### ORAL HEALTH AND NUTRITIONAL INTAKE IN COMMUNITY-DWELLING 90-YEAR-OLD JAPANESE PEOPLE: A CROSS-SECTIONAL STUDY

by

Raksanan Karawekpanyawong

A dissertation submitted to Graduate School of Medical and Dental Sciences,

Faculty of Dentistry, Niigata University in conformity with the requirements

for the degree of Doctor of Philosophy.

Niigata Prefecture, Japan

March, 2022

© Raksanan Karawekpanyawong 2022

All rights reserved

## Abstract

**Introduction:** This study aimed to determine the relationship between dentition, mastication, salivation, and nutritional intake in 90-year-old Japanese people.

**Methods:** This cross-sectional study included 84 participants (39 men and 45 women) aged 90 years. We used questionnaires to collect demographic information, smoking status, nutritional intake, and higher-level functional capacity. Nutritional intake was assessed using the validated Brief-Type Self-Administered Diet History Questionnaire, and higher-level functional capacity was assessed using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC) questionnaire. Oral examinations, masticatory performance tests, stimulated salivary flow rate (SSF) tests, blood tests, blood pressure tests, and body mass index (BMI) assessments were conducted. Univariable and multivariable linear regression analyses were performed.

**Results:** Multivariable linear regression analyses adjusted for sex, education, TMIG-IC, and BMI  $\leq 20$  kg/m2 showed that participants with masticatory performance < 173 mg/dL had lower intake of folic acid and vitamin A than those with masticatory performance  $\geq 173$  mg/dL. SSF was positively associated with intake of vitamin A. The number of teeth was positively associated with the intake of  $\beta$ -carotene. With smaller effect sizes, masticatory performance, SSF and number of teeth were also associated with the intake of various micronutrients and carbohydrates.

**Conclusion:** Lower masticatory performance, lower SSF, and fewer teeth were associated with a lower intake of several micronutrients, such as vitamin A,  $\beta$ -carotene, and folic acids, in Japanese individuals of advanced age. Oral health practitioners should pay careful attention to the nutritional intake of older people with poor mastication, dry mouth, and severe tooth loss.

# Contents

Chapter 1 Introduction	1
Chapter 2 Materials & Methods	3
2.1 Sample	3
2.2 Higher-Level Functional Capacity	4
2.3 Nutritional Intake Assessment	4
2.4 Oral Health Assessment	5
2.5 Other Assessments	5
2.6 Statistical Analysis	6
Chapter 3 Results	8
Chapter 4 Discussion/Conclusion	19
4.1 Main Findings	19
4.2 Strengths and Limitations	21
4.3 Generalizability and Biases	22
4.4 Mastication, Salivation, Dentition, and Vitamin A	23
4.5 Other Findings	24
4.6 Implications for Future Research	26
4.7 Conclusion	26
Appendix 1 Nutritional intake of 90-year-old Japanese people in 2018 (n=83)	27
Appendix 2 Plots of the unstandardized correlation coefficients (B) and compatib	oility
intervals (CI) of the results from multivariable linear regression analyses	31
Acknowledgements	37
Statement of Ethics	37
Conflict of Interest Statement	37
Funding Sources	37
Author Contributions	37
Data Availability Statement	38
References	39
List of Tables	43
List of Figures	43

## **Chapter 1**

#### Introduction

Age-related deterioration of oral health conditions can affect nutritional intake in older adults (1) because it can alter appetite (2), food enjoyment (3), and food choices (1); it can also diminish their overall well-being and quality of life (4). Physiological changes due to aging, such as reduced vitamin D synthesis from the skin, decreased immune function, and increased gastric pH, raise older people's nutritional requirements (5). Because older people require increased nutrition to maintain their functional health, inadequate macronutrient and micronutrient intake can contribute to frailty syndrome, which may result in increased mortality (6), and can conversely worsen oral health conditions (7-9).

Oral health components, such as dentition (1, 10), mastication (1), and salivation (11), are associated with nutrition. People with reduced dentition consume fewer fruits (12) and vegetables (13) and frequently have lower concentrations of vitamin C (14), A (14), and carotenes (12) in their blood. Poor masticatory performance is associated with malnutrition (15) and obesity (1), whereas hyposalivation is associated with deficient nutritional status (11).

Japanese people have been reported to have the longest average life expectancy because of their comparatively lower mortality rates due to ischemic heart diseases and cancers (16). It is believed that their unique eating patterns may be resulting in the low prevalence of obesity, contributing to their longevity (16).

Although the relationships between dentition, mastication, and nutrition have been widely studied, the measurements and results have been heterogeneous (17). The relationship between salivation and nutritional intake is poorly reported. Among the oldest-old populations (>80 years), information on the association between various aspects of oral health and intake of each nutrient remains insufficient. We hypothesized

that the conditions of dentition, salivation, and mastication among these individuals are associated with the intake of essential nutrients. Accordingly, this study aimed to determine whether there are associations between dentition, mastication, and salivation, and nutrient intake among 90-year-old Japanese people.

## Chapter 2

#### Materials & Methods

#### 2.1 Sample

This cross-sectional study used data from the Niigata cohort study in 2018. The Niigata cohort study is an interdisciplinary study of the aging population initiated in 1998. Figure 1 shows the flow chart of sample selection. We sent a questionnaire regarding health conditions and intention to join further examination to 4,542 older people residing in Niigata city, Japan, who were born in 1927 (70 years old) and did not require any special care for their daily activities. Among the 3,695 responses, 1,479 residents (859 men, 620 women) agreed to participate in the examination. Since we aimed for a 1:1 men to women ratio, we also included female participants who were unsure of their intention to join the examination (n = 190). In addition, we randomly selected 730 out of 859 men. After that, we sent the final invitations to 1,540 prospective participants and asked them to come to the designated survey sites. At the baseline (1998), 600 participants (306 men and 294 women) joined the study (18). From 1998 to 2008, the surveys were conducted yearly at the community halls. However, during 2009–2017, home visiting surveys were conducted annually.

