

# Plasma levels of various amino acids and their changes upon protein uptakes in Japanese young and old men and women

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## Abstract

**Background:** It is not known whether plasma amino acids levels are different between young and old men and women in Japan. No studies have been reported whether protein uptakes influence plasma levels of amino acids, lipids, insulin and glucose.

**Methods:** Self-administered diet history questionnaires were given to young, old men and women. Blood samples were taken early morning to determine plasma levels of various factors.

**Results:** Women and old people had lower plasma levels of most of amino acids, especially essential and branched-chain amino acids than men and young people. The uptakes of total, animal or vegetable proteins did not affect plasma levels of total amino acids. The uptakes of total protein did not affect plasma levels of lipids (triglycerides (TG), or LDL cholesterol) except for young men and old women whose plasma levels of HDL-cholesterol increased upon the uptake of total protein. Plasma levels of BCAAs did not affect plasma levels of insulin, glucose, or lipids. Increase in plasma BCAAs levels results in increase in plasma insulin levels.

**Conclusion:** old people or women have lower plasma levels of essential amino acids and BCAAs than young people or women, respectively. Increase in plasma levels of BCAAs increased only plasma levels of insulin, no increase being shown in plasma levels of glucose, TG, HDL- and LDL-cholesterol. In healthy men and women, the metabolisms of BCAAs and their effects on glucose homeostasis are not impaired.

**Abbreviations:** AAs: Amino acids; BCAAs: Branched-chain amino acids; TG: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein

## Introduction

Amino acids are important factors involved in the metabolism of proteins in all the tissues. A number of studies have indicated that branched -chain amino acids (BCAAs) such as leucine, isoleucine and valine are important in the regulation of protein synthesis [1]. Unlike other essential amino acids, BCAAs are degraded in skeletal muscle, and their circulating levels are higher postprandially [2], thus may influence glucose homeostasis. Branched-chain amino acids concentrations have been shown to be elevated in response to overnutrition and can affect insulin sensitivity and secretion [3]. Number of human studies have consistently indicated that concentrations of BCAAs in plasma and urine are associated with insulin resistance [4-7]. We recently examined relationship between various foods uptakes and body mass index (BMI) and various plasma parameters in Japanese young and old men and women [8]. We now report plasma levels of amino acids and their changes upon protein uptakes in young and old men and women in Japan.

## Ethics

This work has been approved by the Ethical committees of Showa Women's University and NPO "International projects on food and health" and has been carried out in accordance with The Code of

Ethics of the World Medical Association (Declaration of Helsinki) for experiments.

## Methods

We asked men and women older than 50 years old and men and women college students to participate in the experiments. checked their health carefully and recruited them if there were no health problems such as diabetes, hypertension nor serious diseases experienced in the past. They did not smoke in the past. We also excluded people who took drugs for dyslipidemia, hyperglycemia, or hypertension.

Participants were asked not to eat anything after 21.00 PM the previous evening. We took blood samples between 9.00 AM and 11.00 AM. Plasma specimens were collected for assays of blood parameters. We obtained an informed consent prior to conducting the protocol which had been approved by the Ethical Committee of Showa Women's University and Saiseikai Shibuya Satellite Clinic.

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Self-administered diet history questionnaires were given to healthy volunteers and were asked to describe answers on each item by recollection of diets they took. From these questionnaires, we calculated the intakes of energy, carbohydrate, fat, and protein.

After plasma was separated from blood, plasma factors were measured. Ethylenediamine tetra acetic acid (EDTA) was used as an anticoagulant. Blood glucose levels were measured by a hexokinase UV method. The CLEIA (chemiluminescent immunoassay) method was used for the measurement of insulin.

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<sup>®</sup> (Wako Pure Chemical Industries, Ltd., Osaka Japan). Target free amino acids as derivatized compounds were separated under a reversed phase UHPLC condition and determined by the liquid chromatograph mass spectrometer.

