only), and dietary factors, including glycemic load and daily intakes (continuous) of saturated fat, dietary fiber, alcohol, and coffee. (Glycemic load is defined as the amount of each carbohydrate consumed multiplied by its respective glycemic index. The glycemic index ranks carbohydrate foods on the basis of their postprandial blood glucose response (24).) The associations of individual BCAAs with the risk of diabetes were evaluated in a similar way. All of the statistical analyses were performed using SAS programs (SAS Institute Inc., Cary, North Carolina). Significance was defined as a 2-sided P value less than 0.05.

RESULTS

Characteristics of the study population, by sex and tertile of total BCAA intake (percentage of total protein intake), are shown in Table 1. Men and women who had a greater intake of total BCAAs were more likely to be older and less educated (<15 years) and to have reported a history of hypertension. They also had lower intakes of dietary fiber, alcohol, and coffee and higher intakes of saturated fat and total protein. In addition, women who had a greater intake of total BCAAs were more likely to be postmenopausal and never smokers. Men who had a greater intake of total BCAAs had a higher dietary glycemic load. Major food groups supplying BCAAs were cereals/potatoes and starches, fish and shellfish, and meats. The contributions (as a percentage of total amount of BCAAs) of these food groups to BCAA intake were 24.6%, 23.2%, and 14.9%, respectively, in men and 22.7%, 20.7%, and 13.7%, respectively, in women. Our questionnaire was designed to measure an individual's relative intake of nutrients rather than absolute values. Although we present the mean values for nutrients in Table 1, some of them may have been overestimated by our questionnaire. The mean values estimated from the FFQ were generally higher than those estimated from 12 daily diet records; for example, the mean estimate of total protein in the former was 8% higher in men and 14% higher in women than in the latter.

During a 10-year follow-up period, 438 participants reported the development of diabetes. The hazard ratios and 95% confidence intervals for diabetes according to tertiles of total BCAAs and constituent amino acids are shown in Table 2. In men, total BCAA intake was not significantly associated with the risk of diabetes after controlling for covariates. However, compared with the lowest intake, the highest tertile of leucine intake was marginally significantly associated with a decrease in the risk of diabetes ($P = 0.06$). The trend toward greater reduction in the risk of diabetes with increasing leucine intake was also of borderline significance ($P = 0.06$). In women, a high intake of total BCAAs was significantly inversely associated with the risk of diabetes, and the trend was also significant. A similar association was observed for each individual BCAA, although the association was somewhat weaker and nonsignificant for isoleucine.

Analysis stratified according to body mass index (<25 or ≥25) showed that this variable did not greatly affect the associations between BCAA intake and diabetes. For example, in men, the hazard ratios for the highest tertile of leucine versus the lowest were 0.73 (95% confidence interval (CI): 0.45, 1.18; P-trend = 0.19) and 0.58 (95% CI: 0.31, 1.09; P-trend = 0.09), respectively, for these 2 body mass index groups. The corresponding values for total BCAAs in women were 0.53 (95% CI: 0.30, 0.96; P-trend = 0.04) and 0.75 (95% CI: 0.35, 1.62; P-trend = 0.45). Exclusion of persons with diabetes that developed during the first 3 years did not alter the results substantially; for example, the hazard ratios for the highest tertile of intake versus the lowest were 0.70 (95% CI: 0.40, 1.08; P-trend = 0.10) for leucine in men and 0.55 (95% CI: 0.34, 0.90; P-trend = 0.02) for total BCAAs in women.