In 2018, all the participants were 90 years old. Eighty-eight participants (41 men and 47 women) participated in the survey at the community halls. They accounted for 14.7% of the participants at baseline. Written informed consent was obtained from all participants at the baseline and in 2018. Participants were asked to complete the questionnaires in advance regarding higher-level functional capacity, nutritional intake, and smoking status and bring the questionnaires to the survey sites. Researchers checked the questionnaires and assisted the participants to fill in information in the incomplete fields. Oral health assessments, height and weight measurements, blood samplings, and blood pressure measurements were conducted.

#### 2.2 Higher-Level Functional Capacity

Higher-level functional capacity was measured using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC) (19), which indicates the ability of a person to live independently and thus access nutritious foods. The TMIG-IC is a 13-item questionnaire that consists of three domains: instrumental activity of daily living, intellectual activity, and social role. Apart from physical function, the TMIG-IC score also reflects dependency, cognitive function, and social health.

#### 2.3 Nutritional Intake Assessment

Nutritional intake was assessed using the validated Brief-Type Self-Administered Diet History Questionnaire (BDHQ) (20). BDHQ is a 58-item food frequency questionnaire that asks participants how frequently they consumed 11 kinds of foods in the past month, including grains, potatoes, meat, seafood, eggs, dairy products, vegetables, seaweeds, beans, nuts, and fruits. We asked the participants to complete the questionnaires by themselves or with the assistance of their family members or caregivers at home. At the survey sites, the researchers checked for unfilled items and asked the participants to answer those questions (if they were comfortable doing that). Additional explanation was provided when needed. After data collection, we sent the data to the experts to calculate the estimated intake of daily energy, macronutrients, and micronutrients through EBNJAPAN (http://www.ebnjapan.org/). The estimated nutritional intake for the 58 food items was calculated using the software developed by Sasaki in 2004 (21). Detailed information on calculation of nutritional intake collected through BDHQ has been published previously (22, 23). The nutrients selected for the data analysis were identified based on the literature review of essential nutrients for older people (5) and nutrients reported to be related to oral health (7). The macronutrients of interest were protein, carbohydrate, fat, and total dietary fiber. The micronutrients of interest were calcium, zinc, folic acid, iron, β-carotene, vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E ( $\alpha$ -tocopherol and  $\gamma$ -tocopherol), vitamin K, n-3 fatty acids, and n-6 fatty acids.

#### 2.4 Oral Health Assessment

The oral health assessment consisted of a dental examination, a masticatory performance test, and a stimulated salivary flow (SSF) test.

In 2018, two dentists conducted the oral examinations using probes and mouth mirrors under artificial lights, following the World Health Organization Basic Methods for Oral Health Survey 5th Edition (24). The numbers of teeth present were recorded.

For assessment of masticatory performance, we asked each participant to chew a glucose-containing gummy jelly for 20 s, rinse with 10 mL of water, and spit out all fragments and water into a filtration tool. The glucose concentration in the solution (mg/dL) was then measured using a special device (GLUCO SENSOR GS-II, GC. Co. Ltd., Tokyo, Japan). A higher glucose concentration in the samples was indicative of superlative masticatory performance. According to the manufacturer's recommendation, a glucose concentration of <100 mg/dL was considered to be indicative of a decline in masticatory performance. The reproducibility and accuracy of this method has been reported elsewhere (25).

SSF was measured by chewing a tasteless gum for 3 min and spitting it out with saliva into a filter; the salivary volume was subsequently recorded (26).

#### 2.5 Other Assessments

The participants' body weights and heights were measured and their body mass index (BMI) was calculated. In line with the criterion of the National Health and Nutritional Survey of Japan participants with a BMI  $\leq 20 \text{ kg/m}^2$  were identified with an undernutrition tendency (27). A low BMI may be indicative of a person's physical and mental barriers to the consumption of a nutritious diet, such as frailty, depression, and related systemic diseases. It was also associated with long-term care needs and mortality in the Japanese population (27). In addition, blood samples were collected at baseline and in 2018 for measurement of serum albumin levels. Blood pressure was measured using a digital sphygmomanometer at baseline and in 2018. We used questionnaires to obtain information regarding sex and education (years) at the baseline in 1998 and reassessed smoking status in 2018.

#### 2.6 Statistical Analysis

This data analysis included 84 participants. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA). Statistical significance was set at p < 0.05 (two-sided). Missing data from all variables were controlled at less than 5%. We were unable to measure the masticatory performance in eight participants, which amounted to about 10% of the total population (n = 88). Four participants did not cooperate with the masticatory performance test owing to low cognitive function. The other four participants refused to take the test. To improve the analysis model, we excluded the latter four participants. The final number of participants included in the analysis was 84, as shown in Figure 1.



Figure 1. Flow chart of participant selection

The characteristics of the sample, similarities between the sample and dropouts, and nutritional intake of the sample were confirmed by descriptive analyses, independent *t*-tests, Mann–Whitney *U* tests, and Pearson chi-square tests.

The estimated intake of each nutrient was adjusted for total daily energy intake of each participant using this formula:

nutritional intake (standard unit/1,000 kcal) =  $\frac{\text{nutritional intake (standard unit/d) x 1,000}}{\text{total energy intake (kcal/d)}}$ 

We used univariable linear regression analysis and multivariable linear regression analysis to determine the association of oral health with each nutrient intake. Dependent variables were the intake of each of the nutrients. Continuous independent variables were masticatory performance (mg/dL), salivary flow rate (mL/3 min), and number of teeth present (teeth). Categorical independent variables were presence of lower masticatory performance (less than the median of 173 mg/dL) and hyposalivation (SSF  $\leq 0.7$  mL/min(28)). All dependent variables and independent variables were entered into both univariable and multivariable models. We performed residual normality tests to confirm normality assumption for linear regression. Independent variables that violated the assumption were excluded from the analyses. For multivariable linear regression, the confounding factors included were sex, education, higher-level functional capacity (TMIG-IC), and undernutrition tendency (BMI  $\leq$  20 kg/m<sup>2</sup>).

To control the false-discovery rate that may arise from multiple testing in linear regression analyses, we estimated the minimum false-discovery rate by calculating q-values, as proposed by Storey in 2002 (29). The minimum false-discovery rate was controlled at 5% (q-values < 0.05). Results of linear regression analyses that gave desirable q-values are identified in the result tables, whereas results with q-values of >0.05 were treated as nonsignificant results.

