose in young or old men. *: $p < 0.05$, **: $p < 0.01$, control vs. glucose, sucrose. #: $p < 0.05$, ##: $p < 0.01$, glucose vs. sucrose.

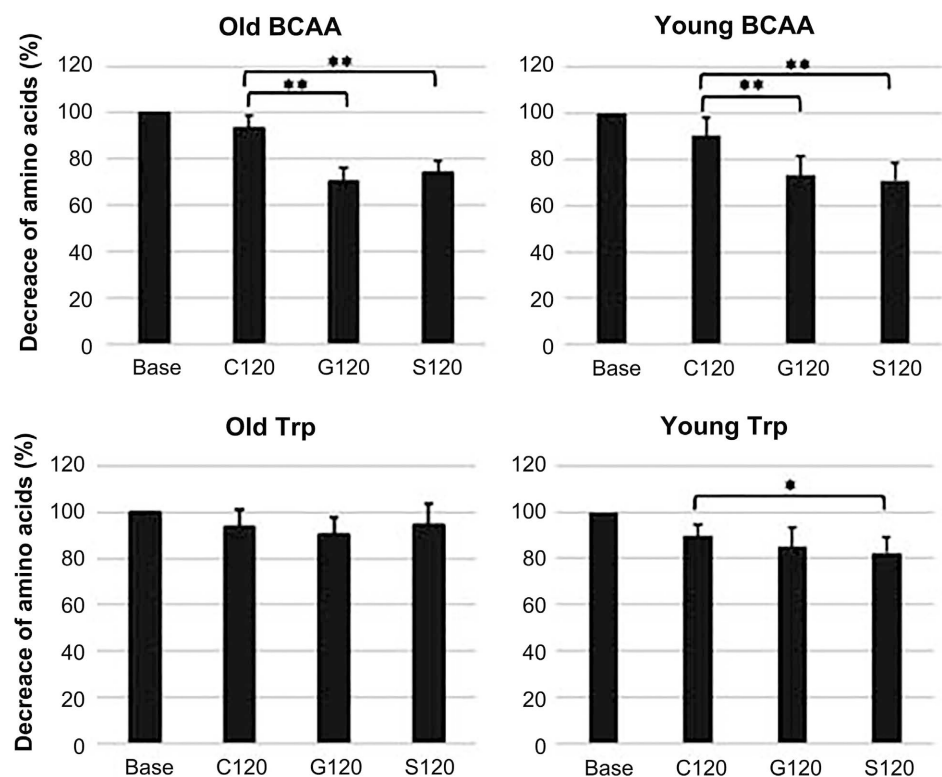


Figure 6. The rate of changes in plasma levels of branched amino acids (BCAA) and tryptophan (Trp) after the administration of glucose or sucrose in young or old men. *: $p < 0.01$. **: $p < 0.01$, control vs. glucose, sucrose. #: $p < 0.05$, ##: $p < 0.01$, glucose vs. sucrose.

of tryptophan levels were higher after the intakes of sucrose than those of glucose.

4. Discussion

As indicated before, for adult persons to keep muscles in good conditions is very important for living a healthy life [6].

An important role for amino acid transporters in the regulation of protein

metabolism has been elucidated over recent years. Many researchers [17]-[23] have analyzed the mechanisms through which specific classes of amino acid transporters facilitate the movement of amino acids across a cell membrane, and it has been shown that the mechanisms through which these transporters and the influx of amino acids into the cell may be linked to the regulation of protein metabolism. Identifying these mechanisms is important for the therapeutic measures of amino acid transport and transporters in the regulation of human skeletal muscle adaptation to increase muscle health and physical function.

Essential amino acids (EAAs) are required for protein synthesis and are provided through the diet or after degradation of endogenous proteins. Raising plasma insulin to 15 to 30 $\mu\text{U/mL}$ suppresses protein degradation with no further suppression at higher concentrations [24] [25]. Accordingly, EAA concentrations decrease during insulin infusion with no stimulation of mixed muscle [26] [27] or mitochondrial protein synthesis [28] [29]. When branched-chain amino acids were infused into humans' skeletal muscle mitochondrial activity increased, which suggests that the infusion causes beneficial roles for mitochondrial function [30].