DISCUSSION

In the present prospective study, high intakes of total BCAAs, leucine, and valine were associated with a decreased risk of diabetes in women. In men, leucine intake was marginally significantly inversely associated with the risk of diabetes. Our results suggest a beneficial effect of dietary leucine or BCAAs in preventing diabetes. However, at present, conflicting data exist on the role of BCAAs as a mechanism for the observed association. Leucine or BCAA supplementation has exerted beneficial effects on the metabolic profiles of obese subjects and patients with diabetes or chronic liver disease (5–7), which supports our findings. However, findings from studies based on plasma BCAA levels have suggested rather that BCAAs may promote insulin resistance (8–13, 25). Plasma BCAA levels were higher in obese subjects than in lean subjects and were positively correlated with insulin resistance (25). A recent nested case-control study in the Framingham Offspring Study (10) showed that plasma BCAA levels were correlated with fasting insulin levels and could predict future risk of diabetes, especially in obese persons and those with elevated fasting glucose levels. Plasma BCAA level was associated with insulin resistance in young normoglycemic adults at baseline and 6-year follow-up (11) and in obese subjects after weight loss (12). However, in general, observed blood amino acid patterns are probably not a direct reflection of diet-derived amino acids. McCormack et al. (13) found that plasma BCAA level, but not dietary BCAA intake, was associated with obesity and insulin resistance measured 18 months later among children and adolescents. Qin et al. (23) reported that a high intake of BCAAs was significantly associated with a lower prevalence of being overweight among apparently healthy middle-aged adults, which contradicts the observations for plasma BCAAs. In fact, we also noted that BCAA intake, either as an absolute measurement or a percentage of protein, was unrelated to plasma BCAA levels ($r = 0.004$ and $r = -0.01$, respectively) and insulin resistance ($r = 0.04$ and $r = -0.01$, respectively) in another sample of women ($n = 850$) enrolled in a health check-up program provided by a general hospital in Gifu (unpublished data). The reasons for the elevated plasma BCAA level among obese or insulin-resistant subjects are unknown. However, several studies found that the activity of the BCAA catabolic enzyme was reduced in obese, insulin-resistant rodents (26, 27). In obese humans, blood BCAA levels have been seen to drop significantly and expression of the enzyme branched-chain α-ketodehydrogenase has been seen to increase following bariatric surgery (28). Thus, it has been proposed that elevated

Table 1. Baseline Characteristics of Study Subjects According to Tertile of Total Branched-chain Amino Acid Intake,[a] Takayama Study, Japan, 1992–2002

Characteristic	Men ($n = 5,885$)							Women ($n = 7,640$)						
	Tertile 1 ($n = 1,962$)		Tertile 2 ($n = 1,962$)		Tertile 3 ($n = 1,961$)		*P* Value[b]	Tertile 1 ($n = 2,547$)		Tertile 2 ($n = 2,547$)		Tertile 3 ($n = 2,546$)		*P* Value[b]
	Mean	%	Mean	%	Mean	%		Mean	%	Mean	%	Mean	%	
Age, years	49.8		51.7		53.9		<0.0001	50.0		51.3		53.1		<0.0001
Years of education														
≤11		46.2		48.6		59.8			55.6		57.0		65.1	
12–14		39.1		39.3		30.7			38.3		37.7		30.9	
≥15		14.7		12.2		9.5	<0.0001		6.1		5.3		4.0	<0.0001
Smoking status														
Never smoker		14.3		16.5		19.2			80.4		86.6		89.2	
Former smoker		27.5		30.4		32.6			5.8		4.7		3.9	
Current smoker		58.2		53.1		48.2	<0.0001		13.8		8.7		6.9	<0.0001
History of hypertension		15.3		17.0		19.3	0.005		12.3		14.5		15.7	0.02
Postmenopausal (women only)									50.3		55.9		64.5	<0.0001
Body mass index[c]	22.7		22.6		22.6		0.21	22.1		22.1		22.1		0.87
Physical exercise, MET-hours/week	28.1		30.3		28.8		0.62	20.8		22.1		21.8		0.33
Daily dietary intake														
Alcohol, g	57.8		40.1		33.9		<0.0001	11.6		6.7		4.9		<0.0001
Coffee, no. of servings	1.1		0.9		0.6		<0.0001	1.0		0.7		0.5		<0.0001
Total energy, kcal	2,702		2,668		2,673		0.28	2,200		2,189		2,190		<0.0001
Protein, g	88.3		94.8		101.5		<0.0001	80.4		84.4		89.6		<0.0001
Saturated fat, g	15.3		16.7		19.2		<0.0001	15.0		16.0		18.1		<0.0001
Fiber, g	16.5		16.4		15.6		<0.0001	18.2		17.0		16.0		<0.0001
Carbohydrate, g	374		376		369		0.18	327		321		313		<0.0001
Glycemic load[d]	223.2		234.9		232.9		<0.0001	195.5		198.3		192.4		0.09

Abbreviation: MET, metabolic equivalent.