## **Chapter 3**

#### Results

The sample consisted of 84 older people (39 men and 45 women). Table 1 shows the characteristics of the sample in 2018. Approximately 16% of the participants presented a decline in masticatory performance (< 100 mg/dL), and about 21% had hyposalivation. The number of remaining teeth ranged from 0 to 28, with a median of 12 teeth. Almost 30% of these older people had >20 teeth in their mouth. Seventeen percent of them were edentulous, and all of the edentulous participants were wearing dentures. The proportion of removable denture wearers in our study was 75% (n = 63). Participants who were not wearing removable dentures had approximately 23 teeth per individual. Among all participants who had 1-19 teeth (n = 45), only one participant had no tooth replacement. Female participants had significantly lower education, less salivary flow, and less daily energy intake than male participants.

According to the descriptive analysis (Table 2), there were no differences at baseline between the sample and the dropout group with respect to sex, education, number of remaining teeth, SSF, blood pressure, or concentration of serum albumin. In the sample, the number of current smokers was significantly lesser than that of the dropouts. The appendix shows the nutritional intake of the sample in 2018.

		Sample	
Variable	Men	Women	Total
	(n = <b>39</b> )	( <b>n</b> = <b>45</b> )	( <b>n</b> = <b>84</b> )
Education (years) <sup>a</sup>	11 (9–14)	10 (8–11)	10 (8–11)
Higher-level functional capacity	11 (9, 12)	11 (0, 12 5)	11 (0, 12)
(TMIG-IC score)	11 (8–12)	11 (8–12.5)	11 (8–12)
Masticatory performance (mg/dL)	$165.19\pm87.4$	$190.07 \pm 80.762$	$178.6\pm84.3$
<173	23 (59.0)	17 (37.8)	40 (47.6)
≥173	14 (35.9)	26 (57.8)	40 (47.6)
Missing data <sup>b</sup>	2 (5.1)	2 (4.4)	4 (4.8)
Stimulated salivary flow (mL/min) <sup>a</sup>	1.5 (1.0-2.0)	1.1 (0.7–1.7)	1.2 (0.8–1.9)
>0.7	33 (84.6)	30 (35.7)	63 (75.0)
≤0.7	5 (12.8)	13 (28.9)	18 (21.4)
Missing data <sup>c</sup>	1 (2.6)	2 (4.4)	3 (3.6)
Number of teeth	12 (4–22)	12 ( <b>3.</b> 5–20)	12 (4–21)
Edentulous	7 (17.9)	7 (15.6)	14 (16.7)
1–19	18 (46.2)	27 (60.0)	45 (53.6)
≥20	14 (35.9)	11 (24.4)	25 (29.7)
Denture use	7(17.0)	7 (15 6)	14 (16 7)
Complete dentures	7(17.9)	7 (13.0)	14(10.7)
Removable partial dentures	22(30.4)	27(00.0) 11(24.4)	49(36.3)
No removable dentures	10 (23.0)	11 (24.4)	21 (25.0)
Deily openay intelse (legel/d) <sup>a</sup>	2087.3	1880.6	1954.3
Dany energy intake (kcal/u)	(1664.2–2914.4)	(1577.3–2122.9)	(1641.2–2533.3)
Undernutrition tendency (BMI, ≤20 kg/m²)	10 (11.9)	11 (13.1)	21 (25.0)
Serum albumin level (g/dL)	4 (3.9–4.2)	4 (3.9–4.3)	4.0 (3.9–4.3)
BP (mmHg)			
Systolic BP $\geq$ 140 or diastolic BP $\geq$ 90	22(50.0)	27(60.0)	50 (50 5)
Systolic BP 130-139 or diastolic BP	23(39.0)	27(00.0)	30(39.3)
80–89	9(23.1) 7(17.0)	9 (20.0) 7 (15.6)	10(21.4) 14(16.7)
Normal	(17.3)	7(13.0)	14(10.7)
Missing data <sup>c</sup>	0 (0.0)	2 (4.4)	2 (2.4)
History of smoking			
Current smoker	3 (7.6)	0 (0.0)	3 (3.6)
Former smoker	18 (46.2)	0 (0.0)	18 (21.4)
Nonsmoker	18 (46.2)	44 (97.8)	62 (73.8)
Missing data <sup>d</sup>	0 (0.0)	1 (2.2)	1 (1.2)

**Table 1:** Characteristics of the sample (2018) (n = 84).

Data were expressed in N (%), mean  $\pm$  SD, and median (25%–75%)

SD: standard deviation; TMIG-IC: Tokyo Metropolitan Institute of Gerontology Index of Competence; BMI: body mass index; BP: blood pressure

<sup>*a*</sup>Mann–Whitney U test showed a significant difference between sexes at p < 0.05 (two-sided)

<sup>b</sup>Due to low cognitive function

<sup>c</sup>Due to refusal to take the test

<sup>d</sup>Due to lack of response

Variable	<b>Sample</b> ( <b>n</b> = <b>84</b> )	Dropout group (n = 516)	<i>p</i> -value
Sex			
Male	39 (46.4)	267 (51.7)	0.366
Female	45 (53.6)	249 (48.3)	
Education (years)	10 (8–11)	10 (8–11)	0.155
Number of teeth	19 (10–25)	22 (13–27)	0.103
Edentulous	5 (5.9)	40 (7.8)	0.682
1–19	34 (40.5)	223 (43.2)	
≥20	45 (53.6)	252 (48.8)	
Missing data <sup>a</sup>	0 (0.0)	1 (0.2)	
Stimulated salivary flow rate (ml/3 min)	3.2 (1.8–4.5)	2.8 (1.6–4.2)	0.147
BMI (kg/m <sup>2</sup> )	$22.3\pm2.9$	$22.8 \pm 2.7$	0.161
BP (mmHg)			
Systolic BP ≥140 or diastolic BP ≥90	39 (46.5)	196 (38.0)	0.339
Systolic BP 130–139 or diastolic BP 80–89	18 (21.4)	129 (25.0)	
Normal	27 (32.1)	191 (37.0)	
Serum albumin level (g/dL)	$4.4\pm0.2$	$4.3\pm0.3$	0.063
Current smoker			
Yes	9 (10.7)	103 (20.0)	0.042*
No	73 (86.9)	399 (77.3)	
Missing data <sup>b</sup>	2 (2.4)	14 (2.7)	

Table 2. Characteristics of the sample at baseline (1998) and the dropout group.