Fernstrom, J.D. and Wurtman R.J. indicated that intakes of tryptophan in foods or injection of insulin increased levels of serotonin and tryptophan in the brain [13] [14]. They indicated that carbohydrate ingestion increased the secretion of insulin which raised plasma levels of tryptophan and lowered the concentrations of the competing amino acids such as branched neutral amino acids in rats [14].

As indicated by them [13] [14], tryptophan is one of the most important substrates for such transmitters as serotonin and melatonin. Since serotonin is known to decrease depression, it is important to know about transport of tryptophan to the brain and tissues.

Tryptophan is transported to the brain competitively with other amino acids [13] [14]. So, we must examine the transportation of tryptophan in conjunction with the transport of other amino acids.

Availability of serotonin as a neurotransmitter is crucial in many physiological processes. Serotonergic neurons in the central nervous system are involved in regular behavioral states and physiological processes including arousal, sleep, appetite, pain, release of hormones and mood [31] [32]. Dysfunction of serotonin neurons may lead to not only depression but other mental disorders.

Several biochemical processes intrinsic to serotonin neurons can be effectively manipulated by actions of chemical substances including the loading of a precursor substance, tryptophan or 5-hydroxytryptophan. Loading means the intakes of tryptophan-rich foods.

As shown in the present research taking tryptophan-rich foods is not enough for increasing tryptophan levels in the brain or tissues. Carbohydrate intakes causing increase in insulin levels in the plasma is necessary [13] [14].

These results seem to indicate that tryptophan is unique and relatively resistant to the stimulation of insulin for the transportation to the brain and muscles.

The function of specific classes of amino acid transporters and the movement of amino acids across the membrane has received much attention as an important cellular mechanism linking amino acid availability and protein metabolism. Specifically, two amino acid transport systems that have been most closely related to mTORC1 signaling are the system L and system A amino acid transporters. System L transporters (*i.e.*, LAT1/SLC7A5) make heterodimers with the production of glycoprotein (CD98/SLC3A2) and are mainly responsible for the influx of branched chain amino acids (Figure 7). Thus, system L transporters can induce intracellular accumulation of a particular amino acid (*i.e.*, leucine) without changing the overall intracellular amino acid concentration. In contrast, system A transporters (*i.e.*, SNAT2) concentrate amino acids within the cell by coupling the influx of amino acids with that of Na⁺ through secondary active transport [32]

Dickinson JM and Rasmussen [33] indicated that there are 4 transporters for amino acids in the muscle and only one transporter is capable of transport tryptophan. It appears that tryptophan is insulin resistant compared to other amino acids (Figure 8). This may explain our results that tryptophan is not effectively transported as other amino acids.