[a] Total branched-chain amino acid intake (sum of leucine, isoleucine, and valine intakes) is expressed as a percentage of total protein intake.

[b] *P* values were based on linear regression analysis for continuous variables and on the χ^2 test for categorical variables.

[c] Weight (kg)/height $(m)^2$.

[d] Glycemic load was calculated by multiplying the carbohydrate content of each food by its glycemic index (24), multiplying this value by the frequency of consumption, and summing these values for all foods. The measure has no units.

Table 2. Hazard Ratios for the Risk of Diabetes According to Tertile of Branched-chain Amino Acid Intake[a] Among Men and Women, Takayama Study, Japan, 1992–2002

BCAA and Tertile of Intake	Men							Women						
	Median Intake	No. of Cases	No. of Subjects	HR[b]	95% CI	HR[c]	95% CI	Median Intake	No. of Cases	No. of Subjects	HR[b]	95% CI	HR[c]	95% CI
Total BCAAs														
1	16.74	106	1,962	1.00		1.00		16.86	64	2,547	1.00		1.00	
2	17.22	76	1,962	0.71	0.53, 0.96	0.75	0.55, 1.03	17.31	58	2,547	0.87	0.61, 1.24	0.75	0.51, 1.10
3	17.69	84	1,961	0.77	0.58, 1.04	0.78	0.54, 1.13	17.76	50	2,546	0.71	0.49, 1.03	0.57	0.36, 0.90
P-trend					0.07		0.17					0.07		0.02
Leucine														
1	7.56	106	1,962	1.00		1.00		7.60	65	2,547	1.00		1.00	
2	7.77	82	1,962	0.77	0.58, 1.03	0.79	0.58, 1.09	7.80	56	2,547	0.84	0.59, 1.20	0.75	0.51, 1.10
3	7.98	78	1,961	0.71	0.53, 0.96	0.70	0.48, 1.02	7.99	51	2,546	0.72	0.50, 1.04	0.62	0.39, 0.97
P-trend					0.02		0.06					0.08		0.04
Isoleucine														
1	4.17	91	1,962	1.00		1.00		4.21	55	2,547	1.00		1.00	
2	4.31	86	1,962	0.94	0.70, 1.27	0.94	0.68, 1.30	4.33	54	2,547	0.93	0.64, 1.36	0.78	0.51, 1.17
3	4.44	89	1,961	0.98	0.72, 1.31	0.93	0.63, 1.39	4.59	63	2,546	1.05	0.73, 1.51	0.77	0.46, 1.25
P-trend					0.87		0.73					0.78		0.31
Valine														
1	5.00	108	1,962	1.00		1.00		5.03	65	2,547	1.00		1.00	
2	5.15	74	1,962	0.68	0.51, 0.92	0.75	0.54, 1.03	5.18	62	2,547	0.90	0.64, 1.28	0.87	0.58, 1.25
3	5.30	84	1,961	0.76	0.57, 1.01	0.87	0.58, 1.24	5.33	45	2,546	0.62	0.42, 9.91	0.61	0.39, 0.94
P-trend					0.05		0.42					0.01		0.03

Abbreviations: BCAA, branched-chain amino acid; CI, confidence interval; HR, hazard ratio.

[a] Total branched-chain amino acid intake (sum of leucine, isoleucine, and valine intakes) is expressed as a percentage of total protein intake.

[b] Adjusted for age.

[c] Additionally adjusted for years of education, body mass index, physical activity, smoking status, history of hypertension, glycemic load, menopausal status (women only), and intakes of total energy, total protein, saturated fat, dietary fiber, alcohol, and coffee.