Lipid and lipoprotein concentrations (total cholesterol, HDL, TG) were determined using a Polychem Chemistry Analyzer (Polymedco Inc.). FFA concentrations were measured by a gas chromatography.

## Statistics

The results are presented as means  $\pm$  SEM. Statistical significance of the differences between groups was calculated according to 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  $P < 0.05$ . Spearman's correlation tests were used to examine statistical significance.

## Results

Table 1 shows the background of participants. We here just mention about ages, heights and body weights because the other parameters were indicated the previous paper [8].

Table 2 shows plasma levels of amino acids, essential, non-essential, and branched chain amino acids of young and old Japanese men and women. We here just pay attention to plasma levels of branched-chain amino acids (BCAAs) such as leucine, isoleucine and valine since their plasma levels are implicated to closely associated with diabetes mellitus. Table 2 shows that the plasma levels of BCAAs are lower in women than in men and lower in older people than younger people. Same relationships hold for essential amino acids (EAAs) such as BCAAs, tryptophan, histidine, phenylalanine, lysine, methionine, and threonine.

Table 3 shows that only in young men, the uptakes of meat and egg protein resulted in significant decrease in total plasma amino acids levels.

Total protein uptakes had nothing to do with plasma levels of glucose, insulin, TG, and LDL-cholesterol. In young men and old women, total protein uptakes resulted in crease in plasma levels of HDL-cholesterol (Table 4).

The data indicate that plasma insulin levels increase with increase in plasma BCAAs levels, most significantly in young men (Table 5).

## Discussion

Protein uptakes not only influence the body growth but the development of the brain. In 1960s, families with young children were given nutritional supplement in rural Guatemala [9]. A team tested

the assumption that giving enough protein in the first few years of life would decrease the incidence of stunted growth. Children who got protein supplements grew 1-2 centimeters taller than a control group. They also found that children who received the protein supplements scored better on reading and knowledge tests as adolescents. These results indicate that protein uptakes are important for the normal development of the body and the brain and intelligence.

Recently, much attention has paid to gender and age differences of diagnoses and treatments of various diseases [10]. In fact, the vast majority of animal studies have been conducted on males, mostly in rodents. For example, the number one killer of US women is heart disease, far exceeding death from breast cancer. Old women and men have a blockade of one or more of coronary arteries, young women are more likely to have diffuse plaques that line and narrow the entire artery [10].

So we thought it is vitally important to examine data using different age and gender groups. So far we examined various foods uptakes in young and old men and women in Japan [8]. We asked young college students and old male and female acquaintances to participate in the study. The number of participants of 4 groups and their ages, heights and weights are shown in Table 1.

There are some papers indicating that insulin regulates the metabolism of carbohydrate, lipid, protein, and amino acid [11]. Proteolysis and associated release of amino acids are inhibited by insulin and insulin stimulates amino acid uptake and protein synthesis in skeletal muscle [12,13]. High insulin levels were shown to stimulate skeletal muscle protein synthesis [14]. As to individual amino acids, the plasma levels of alanine, phenylalanine, valine, leucine, isoleucine, and tyrosine were shown to increase and the plasma levels of histidine and glutamine were shown to decrease in hyperglycemia [15].

There has been no research investigation concerning the effects of glucose or sucrose administration on plasma levels of various amino acids in young and aged male adults.

Fernstrom, J.D. and Wurtman, R.J. indicated that when plasma tryptophan concentrations were raised by receiving tryptophan in foods [16] or injection of insulin [17], the brain serotonin and tryptophan concentration increased. They indicated that carbohydrate ingestion increased insulin secretion which raised plasma tryptophan and lowered the concentration of the competing amino acids such as branched neutral amino acids in rats [18]. Carbohydrate ingestion was shown to decrease plasma free amino acid levels [19], and glucose intake resulted in a decrease in large neutral amino acids such as methionine, phenylalanine, tyrosine, and tryptophan [20]. These results suggested that an increase in plasma levels of glucose or insulin may increase the transport of some amino acids using various transporters, thus decreasing the concentration of such amino acids.