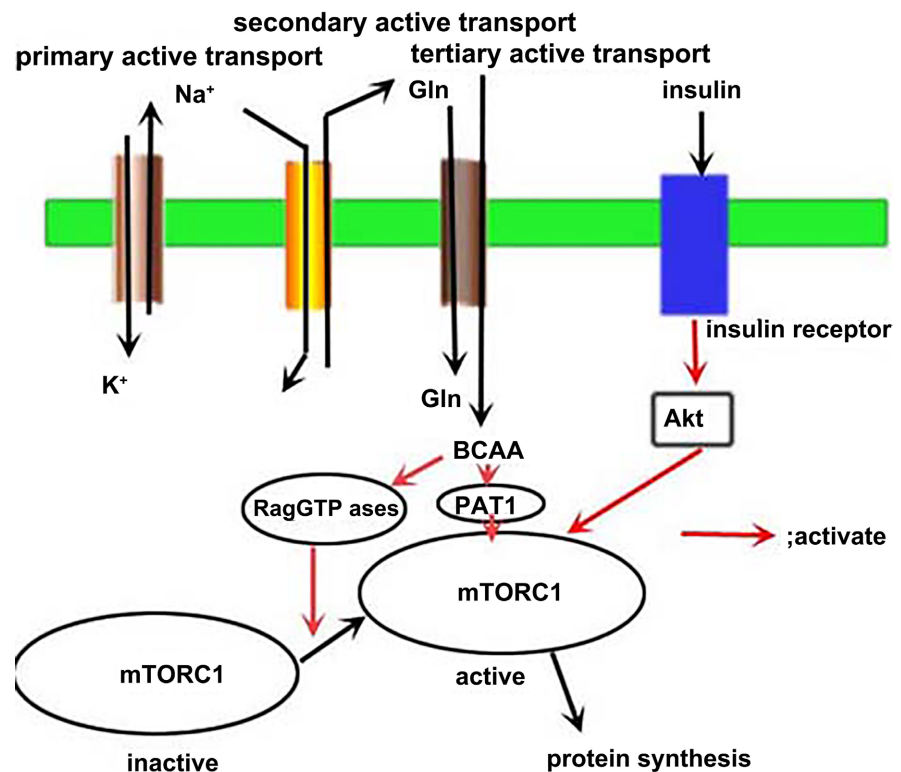


Figure 7. Schematic of potential cellular mechanisms responsible for amino acid control of muscle protein synthesis. Gln, glutamine; BCAA, branched chain amino acids; Rag, Ras-related GTPase; PAT1, proton-assisted amino acid transporter 1; Akt, protein kinase B; mTORC1, mammalian target of rapamycin complex 1; Rab, member of the Rab family of GTPases; Rheb, ras-homologue enriched in brain; MAP4K3, mitogen activated protein kinase.

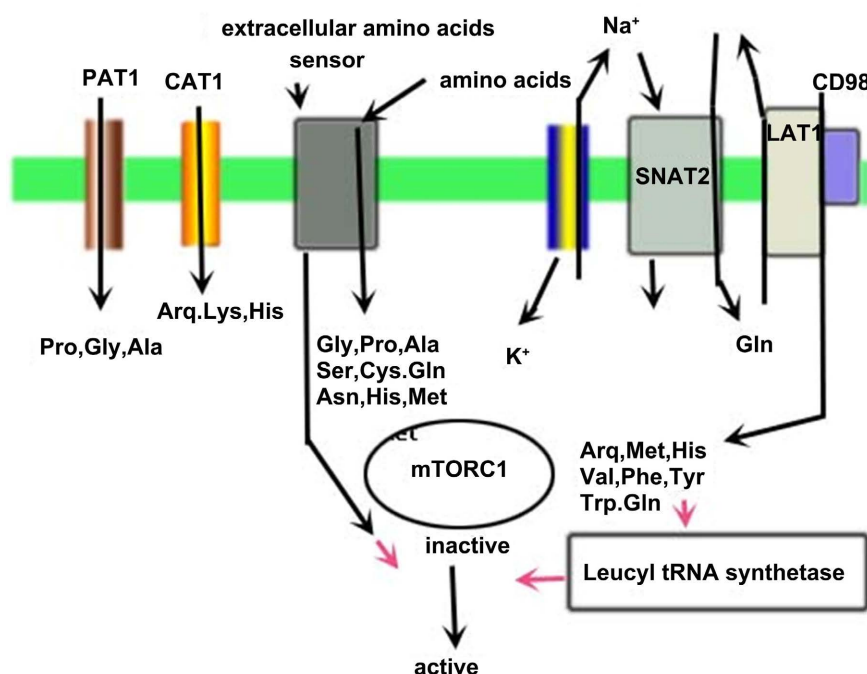


Figure 8. Schematic of the mechanisms regulating protein metabolism in response to increased amino acid availability and the role of amino acid transporters. GATOR, GTPase activating protein [GAP] activity toward Rags; Gln, glutamine; LAT1, system L amino acid transporter; MLCK II, myosin light-chain kinase II; mTORC1, mechanistic target of rapamycin complex 1; PAT1, proton-assisted amino acid transporter 1; RAGs, Ras-related GTPases; SNAT2, system A amino acid transporter.