Data were expressed in N(%), mean  $\pm$  SD, and median (25%–75%).

Continuous variables were analyzed using independent t- and Mann–Whitney U tests, whereas categorical variables were analyzed using Pearson's chi-square tests.

SD: standard deviation; BMI: body mass index; BP: blood pressure

\*p < 0.05 (two-sided) <sup>a</sup>Due to refusal to undergo oral examinations

<sup>b</sup>Due to lack of response

Table 3 displays the results from the univariable linear regression analyses. We found positive associations between masticatory performance, SSF rate, number of teeth and the intake of several micronutrients. The participants who had masticatory performance <173 mg/dL consumed less micronutrients than those who had masticatory performance  $\geq 173$  mg/dL. Among macronutrients, only the intake of carbohydrates had a negative association with SSF rate. After adjustment for confounders, the associations between the oral health-related variables and the intake of some micronutrients were no longer statistically significant; however, the associations between number of teeth and the intake of  $\beta$ -carotene and vitamin C became statistically significant in multivariable models (Table 4). We failed to identify any statistically significant associations between hyposalivation and nutritional intake both in univariable and multivariable models (Tables 3-4).

	Masticatory performance (mg/dL)	Lower masticatory performance group <sup>a,b</sup> (n = 40)	SSF rate (mL/3 min)	Hyposalivation (SSF rate, ≤0.7 mL/min) <sup>b</sup> (n = 18)	Number of teeth
Nutrient	B	B	B	B	B
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
Energy	-0.982	153.444	4.517	-127.701	-2.086
(kcal/d)	(-3.489, 1.526)	(-189.935, 496.823)	(-67.535, 76.570)	(-542.314, 286.911)	(-19.936, 15.765)
Protein	0.010	-2.274	0.441	-2.652	0.087
(g/1000 kcal)	(-0.022, 0.043)	(-6.652, 2.102)	(-0.445, 1.328)	(-7.763, 2.460)	(-0.133, 0.307)
Carbohydrate	0.004	0.668	- <b>1.068</b> * <sup>#</sup>	3.807	-0.109
(% energy)	(-0.022, 0.030)	(-2.861, 4.197)	(-1.759, -0.377)	(-0.329, 7.942)	(-0.289, 0.071)
Fat	-0.006	-0.015	0.319	-1.549	0.022
(% energy)	(-0.024, 0.011)	(-2.387, 2.356)	(-0.167, 0.805)	(-4.363, 1.265)	(-0.100, 0.144)
Dietary fiber	0.005	-0.930	$-0.116 \\ (-0.318, 0.086)$	0.258	0.037
(g/1000 kcal)	(-0.002, 0.013)	(-1.916, 0.056)		(-0.917, 1.433)	(-0.013, 0.087)
Calcium	0.386	<b>-66.861</b> * <sup>#</sup>	9.009	-41.839	<b>2.939</b> *#
(mg/1000 kcal)	(-0.005, 0.777)	(-119.637, -14.086)	(-1.940, 19.958)	(-105.391, 21.713)	(0.257, 5.621)
Vitamin D	0.005	-1.264	0.321	-1.735	0.073
(µg/1000 kcal)	(-0.019, 0.029)	(-4.521, 1.993)	(-0.334, 0.976)	(-5.515, 2.045)	(-0.089, 0.236)
Zinc	0.001	-0.247	0.021	-0.071	0.007
(mg/1000 kcal)	(-0.001, 0.004)	(-0.588, 0.094)	(-0.050, 0.091)	(-0.479, 0.337)	(-0.010, 0.025)
Folic acid	<b>0.308</b> * <sup>#</sup>	- <b>48.268*</b> <sup>#</sup>	3.332	-4.072	<b>2.091</b> * <sup>#</sup> (0.535, 3.648)
(µg/1000 kcal)	(0.081, 0.535)	(-78.924, -17.613)	(-3.176, 9.840)	(-41.842, 33.699)	
Iron	<b>0.004</b> *#	- <b>0.742</b> * <sup>#</sup>	$\begin{array}{c} 0.070 \\ (-0.040,  0.180) \end{array}$	-0.196	0.026
(mg/1000 kcal)	(0.000, 0.008)	(-1.267, -0.217)		(-0.834, 0.442)	(-0.001, 0.053)

**Table 3.** Results of univariable linear regression analysis for intake of each nutrient as the dependent variable against each oral health-related factor as the independent variable (n = 84).

	Masticatory performance (mg/dL)	Lower masticatory performance group <sup>a,b</sup> (n = 40)	SSF rate (mL/3 min)	Hyposalivation (SSF rate, ≤0.7 mL/min) <sup>b</sup> (n = 18)	Number of teeth
Nutrient	B	B	B	B	B
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
β-carotene	3.455	-582.126	-42.886	179.671	27.656
(µg/1000 kcal)	(-1.275, 8.186)	(-1226.034, 61.781)	(-174.619, 88.848)	(-581.048, 940.391)	(-4.500, 59.811)
Vitamin A	<b>1.395</b> * <sup>#</sup>	<b>-164.204</b> *#	<b>35.600</b> * <sup>#</sup>	-89.269	5.426
(µg RAE <sup>c</sup> /1000 kcal)	(0.270, 2.520)	(-320.084, -8.325)	(4.321, 66.879)	(-274.448, 95.910)	(-2.485, 13.337)
Vitamin C	0.075	- <b>15.756</b> *#	0.177	1.256	0.667
(mg/1000 kcal)	(-0.032, 0.182)	(-30.142, -1.371)	(-2.801, 3.155)	(-15.919, 18.432)	(-0.056, 1.390)
Vitamin E (mg α-tocopherol/1000 kcal)	0.001 (-0.003, 0.005)	-0.325 (-0.829, 0.179)	0.038 (-0.065, 0.141)	-0.144 (-0.740, 0.452)	0.020 (-0.005, 0.046)
γ-tocopherol	-0.002	-0.164	<b>0.199*</b> <sup>#</sup>	-0.640	0.034
(mg/1000 kcal)	(-0.008, 0.005)	(-1.031, 0.703)	(0.026, 0.371)	(-1.658, 0.379)	(-0.010, 0.077)
Vitamin B1	0.000	-0.032	0.002	-0.023	0.001
(mg/1000 kcal)	(0.000, 0.000)	(-0.078, 0.014)	(-0.007, 0.012)	(-0.077, 0.031)	(-0.001, 0.003)
Vitamin B <sub>2</sub>	<b>0.001*</b> <sup>#</sup>	- <b>0.111</b> *#	<b>0.022</b> * <sup>#</sup>	-0.073	0.004
(mg/1000 kcal)	(0.000, 0.001)	(-0.209, -0.013)	(0.002, 0.042)	(-0.190, 0.045)	(-0.001, 0.009)
Vitamin B3	0.001	-0.465	0.197	-1.106	0.018
(mg/1000 kcal)	(-0.010, 0.012)	(-1.985, 1.056)	(-0.109, 0.502)	(-2.870, 0.658)	(-0.058, 0.095)
Vitamin B5	0.001	-0.229	0.027	-0.105	0.009
(mg/1000 kcal)	(-0.001, 0.004)	(-0.589, 0.132)	(-0.046, 0.101)	(-0.530, 0.321)	(-0.009, 0.027)
Vitamin B6	0.000	-0.065	0.014	-0.060	0.003
(mg/1000 kcal)	(0.000, 0.001)	(-0.166, 0.037)	(-0.006, 0.035)	(-0.179, 0.059)	(-0.003, 0.008)
Vitamin B12	0.007	-1.311	<b>0.395</b> * <sup>#</sup>	-1.547	0.030
(µg/1000 kcal)	(-0.007, 0.022)	(-3.258, 0.635)	(0.007, 0.784)	(-3.820, 0.727)	(-0.069, 0.128)