We reported that the plasma levels of EAAs, such as phenylalanine, leucine, isoleucine, valine, and tryptophan, decreased after the administration of glucose in humans [21]. We also found that both EAA and BCAA levels after sucrose administration significantly decreased in both young and aged men. Probably changes in the plasma levels of EAAs and BCAAs are sensitive to an increase in the insulin levels because sucrose administration resulted in lower levels of insulin than glucose administration.

These results suggest that there were significant decreases in the plasma levels of total amino acids, EAAs, and NEAAs after glucose administration in both old and young men.

**Table 1.** The background parameters of participants

	<b>a</b>	<b>b</b>	<b>c</b>	<b>d</b>	<b>Statistical Significances</b>
	Young men (n=49)	Young women (n=47)	Old men (n=25)	Old women (n=39)	
Age	20.7 ± 1.5	21.2 ± 0.7	60.8 ± 9.9	67.4 ± 7.5	a vs. c; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Height (m)	1.72 ± 0.06	1.58 ± 0.05	1.69 ± 0.07	1.57 ± 0.06	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
Weight (kg)	65.1 ± 8.9	51.4 ± 5.8	71.1 ± 13.1	50.6 ± 6.8	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01

a,b,c,d indicate plasma values of ages, heights and weights of participants, respectively.

**Table 2.** Various amino acids levels in plasma20 in Japanese young and old men and women

<b>Amino acid</b>	<b>Plasma amino acids levels of 12 young and old men and women</b>				<b>Statistical significance</b>
Histidine	78.5 ± 7.7	74.1 ± 6.0	78.7 ± 7.5	72.2 ± 6.6	a vs. d; p<0.01, c vs. d; p<0.01
Lysine	185.9 ± 28.6	154.0 ± 23.7	190.9 ± 28.7	170.0 ± 20.4	a vs. b; p<0.01, a vs. d; p<0.05, b vs. c; p<0.01, c vs. d; p<0.05
Methionine	27.3 ± 3.6	25.4 ± 3.7	27.6 ± 4.9	20.6 ± 3.3	a vs. d; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Phenylalanine	56.9 ± 7.0	54.2 ± 5.5	65.4 ± 8.8	51.9 ± 6.0	a vs. c; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
Threonine	131.4 ± 21.5	133.5 ± 43.9	136.5 ± 27.4	121.8 ± 41.9	No significance
Tryptophan	65.8 ± 9.1	65.4 ± 7.1	58.7 ± 9.2	51.4 ± 6.4	a vs. c; p<0.01, a vs. d; p<0.01, b vs. c; p<0.05, b vs. d; p<0.01, c vs. d; p<0.01
Isoleucine	67.9 ± 11.2	56.6 ± 7.9	65.7 ± 8.5	49.9 ± 7.4	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, b vs. d; p<0.05, c vs. d; p<0.01
Leucine	129.8 ± 16.3	104.6 ± 10.3	133.8 ± 17.5	100.3 ± 12.6	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
Valine	221.9 ± 23.8	203.9 ± 25.1	228.1 ± 30.6	186.5 ± 32.4	a vs. d; p<0.01, b vs. c; p<0.05, c vs. d; p<0.01