Acknowledgements

Experiments were designed and performed by all of the authors. AT wrote a manuscript. Statistical analyses were done by TT. All authors read the manuscript and approved the final version. All the authors had responsibilities for the final content.

Financial Support

This study was supported by grants by Ito Memorial Foundation, grant in aid of Showa Women's University funds.

Conflicts of Interest

No conflicts of interest for any author.

References

- [1] Evans, W.J. (1995) What Is Sarcopenia? *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, **50A**, 5-8.
https://doi.org/10.1093/gerona/50A.Special_Issue.5
- [2] Dutta, C. and Hadley, E.C. (1995) The Significance of Sarcopenia in Old Age. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, **50A**, 1-4.
https://doi.org/10.1093/gerona/50A.Special_Issue.1

- [3] Volpi, E., Mittendorfer, B., Rasmussen, B.B. and Wolfe, R.R. (2000) The Response of Muscle Protein Anabolism to Combined Hyperaminoacidemia and Glucose-Induced Hyperinsulinemia Is Impaired in the Elderly. *Journal of Clinical Endocrinology and Metabolism*, **85**, 4481-4490. <https://doi.org/10.1210/jc.85.12.4481>
- [4] Guillet, C., Prod'homme, M., Balage, M., Gachon, P., Giraudet, C., Morin, L., Grizard, J. and Boirie, Y. (2004) Impaired Anabolic Response of Muscle Protein Synthesis Is Associated with S6K1 Dysregulation in Elderly Humans. *The FASEB Journal*, **18**, 1586-1587. <https://doi.org/10.1096/fj.03-1341fje>
- [5] Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., Wackerhage, H., Taylor, P.M. and Rennie, M.J. (2005) Anabolic Signaling Deficits Underlie Amino Acid Resistance of Wasting, Aging Muscle. *The FASEB Journal*, **19**, 422-424. <https://doi.org/10.1096/fj.04-2640fje>
- [6] Rasmussen, B.B., Fujita, S., Wolfe, R.R., Mittendorfer, B., Roy, M., Rowe, V.L. and Volpi, E. (2006) Insulin Resistance of Muscle Protein Metabolism in Aging. *The FASEB Journal*, **20**, 768-769. <https://doi.org/10.1096/fj.05-4607fje>
- [7] Dardevet, D., Sornet, C., Bayle, G., Prugnaud, J., Pouyet, C. and Grizard, J. (2002) Postprandial Stimulation of Muscle Protein Synthesis in Old Rats Can Be Restored by a Leucine-Supplemented Meal. *The Journal of Nutrition*, **132**, 95-100. <https://doi.org/10.1093/jn/132.1.95>
- [8] Kimball, S.R., Jefferson, L.S., Fadden, P., Haystead, T.A. and Lawrence Jr, J.C. (1996) Insulin and Diabetes Cause Reciprocal Changes in the Association of eIF-4E and PHAS-I in Rat Skeletal Muscle. *American Journal of Physiology-Cell Physiology*, **270**, C705-C709. <https://doi.org/10.1152/ajpcell.1996.270.2.C705>
- [9] Timmerman, K.L., Lee, J.L., Dreyer, H.C., Dhanani, S., Glynn, E.L., Fry, C.S., Drummond, M.J., Sheffield-Moore, M., Rasmussen, B.B. and Volpi, E. (2010) Insulin Stimulates Human Skeletal Muscle Protein Synthesis via an Indirect Mechanism Involving Endothelial-Dependent Vasodilation and Mammalian Target of Rapamycin Complex 1 Signaling. *Journal of Clinical Endocrinology and Metabolism*, **95**, 3848-3857. <https://doi.org/10.1210/jc.2009-2696>
- [10] Fujita, S., Rasmussen, B.B., Cadenas, J.G., Grady, J.J. and Volpi, E. (2006) Effect of Insulin on Human Skeletal Muscle Protein Synthesis Is Modulated by Insulin-Induced Changes in Muscle Blood Flow and Amino Acid Availability. *American Journal of Physiology-Endocrinology and Metabolism*, **291**, E745-E754. <https://doi.org/10.1152/ajpendo.00271.2005>
- [11] Boirie, Y., Short, K.R., Ahlman, B., Charlton, M. and Nair, K.S. (2001) Tissue-Specific Regulation of Mitochondrial and Cyto-Plasmic Protein Synthesis Rates by Insulin. *Diabetes*, **50**, 2652-2658. <https://doi.org/10.2337/diabetes.50.12.2652>
- [12] Gelfand, R.A. and Barrett, E.J. (1987) Effect of Physiologic Hyperinsulinemia on Skeletal Muscle Protein Synthesis and Breakdown in Man. *Journal of Clinical Investigation*, **80**, 1-6. <https://doi.org/10.1172/JCI113033>
- [13] Fernstrom, J.D. and Wurtman, R.J. (1971) Brain Serotonin Content: Increase Following Ingestion of Carbohydrate Diet. *Science*, **174**, 1023-1025. <https://doi.org/10.1126/science.174.4013.1023>
- [14] Fernstrom, J.D. and Wurtman, R.J. (1972) Brain Serotonin Content: Physiological Regulation by Plasma Neutral Amino Acids. *Science*, **178**, 414-416. <https://doi.org/10.1126/science.178.4059.414>
- [15] Lipsett, D., Madras, B.K., Wurtman, R.J. and Munro, H.N. (1973) Serum Tryptophan Level after Carbohydrate Ingestion: Selective Decline in Non-Albumin-Bound Tryptophan Coincident with Reduction in Serum Free Fatty Acids. *Life Sciences*, **12**, 57-64. [https://doi.org/10.1016/0024-3205\(73\)90027-1](https://doi.org/10.1016/0024-3205(73)90027-1)