	Masticatory performance (mg/dL)	Lower masticatory performance group <sup>a,b</sup> (n = 40)	SSF rate (mL/3 min)	Hyposalivation (SSF rate, ≤0.7 mL/min) <sup>b</sup> (n = 18)	Number of teeth
Nutrient	B	B	B	B	B
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
Vitamin K	0.212	-29.683	-3.223	27.389	1.774
(µg/1000 kcal)	(-0.056, 0.479)	(-66.292, 6.926)	(-10.664, 4.219)	(-15.302, 70.080)	(-0.037, 3.584)
n-3 fatty acids	0.000	-0.038	0.042	-0.158	0.007
(% energy)	(-0.002, 0.002)	(-0.307, 0.231)	(-0.012, 0.095)	(-0.472, 0.155)	(-0.007, 0.020)
n-6 fatty acids	-0.001	-0.086	<b>0.108</b> * <sup>#</sup>	-0.378	0.016
(% energy)	(-0.005, 0.003)	(-0.588, 0.417)	(0.006, 0.210)	(-0.975, 0.220)	(-0.010, 0.042)

SSF: stimulated salivary flow; B: unstandardized correlation coefficient; CI: compatibility interval; RAE: retinol activity equivalent

<sup>a</sup>Masticatory performance is less than the median (<173 mg/dL)

<sup>b</sup>Reference group for categorical varibles: higher masticatort performance ( $\geq 173$ mg/dL), normal SSF rate (>0.7 mL/min) <sup>c</sup>µg RAE = retinol (µg) +  $\beta$ -carotene (µg) × 1/12 +  $\alpha$ -carotene (µg) × 1/24 +  $\beta$ -cryptoxanthin (µg) × 1/24 + other provitamin A carotenoids (µg) × 1/24 \**p* < 0.05 (*two-sided*)

<sup>#</sup>q (minimum false-discovery rate) < 0.05

After adjustment for sex, education, higher-level functional capacity (TMIG-IC), and undernutrition tendency (BMI  $\leq 20 \text{ kg/m}^2$ ), as shown in Table 4, masticatory performance was found to be positively associated with vitamin A intake. Participants with masticatory performance < 173 mg/dL had a significantly lower intake of folic acid, iron, and vitamin A, as compared to those with masticatory performance  $\geq 173$ mg/dL. SSF was found to be positively associated with the intake of vitamin A, B2,  $\gamma$ tocopherol, and n-6 fatty acids but negatively associated with the intake of carbohydrates. The number of remaining teeth was positively associated with the intake of folic acid, β-carotene, and vitamin C. With large effect sizes, our results demonstrate that participants with lower masticatory performance (<173 mg/dL) compared to those with higher masticatory performance ( $\geq 173 \text{ mg/dL}$ ), had lower intake of folic acid and vitamin A; SSF was associated with vitamin A intake; and number of teeth was associated with  $\beta$ -carotene (a provitamin A) intake. We were also able to observe some associations with large effect sizes that were not statistically significant, such as masticatory performance and  $\beta$ -carotene intake; masticatory performance < 173 mg/dL and a lower  $\beta$ -carotene intake; hyposalivation and vitamin A intake; and number of remaining teeth and vitamin A intake.