Tyrosine	58.2 ± 8.7	56.8 ± 8.4	70.3 ± 12.0	54.1 ± 8.4	a vs. c; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
Alanine	356.0 ± 68.9	346.1 ± 82.3	415.9 ± 75.7	326.1 ± 64.4	a vs. c; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
A-Amino Butyric Acid	20.9 ± 5.3	19.0 ± 6.1	21.7 ± 6.5	17.9 ± 4.1	c vs. d; p<0.01
Arginine	77.6 ± 15.0	65.4 ± 14.1	79.3 ± 17.6	82.4 ± 17.5	a vs. b; p<0.05, b vs. c; p<0.05, b vs. d; p<0.01
Asparagine	45.0 ± 6.1	47.9 ± 7.9	47.3 ± 5.7	30.1 ± 8.1	a vs. d; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Aspartic Acid	3.4 ± 1.4	2.5 ± 0.4	3.4 ± 0.7	9.7 ± 2.5	a vs. d; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Citrulline	22.2 ± 3.6	23.2 ± 4.0	27.0 ± 6.2	35.8 ± 8.5	a vs. c; p<0.01, a vs. d; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Cystine	14.1 ± 4.1	27.1 ± 5.5	23.6 ± 6.7	3.7 ± 1.6	a vs. b; p<0.01, a vs. c; p<0.01, a vs. d; p<0.01, b vs. c; p<0.05, b vs. d; p<0.01, c vs. d; p<0.01
Glutamic Acid	37.0 ± 11.6	23.5 ± 6.1	45.0 ± 16.5	163.8 ± 81.7	a vs. d; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Glutamine	548.5 ± 64.0	534.4 ± 70.0	575.6 ± 52.0	349.6 ± 87.6	a vs. d; p<0.01, b vs. d; p<0.01, c vs. d; p<0.01
Glycine	213.5 ± 25.9	217.3 ± 56.8	198.5 ± 28.6	236.7 ± 65.3	c vs. d; p<0.05
Mono Ethanolamine	8.5 ± 1.2	7.0 ± 0.9	8.8 ± 1.3	7.6 ± 1.0	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.05, c vs. d; p<0.01
Ornithine	59.4 ± 12.5	61.7 ± 17.3	72.5 ± 13.9	61.3 ± 15.3	a vs. c; p<0.01, c vs. d; p<0.01
Proline	172.8 ± 58.4	139.9 ± 40.7	169.2 ± 56.9	116.7 ± 39.5	a vs. d; p<0.01, c vs. d; p<0.01
Serine	124.6 ± 17.1	129.3 ± 25.9	105.5 ± 18.2	118.3 ± 22.2	a vs. c; p<0.01, b vs. c; p<0.01
Taurine	49.9 ± 1.5	50.9 ± 1.7	67.7 ± 3.1	61.2 ± 1.8	a vs. c; p<0.01, a vs. d; p<0.01, b vs. c; p<0.01, b vs. d; p<0.01
Total Amino Acids	2751.7 ± 215.0	2602.8 ± 295.2	2891.4 ± 201.6	2476.9 ± 233.2	a vs. d; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
Total Essential Amino Acids	965.3 ± 89.0	871.6 ± 84.1	985.4 ± 94.9	824.8 ± 92.9	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.05, c vs. d; p<0.01
Total Non-Essential Amino Acids	1786.4 ± 153.3	1731.2 ± 242.8	1906.0 ± 166.0	1652.1 ± 168.4	a vs. c; p<0.05, a vs. d; p<0.01, b vs. c; p<0.01, c vs. d; p<0.01
Total Branched Chain Amino Acids	419.5 ± 48.3	365.1 ± 38.9	427.5 ± 52.3	336.8 ± 49.2	a vs. b; p<0.01, a vs. d; p<0.01, b vs. c; p<0.05, c vs. d; p<0.01
Fisher Ratio	3.7 ± 0.4	3.3 ± 0.5	3.2 ± 0.4	3.2 ± 0.4	a vs. b; p<0.01, a vs. c; p<0.01, a vs. d; p<0.01

**Table 3.** Relationships between the uptakes of proteins obtained from various foods sources and plasma levels of amino acid