- [16] Pan, R.M., Mauron, C., Glaeser, B. and Wurtman, R.J. (1982) Effect of Various Oral Glucose Doses on Plasma Neutral Amino Acid Levels. *Metabolism*, **31**, 937-943. [https://doi.org/10.1016/0026-0495\(82\)90185-8](https://doi.org/10.1016/0026-0495(82)90185-8)
- [17] Sancak, Y., Peterson, T.R., Shaul, Y.D., *et al.* (2008) The Rag GTPases Bind Raptor and Mediate Amino Acid Signaling to mTORC1. *Science*, **320**, 1496-1501. <https://doi.org/10.1126/science.1157535>
- [18] Heublein, S., Kazi, S., Ogmundsdottir, M.H., *et al.* (2010) Proton-Assisted Amino-Acid Transporters Are Conserved Regulators of Proliferation and Amino-Acid-Dependent mTORC1 Activation. *Oncogene*, **29**, 4068-4079. <https://doi.org/10.1038/onc.2010.177>
- [19] Baird, F.E., Bett, K.J., MacLean, C., *et al.* (2009) Tertiary Active Transport of Amino Acids Reconstituted by Coexpression of System A and L Transporters in *Xenopus* Oocytes. *The American Journal of Physiology-Endocrinology and Metabolism*, **297**, E822-E829. <https://doi.org/10.1152/ajpendo.00330.2009>
- [20] Nicklin, P., Bergman, P., Zhang, B., *et al.* (2009) Bidirectional Transport of Amino Acids Regulates mTOR and Autophagy. *Cells*, **136**, 521-534. <https://doi.org/10.1016/j.cell.2008.11.044>
- [21] Zoncu, R., Bar-Peled, L., Efeyan, A., *et al.* (2011) mTORC1 Senses Lysosomal Amino Acids through an Inside-Out Mechanism That Requires the Vacuolar H⁺-ATPase. *Science*, **334**, 678-683. <https://doi.org/10.1126/science.1207056>
- [22] Sancak, Y., Bar-Peled, L., Zoncu, R., *et al.* (2010) Ragulator-Rag Complex Targets mTORC1 to the Lysosomal Surface and Is Necessary for Its Activation by Amino Acids. *Cells*, **141**, 290-303. <https://doi.org/10.1016/j.cell.2010.02.024>
- [23] Saltin, B. and Gollnick, P.D. (2011) Skeletal Muscle Adaptability: Significance for Metabolism and Performance. In: Peachy, L.D., Eds., *Handbook of Physiology, Skeletal Muscle*, American Physiological Society, Bethesda, 555-630.
- [24] Greenhaff, P.L., Karagounis, L.G., Peirce, N., *et al.* (2008) Disassociation between the Effects of Amino Acids and Insulin on Signaling, Ubiquitin Ligases, and Protein Turnover in Human Muscle. *The American Journal of Physiology-Endocrinology and Metabolism*, **295**, E595-E604. <https://doi.org/10.1152/ajpendo.90411.2008>
- [25] Wilkes, E.A., Selby, A.L., Atherton, P.J., *et al.* (2009) Blunting of Insulin Inhibition of Proteolysis in Legs of Older Subjects May Contribute to Age Related Sarcopenia. *The American Journal of Clinical Nutrition*, **90**, 1343-1350. <https://doi.org/10.3945/ajcn.2009.27543>
- [26] Chow, L.S., Albright, R.C., Bigelow, M.L., Toffolo, G., Cobelli, C. and Nair, K.S. (2006) Mechanism of Insulin's Anabolic Effect on Muscle: Measurements of Muscle Protein Synthesis and Breakdown Using Aminoacyl-tRNA and Other Surrogate Measures. *The American Journal of Physiology-Endocrinology and Metabolism*, **291**, E729-E736. <https://doi.org/10.1152/ajpendo.00003.2006>
- [27] Nygren, J. and Nair, K.S. (2003) Differential Regulation of Protein Dynamics in Splanchnic and Skeletal Muscle Beds by Insulin and Amino Acids in Healthy Human Subjects. *Diabetes*, **52**, 1377-1385. <https://doi.org/10.2337/diabetes.52.6.1377>
- [28] Barazzoni, R., Short, K.R., Asmann, Y., Coenen-Schimke, J.M., Robinson, M.M. and Nair, K.S. (2012) Insulin Fails to Enhance mTOR Phosphorylation, Mitochondrial Protein Synthesis, and ATP Production in Human Skeletal Muscle without Amino Acid Replacement. *American Journal of Physiology Endocrinology and Metabolism*, **303**, E1117-E1125. <https://doi.org/10.1152/ajpendo.00067.2012>
- [29] Stump, C.S., Short, K.R., Bigelow, M.L., Schimke, J.M. and Nair, K.S. (2003) Effect of Insulin on Human Skeletal Muscle Mitochondrial ATP Production, Protein Syn-