	Masticatory performance (mg/dL)	Lower masticatory performance group <sup>a,b</sup> (n = 40)	SSF rate (mL/3 min)	Hyposalivation (SSF rate, ≤0.7 mL/min) <sup>b</sup> (n = 18)	Number of teeth
Nutrient	B	B	B	B	B
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
Energy	-0.380	42.446	-17.243	-29.855	-1.539
(kcal/d)	(-2.924, 2.164)	(-316.637, 401.528)	(-92.067, 57.581)	(-457.899, 398.188)	(-20.599, 17.520)
Protein	$0.008 \\ (-0.025, 0.040)$	-1.861	0.546	-3.129	0.109
(g/1000 kcal)		(-6.466, 2.743)	(-0.390, 1.481)	(-8.472, 2.214)	(-0.130, 0.348)
Carbohydrate	0.002	1.020	- <b>1.140</b> * <sup>#</sup>	3.632	-0.114
(% energy)	(-0.024, 0.029)	(-2.732, 4.772)	(-1.875, -0.404)	(-0.753, 8.017)	(-0.311, 0.083)
Fat	-0.009	0.454	0.429	-1.922	0.023
(% energy)	(-0.026, 0.008)	(-1.971, 2.878)	(-0.074, 0.932)	(-4.817, 0.974)	(-0.107, 0.153)
Dietary fiber	0.003	-0.550	-0.062	-0.104	0.046
(g/1000 kcal)	(-0.004, 0.010)	(-1.536, 0.436)	(-0.262, 0.138)	(-1.249, 1.041)	(-0.004, 0.095)
Calcium	0.323	-56.258*	11.007	-51.171	3.284*
(mg/1000 kcal)	(-0.074, 0.721)	(-111.800, -0.716)	(-0.361, 22.375)	(-116.684, 14.342)	(0.418, 6.150)
Vitamin D	0.005 (-0.019, 0.030)	-1.481	0.367	-1.782	0.089
(µg/1000 kcal)		(-4.870, 1.907)	(-0.315, 1.049)	(-5.687, 2.123)	(-0.085, 0.262)
Zinc	0.001	-0.177	0.032	-0.127	0.009
(mg/1000 kcal)	(-0.002, 0.003)	(-0.533, 0.179)	(-0.042, 0.106)	(-0.552, 0.297)	(-0.010, 0.028)
Folic acid	0.261*	<b>-41.449</b> * <sup>#</sup>	4.869	-12.126	<b>2.514</b> * <sup>#</sup> (0.909, 4.118)
(µg/1000 kcal)	(0.034, 0.489)	(-73.231, -9.667)	(-1.740, 11.477)	(-50.326, 26.074)	
Iron	0.004	- <b>0.660</b> * <sup>#</sup>	0.095	-0.304	0.031*
(mg/1000 kcal)	(0.000, 0.007)	(-1.208, -0.113)	(-0.017, 0.208)	(-0.957, 0.349)	(0.002, 0.059)
β-carotene	2.619	-493.228	-16.748	2.385	<b>39.268</b> * <sup>#</sup> (6.304, 72.232)
(μg/1000 kcal)	(-2.071, 7.309)	(-1150.590, 164.135)	(-151.039, 117.544)	(-765.179, 769.948)	

**Table 4.** Results of multivariable linear regression analysis for intake of each nutrient as the dependent variable against each oral health-related factor as the independent variable (n = 84).

	Masticatory performance (mg/dL)	Lower masticatory performance group <sup>a,b</sup> (n = 40)	SSF rate (mL/3 min)	Hyposalivation (SSF rate, ≤0.7 mL/min) <sup>b</sup> (n = 18)	Number of teeth
Nutrient	B	B	B	B	B
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
Vitamin A	<b>1.500</b> * <sup>#</sup>	- <b>199.522</b> * <sup>#</sup>	<b>40.451</b> * <sup>#</sup> (6.891, 74.011)	-103.303	7.638
(µg RAE <sup>c</sup> /1000 kcal)	(0.329, 2.670)	(-365.524, -33.520)		(-300.949, 94.343)	(-1.050, 16.327)
Vitamin C (mg/1000 kcal)	$0.049 \\ (-0.055, 0.154)$	-11.799 (-26.348, 2.750)	0.572 (-2.416, 3.560)	-2.091 (-19.174, 14.991)	<b>0.866</b> * <sup>#</sup> (0.132, 1.601)
Vitamin E	0.000	-0.187	0.059	-0.239	0.022
(mg α-tocopherol/1000 kcal)	(-0.003, 0.004)	(-0.711, 0.337)	(-0.048, 0.167)	(-0.855, 0.377)	(-0.005, 0.049)
γ-tocopherol	-0.002	0.056	<b>0.225</b> * <sup>#</sup> (0.048, 0.402)	-0.583	0.018
(mg/1000 kcal)	(-0.009, 0.004)	(-0.830, 0.942)		(-1.627, 0.461)	(-0.029, 0.065)
Vitamin B1	0.000	-0.024	0.003	-0.032	0.002
(mg/1000 kcal)	(0.000, 0.000)	(-0.070, 0.022)	(-0.006, 0.013)	(-0.085, 0.022)	(-0.001, 0.004)
Vitamin B <sub>2</sub>	0.001	-0.105*	<b>0.025</b> * <sup>#</sup> (0.004, 0.046)	-0.088	0.005
(mg/1000 kcal)	(0.000, 0.001)	(-0.209, 0.000)		(-0.211, 0.035)	(0.000, 0.011)
Vitamin B3	$0.000 \\ (-0.011, 0.012)$	-0.476	0.227	-1.253	0.033
(mg/1000 kcal)		(-2.056, 1.104)	(-0.092, 0.546)	(-3.077, 0.571)	(-0.048, 0.115)
Vitamin B5	$0.001 \\ (-0.002, 0.003)$	-0.136	0.047	-0.220	0.014
(mg/1000 kcal)		(-0.502, 0.230)	(-0.028, 0.122)	(-0.648, 0.209)	(-0.005, 0.033)
Vitamin B6	0.000	-0.063	0.016	-0.071	0.004
(mg/1000 kcal)	(0.000, 0.001)	(-0.167, 0.041)	(-0.005, 0.037)	(-0.191, 0.050)	(-0.001, 0.009)
Vitamin B <sub>12</sub>	$\begin{array}{c} 0.009 \\ (-0.006,  0.023) \end{array}$	-1.631	0.397	-1.375	0.033
(µg/1000 kcal)		(-3.663, 0.401)	(-0.012, 0.805)	(-3.748, 0.998)	(-0.073, 0.139)
Vitamin K	0.115	-12.938	-0.141	12.168	2.027*
(µg/1000 kcal)	(-0.142, 0.372)	(-49.294, 23.418)	(-7.530, 7.248)	(-29.954, 54.290)	(0.206, 3.848)
n-3 fatty acids	0.000	-0.058	0.047	-0.157	0.007
(% energy)	(-0.002, 0.002)	(-0.341, 0.225)	(-0.010, 0.104)	(-0.485, 0.171)	(-0.007, 0.022)

	Masticatory performance (mg/dL)	Lower masticatory performance group <sup><math>a,b</math></sup> ( $n = 40$ )	SSF rate (mL/3 min)	Hyposalivation (SSF rate, ≤0.7 mL/min) <sup>b</sup> (n = 18)	Number of teeth
Nutrient	B	B	B	B	B
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
n-6 fatty acids	-0.001	0.053	<b>0.126</b> * <sup>#</sup> (0.021, 0.231)	-0.372	0.008
(% energy)	(-0.005, 0.002)	(-0.463, 0.570)		(-0.991, 0.246)	(-0.020, 0.036)

The results were adjusted for sex, education, higher-level functional capacity (Tokyo Metropolitan Institute of Gerontology Index of Competence score), and undernutrition tendency (body mass index,  $\leq 20 \text{ kg/m}^2$ ).