Correlation coefficients				
	Young men	Young women	Old men	Old women
Total protein uptake vs. Total plasma amino acids levels	-0.14	0.17	-0.26	0.21
Animal protein uptake vs. Total plasma amino acids levels	-0.17	0.08	-0.21	0.16
Vegetable protein uptakes vs. total plasma amino acids levels	-0.02	0.24	-0.32	0.22
Bean uptake vs. Total plasma amino acids levels	-0.2	0	0.07	0.24
Fish uptake vs. Total plasma amino acids levels	0.12	0.08	-0.18	0.27
Meat uptake vs. Total plasma amino acids levels	-0.31*	0.19	-0.15	-0.24
Egg uptake vs. Total plasma amino acids levels	-0.37**	-0.12	-0.05	-0.25
Milk uptake vs. Total plasma amino acids levels	0.05	-0.18	-0.07	0.2

\*\*p&lt;0.01, \*p&lt;0.05

**Table 4.** Relationship between total protein uptake and various plasma factors

Total protein uptake vs.	Young men	Young women	Old men	Old women
Fasting glucose levels	0.17	0.01	-0.02	-0.26
Plasma insulin levels	-0.18	0.06	0.12	0.06
Plasma TG levels	-0.06	0.07	0	-0.09
Plasma HDL-cholesterol levels	0.29*	-0.04	0.09	0.33*
Plasma LDL-cholesterol levels	-0.18	0.14	0.03	-0.26

\*p&lt;0.05

**Table 5.** Correlation between BCAAs and plasma levels of various factors

	Young men	Young women	Old men	Old women
Valine vs. Plasma fasting glucose levels	0.11	-0.10	-0.28	0.12
Valine vs. Plasma insulin levels	0.63**	0.22	0.22	0.19
Valine vs. HDL-Chol.	0.00	0.44*	-0.25	0.01
Valine vs. LDL-Chol.	0.18	0.24	0.18	-0.04
Valine vs. TG	0.24	0.21	0.34	-0.17
Leucine vs. plasma fasting glucose levels	0.09	0.06	-0.07	0.15
Leucine vs. Plasma levels of insulin	0.55**	0.28	0.23	0.31
Leucine vs. HDL-Chol.	-0.03	0.28	-0.26	-0.11
Leucine vs. LDL-Chol.	0.10	0.31	0.10	0.09
Leucine vs. TG	0.16	0.31	0.27	-0.11
Isoleucine vs. Plasma fasting glucose levels	0.24	0.09	-0.05	0.17
Isoleucine vs. Plasma insulin levels	0.47**	0.16	0.27	0.11
Isoleucine vs. HDL-Chol.	-0.05	0.38	-0.26	-0.04
Isoleucine vs. LDL-Chol.	0.10	0.34	-0.05	0.06
Isoleucine vs. TG	0.13	0.14	0.36	-0.19

\*\*p&lt;0.01, \*p&lt;0.05

Thus it is quite natural that plasma levels of BCAAs increase in patients of type 2 diabetes [4-7] because the metabolism of such amino acids are impaired.

In the present studies we report that plasma levels of essential amino acids are lower in women than in men, and that their levels are lower in old people than in young people (Table 2). These results may mean that in healthy old men and women levels of BCAAs and EAAs are not high, thus indicating normal amino acids metabolism.

The uptakes of total, animal, and vegetable proteins did not increase total amino acids levels in the plasma (Table3), which may mean that

the degradation and synthesis of amino acids are well regulated and not influenced by some extent of the increased uptake of proteins in healthy people.

It is also interesting that no correlations existed between protein uptakes and plasma insulin or glucose levels (Table 4). It is extremely interesting that increase in plasma levels of BCAAs increased plasma insulin levels (Table 5). These results indicate that although insulin levels are influenced by plasma levels of BCAAs, in healthy people the metabolism of BCAAs and their effects on glucose homeostasis are not impaired.