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plasma BCAA levels in obese or diabetic subjects are caused, in part, by reduced BCAA catabolism (29). Although stimulation of insulin secretion by BCAA is expected to prevent hyperglycemia, this process might not sufficiently compensate for impaired insulin secretion. In this context, it is possible that BCAAs play different roles in glucose metabolism among persons with insulin-resistant and non-insulin-resistant conditions. We did not include the measurement of blood glucose or insulin level. However, body mass index, which is generally correlated with insulin resistance, did not greatly modify the association between dietary BCAA intake and the risk of diabetes. Although it is not known whether circulating BCAAs are causes/mediators of insulin resistance or by-products of the associated metabolic dysfunction, the present study highlights the need for researchers to consider dietary intake of BCAAs.

Among the individual BCAAs, leucine shows great potency in stimulating the secretion of insulin (30). We observed that leucine, as well as total BCAAs, was significantly inversely associated with the risk of diabetes in women. Although these associations were not significant in men, inverse associations were suggested. Obayashi et al. (31) reported that estradiol increased the activity of the BCAA catabolism enzyme in ovariectomized rats, suggesting control of BCAA catabolism by estrogen. Hormonal status in women may favor the potentially beneficial effect of dietary BCAAs on the risk of diabetes.

Strengths of our study include the prospective design, validation of the dietary questionnaire, representation of the general population, and information on potential confounders. Several limitations should also be considered. The identification of cases of diabetes was based on self-reports. In a previous study conducted in Japan, relatively high sensitivity and specificity were reported for self-reported diabetes relative to physician-reported diabetes; the sensitivity and specificity were 80.8% and 99.3%, respectively (32). However, no screening for undiagnosed diabetes was done. The sensitivity of self-reported diabetes as compared with the criterion defined by hemoglobin A_{1c} level was low in our subsample, and low sensitivity of self-reported diabetes in comparison with biomarkers has been reported from other studies (33, 34). If subjects who had diabetes but were misclassified as nondiabetic were more likely to have had a higher intake of BCAAs than those who were correctly classified as diabetic, the results found in the present study would have been affected. Considering that the rate of response to the follow-up questionnaire was not high, the possibility that subjects who had diabetes participated in the study only when they had a low intake of BCAAs or that those who had no diabetes participated in the study only when they had a high intake of BCAAs should also be considered. However, BCAAs are present in various foods, and their intake was expressed as a percentage of total protein intake. In addition, baseline BCAA intakes were similar between respondents and nonrespondents to the follow-up questionnaire. Therefore, it is not likely that BCAA intake was dependent on the diagnosis of diabetes or participation in the study. Despite the use of a validated FFQ, some degree of misclassification of dietary intake is to be expected, just as in other nutritional epidemiologic studies. However, it is unlikely that incident diabetes cases would be systematically underestimated in our FFQ at baseline. Underlying diseases or preclinical signs at baseline may have affected diet, but it is unlikely that such conditions induced lower consumption of BCAAs without affecting total protein or total energy intake. In addition, exclusion of the first 3 years of follow-up did not substantially change the results. Adjustment for numerous lifestyle and dietary factors did not appreciably affect the results. However, we could not fully establish whether the observed reduction in the risk of diabetes was attributable to other nutrient parameters. We could not obtain information on family history of diabetes.

In conclusion, our findings suggest that dietary leucine or BCAA intake might be associated with the risk of diabetes in adults. Studies focusing on the relationship between dietary intake of BCAAs, especially long-term intake, and diabetes are needed. Because this is, to our knowledge, the first study to have examined the association between BCAA intake and risk of diabetes, replication of these results is required.

ACKNOWLEDGMENTS

Author affiliations: Department of Epidemiology and Preventive Medicine, Graduate School of Medicine, Gifu University, Gifu, Japan (Chisato Nagata, Kozue Nakamura, Keiko Wada, Michiko Tsuji, Yuya Tamai, and Toshiaki Kawachi).

This study was supported by grants from the Japanese Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology (Tokyo, Japan).

Conflict of interest: none declared.

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