SSF: stimulated salivary flow; B: unstandardized correlation coefficient; CI: compatibility interval; RAE: retinol activity equivalent

<sup>a</sup>Masticatory performance is less than the median (<173 mg/dL)

<sup>b</sup> Reference group for categorical varibles: higher masticatort performance ( $\geq 173$ mg/dL), normal SSF rate (>0.7 mL/min)

<sup>c</sup> $\mu g RAE = retinol (\mu g) + \beta$ -carotene ( $\mu g$ ) × 1/12 +  $\alpha$ -carotene ( $\mu g$ ) × 1/24 +  $\beta$ -cryptoxanthin ( $\mu g$ ) × 1/24 + other provitamin A carotenoids ( $\mu g$ ) × 1/24 +  $\beta$ -cryptoxanthin ( $\mu g$ ) × 1/24 + other provitamin A carotenoids ( $\mu g$ ) × 1/24 +  $\beta$ -cryptoxanthin ( $\mu g$ ) × 1/24 + other provitamin A carotenoids ( $\mu g$ ) × 1/24 +  $\beta$ -cryptoxanthin ( $\mu g$ ) × 1/24 + other provitamin A carotenoids ( $\mu g$ ) × 1/24 +  $\beta$ -cryptoxanthin ( $\mu g$ ) × 1/24 +  $\beta$ -cryptoxant

<sup>#</sup>q (minimum false-discovery rate) < 0.05

## **Chapter 4**

#### **Discussion/Conclusion**

#### 4.1 Main Findings

This study shows significant associations between SSF and vitamin A intake and number of teeth and  $\beta$ -carotene intake. Participants with a lower masticatory performance (<173 mg/dL) consumed less folic acid and vitamin A, compared with the reference group (masticatory performance  $\geq 173$  mg/dL). With smaller effect sizes, there were positive associations between masticatory performance and vitamin A intake; masticatory performance < 173 mg/dL and lower iron intake; SSF and vitamin B2,  $\gamma$ -tocopherol, and n-6 fatty acid intake; and number of teeth and folic acid and vitamin C intake. However, there was a negative association between SSF and carbohydrate intake (Table 5).

#### Table 5. Summary of the main findings

Oral health-related factor	Associated nutritional intake <sup>a,b</sup>	Associated nutritional intake <sup>a,b</sup> with large effect sizes <sup>c</sup>
Masticatory performance (mg/dL)	Vitamin A	-
Presence of lower masticatory performance (<173mg/dL)	Folic acid, iron, and vitamin A	Folic acid and vitamin A
Stimulated salivary flow rate (ml/3 min)	Carbohydrate <sup>d</sup> , vitamin A, $\gamma$ -tocopherol, vitamin B <sub>2</sub> , and n-6 fatty acids	Vitamin A
Hyposalivation $(SSF \le 0.7 mL/min)$	-	-
Number of teeth (teeth)	Folic acid, $\beta$ -carotene, and vitamin C	β-carotene

<sup>a</sup> p < 0.05 (two-sided) <sup>b</sup> q (minimal false discovery rate) < 0.05 <sup>c</sup> Large effect size refers to the absolute value of unstandardized correlation coefficient (B) more than 10 as relatively compared within the study

<sup>d</sup>Negative correlation

#### 4.2 Strengths and Limitations

The strengths of our study are that we targeted 90-year-old people, for whom findings on oral health and nutritional intake are limited. Furthermore, we used clinical measurements for all oral health parameters; unlike other studies, we used validated methods to objectively measure masticatory performance. Finally, we investigated other aspects of oral health such as salivary flow rate, which are not frequently studied.

The limitations of this study include its cross-sectional design, small sample size, incomprehensive dimensions of oral health, and exclusion of the interrelationship between the three oral health factors.

The cross-sectional design is not helpful in elucidating causational relationship. Thus, one should interpret the findings with caution.

Based on the small sample size, only a few covariates could be added to the analyses. We had to omit some confounders, such as medications. This small sample size resulted in insufficient powers of the tests to detect any relationships between oral health factors and intake of most macronutrients except carbohydrates.

We selected masticatory performance, SSF, and the number of teeth from all dimensions of oral health because they provided higher coefficients of determination in the linear regression models than other available information did (e.g., self-reported swallowing problems, dental caries, and periodontal status). In our analysis we did not consider the effects of denture use for two reasons: there was only one participant who had less than 20 teeth and did not have tooth replacement, and the directions for impact of wearing dentures on dietary intake are still unclear (30).

Interrelationships between the three oral variables through diet modification (31) can complicate the interpretation. These interrelationships were not considered because an appropriate examination would have required a variety of analyses that would overwhelm the primary analyses. Furthermore, univariable linear regression showed that SSF of the participants was not associated with their masticatory performance or their number of teeth.

#### 4.3 Generalizability and Biases

Because of the dropouts, the generalizability of our findings is reduced. During the past 20 years, many of the participants have passed away, which may have resulted in *survival bias*. Hence, our findings can be generalized to 90-year-olds living in Central Japan, who are less likely to smoke. However, in most aspects, the characteristics of the sample were not statistically different from the dropout group.

At baseline, the characteristics of the participants were compared with those of the nonparticipants. Participants had approximately 2.6–3.9 teeth more than the nonparticipants (18). We assume that our participants had better oral health conditions than typical older people. The loss of participants from the test and comparison groups may also be unequal, resulting in *attrition bias*.