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## References

- Kolanu BR, Boddula V, Vadakedath S, Kandi V (2017) Amino Acid (Leucine) Chromatography: A Study of Branched-Chain Aminoaciduria in Type 2 Diabetes. *Cureus* 9: e1091. [Crossref]
- Buse MG, Biggers JF, Friderici KH, Buse JF (1972) Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat. The effect of fatty acids, glucose, and pyruvate respiration. *J Biol Chem* 247: 8085-8096.
- McCormack SE, Shaham O, McCarth MA, Deik AA, Wang TJ, et al. (2013) Circulating Branched-chain Amino Acid Concentrations Are Associated with Obesity and Future Insulin Resistance in Children and Adolescents. *Pediatr Obes* 8: 52-61. [Crossref]
- Adeva MM, Calviño J, Souto G, Donapetry C (2012) Insulin resistance and the metabolism of branched-chain amino acids in humans. *Amino Acids* 43:171-81.
- Giesbertz P, Daniel H (2016) Branched-chain amino acids as biomarkers in diabetes. *Curr Opin Clin Nutr Metab* 19: 48-54.
- Okekunle AP, Li Y, Liu L, Du S, Wu X, et al. Abnormal circulating amino acid profiles in multiple metabolic disorders. *Diabetes Res Clin Pract* 132: 45-58. [Crossref]
- Connelly MA, Wolak-Dinsmore J, Dullaart RPF (2017) Branched Chain Amino Acids Are Associated with Insulin Resistance Independent of Leptin and Adiponectin in Subjects with Varying Degrees of Glucose Tolerance. *Metab Syndr Relat Disord* 15: 183-186.
- Shimizu F, Ishii Y, Ogawa M, Takao T, Matsuoka K, et al. (2017) Relationship between Various Food Uptakes and Body Mass Index (BMI) in Japanese Young and Old Men and Women. *J Clin Nutr Diet* 3: 2.
- Storrs C (2017) How poverty affects the brain. *Nature* 547: 150-152. [Crossref]
- Stefanick ML (2017) Not just for men. *Scientific American* 317: 48-53.
- Felig P (1975) Amino acid metabolism in man. *Annu Rev Biochem* 44: 933-955. [Crossref]
- Jefferson LS, Li JB, Rannels SR (1977) Regulation by insulin of amino acid release and protein turnover in the perfused rat hemiscorpus. *J Biol Chem* 252: 1476-1483. [Crossref]
- Biolo G, Williams BD, Fleming RY, Wolfe RR (1999) Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes* 48: 949-957
- Robinson MM, Soop M, Sohn TS, Morse DM, Schimke JM, et al. (2014) High insulin combined with essential amino acids stimulates skeletal muscle mitochondrial protein synthesis while decreasing insulin sensitivity in healthy humans. *Clin Endocrinol Metab* 99: E2574-E2583.
- Stancáková A, Civelek M, Saleem NK, Soinen P, Kangas AJ, et al. (2012) Hyperglycemia and a common variant of GSKR are associated with the levels of eight amino acids in 9,369 Finnish men. *Diabetes* 61: 1895-1902.

16. Fernstrom JD, Wurtman RJ (1971) Brain serotonin content: increase following ingestion of carbohydrate diet. *Science* 174: 1023-1025.
17. Fernstrom JD, Wurtman RJ (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* 178: 414-416
18. Lipsett D, Madras BK, Wurtman RJ, Munro HN (1973) Serum tryptophan level after carbohydrate ingestion: selective decline in non-albumin-bound tryptophan coincident with reduction in serum free fatty acids. *Life Sci* 12:57-64.
19. Pan RM, Mauron C, Glaeser B, Wurtman RJ (1982) Effect of various oral glucose doses on plasma neutral amino acid levels. *Metabolism* 31: 937-943.
20. Shimbo K1, Kubo S, Harada Y, Oonuki T, Yokokura T, et al. (2010) Automated precolumn derivatization system for analyzing physiological amino acids by liquid chromatography/mass spectrometry. *Biomed Chromatogr* 24: 683-691. [[Crossref](#)]
21. Ogawa M, Takao T, Ishii Y, Shimizu F, Takada A, et al. 2016. Changes in Plasma Amino Acid Levels After the Administration of Glucose or Sucrose to Healthy Young and Aged Males. *M J Nutr* 1: 008.