thesis, and mRNA Transcripts. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 7996-8001.

<https://doi.org/10.1073/pnas.1332551100>

- [30] Tatpati, L.L., Irving, B.A., Tom, A., *et al.* (2010) The Effect of Branched Chain Amino Acids on Skeletal Muscle Mitochondrial Function in Young and Elderly Adults. *The Journal of Clinical Endocrinology and Metabolism*, **95**, 894-902.
<https://doi.org/10.1210/jc.2009-1822>
- [31] Jacobs, B.L. and Fornal, C.A. (1991) Activity of Brain Serotonergic Neurons in the Behaving Animal. *Pharmacological Reviews*, **43**, 563-578.
- [32] Delgado, P.L., Charney, D.S., Price, L.H., Aghajanian, G.K., Landis, H. and Heninger, G.R. (1990) Serotonin Function and the Mechanism of Antidepressant Action. Reversal of Antidepressant-Induced Remission by Rapid Depletion of Plasma Tryptophan. *Archives of General Psychiatry*, **47**, 411-418.
<https://doi.org/10.1001/archpsyc.1990.01810170011002>
- [33] Dickinson, J.M. and Rasmussen, B.B. (2011) Essential Amino Acid Sensing, Signaling, and Transport in the Regulation of Human Muscle Protein Metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care*, **14**, 83-88.
<https://doi.org/10.1097/MCO.0b013e3283406f3e>