As we used BDHQ, a self-report assessment, older people with inferior oral health conditions may recall more unhealthy food than they actually consumed in reality. Moreover, older people tend to have memory loss, which can reduce the reliability of their responses. We reduced *recall bias* by sending questionnaires in advance, providing the participants with sufficient time to complete or recheck the questionnaires prospectively. In the case that the participants could not complete the questionnaires by themselves, we asked their family members or caregivers to complete the questionnaires on their behalf. Moreover, the study hypothesis was blinded to the participants or the questionnaire respondents. We also found that the participants left some questions unfilled. There were no distinct patterns of unfilled items that we could observe. We were able to get answers for all unfilled items by asking. We could not get the information from one participant who had low cognitive function and did not have anyone to help complete the questionnaire. BDHQ did not include consumption of nonalcoholic beverages or supplements in the calculation of nutritional intake. For this reason, the validity may be lower in the case of some nutrients; however, researchers confirmed that the relative validity as compared to semi-weighed dietary records among the individuals of advanced age remained acceptable (32). Although BDHQ has been validated against biomarkers, its validation among the individuals of advanced age has not been performed. The estimated nutrient intake in our study may not represent nutrient concentration in blood or nutritional status of the participants.

# 4.4 Mastication, Salivation, Dentition, and Vitamin A

Our findings demonstrate that the three oral health-related variables were highly associated with vitamin A intake. Participants with masticatory performance of <173 mg/dL or less SSF consumed significantly less vitamin A and its equivalences; participants with fewer teeth also consumed less  $\beta$ -carotene, an important provitamin A. Despite the large 95% CIs of these findings (Table 4), the minimum estimated effects were convincing.

Our findings are consistent with large surveys conducted in Japan (13), the United States (12, 33, 34), and the United Kingdom (14). Studies have shown that people with fewer teeth have a lower intake of vitamin A (13) and  $\beta$ -carotene (33). Edentulous people and partially dentate people are more likely to have lower serum retinol (14) and  $\beta$ -carotene (12, 34) levels, respectively.

The mechanism for this association remains unclear, but it is possibly bidirectional. Firstly, diminished oral conditions may reduce the intake of foods containing vitamin A and  $\beta$ -carotene, as older people with tooth loss often eat fewer dairy products (13), green–yellow vegetables (13), total vegetables (13), and carrots (34) due to their difficulties in chewing (35). This adaptation to eating softer and less fibrous foods reduces salivary gland stimulation, aggravates further tooth loss, and avoidance of chewy foods as a vicious circle (31).

Conversely, a lower intake of vitamin A and  $\beta$ -carotene can cause gingival hyperplasia (7), worsen periodontal disease (36), and decrease parotid gland secretion in rats (37) since vitamin A plays roles in immune function (38) and maintenance of epithelial cell health (7).

#### 4.5 Other Findings

Consistent with our findings, lower folic acid (vitamin B9) intake was associated with tooth loss (34). This might result from a lower intake of vegetables among older adults with tooth loss (13). Inversely, lower folate intake is known to affect the synthesis of macromolecules, such as DNA, in humans. (9, 39)Folate deficiency is associated with oropharyngeal cancers (39) and periodontal disease (9).

A lower intake of vitamin C or serum ascorbate level has been reported to accompany reduced dentition (13, 33, 34), denture use (33), decreased posterior occlusion (12, 40), and edentulism (14). Consistent with previous findings, we found that tooth loss was associated with lower vitamin C intake. The mechanism might be that people with tooth loss consume fewer fruits (12) and vegetables (13); conversely, vitamin C is also known to affect periodontal health in several ways (8).

Our findings on the association between impaired dentition and lower intake of iron are consistent with those of previous studies (14, 40, 41). Edentulous patients consumed lower amounts of various micronutrients including nonheme iron than dentate patients (41). This phenomenon might come from a lower consumption of meat, which is the main source of iron intake in the Japanese population(42).

Researchers have reported that tooth loss and increased carbohydrate intake are related (13). Conversely, we found that less saliva and not tooth loss, was associated with a higher intake of carbohydrates. A potential explanation may be the lack of sufficient statistical powers of the tests  $(1 - \beta)$  to detect the association between other oral factors and intake of carbohydrates. Evidence of the association between SSF and carbohydrate intake is lacking. However, one study reported poor nutritional status among people with SSF < 0.5 mL/min (43). As saliva lubricates and protects the oral mucosa from physical trauma (31), people with reduced saliva may avoid oral discomfort by eating soft, high-carbohydrate foods (e.g., cakes, bread) and may consume fewer chewy, fibrous foods that are needed to stimulate the salivary glands. Some researchers reported that reduced salivary flow may affect eating because of its vital role in swallowing (44); however, this assumption remains controversial (45).

No previous studies have reported the association of SSF with the intake of  $\gamma$ tocopherol, vitamin B2, and n-6 fatty acids. This may be due to the lack of research on the association between salivary flow rate and nutritional intake. Although all forms of vitamin E are anti-oxidants, unlike  $\alpha$ -tocopherol,  $\gamma$ -tocopherol given its antiinflammatory properties can inhibit cyclooxygenase activity (46). Moreover,  $\gamma$ tocopherol is the major form of vitamin E in plant seeds and products such as nuts, which may be difficult to eat with less saliva. Researchers have reported that deficiency of vitamin B2 was prevalent among patients with burning mouth syndrome (47) and angular stomatitis(48) since it helps to maintain the function of keratinocytes. A crosssectional study reported a significantly lower intake of n-3 and n-6 fatty acids among patients with Sjögren's syndrome (49). However, in our study, participants did not reduce their n-3 fatty acids consumption in spite of their decreased SSF. This may be because of the extensive consumption of fish, an abundant source of n-3 fatty acids, among the Japanese.

Some studies have reported a contrasting list of oral health-related nutrients (50) or weak associations between dental status and nutritional intake (51). Differences in measurement of masticatory performance and nutritional intake and differences in ages of the sample may explain this divergence. Yoshihara A., et al. (50) used three-day food diary to measure nutritional intake in the same population when they were much younger. The explanation might be that the participants may have changed their eating habits as a consequence of a variety of social, physiological, and psychological changes (52). Osterberg T., et al. used a self-assessed masticatory ability questionnaire, which is less sensitive than our measurement (51).

#### **4.6 Implications for Future Research**

The self-reported food frequency questionnaire does not consider the softness of the food items. Different food preparation methods, such as boiling, may alter the interpretation of our findings. Further studies that focus on a few important nutrients with the consideration given to cooking methods may help explain the relationship between dietary intake and oral health conditions.

It is essential to note that there is no strong evidence that tooth loss can affect diet or nutrition as reported in a systematic review of longitudinal studies (17). Poor dentition, mastication, and dry mouth are only three factors among a wide range of factors that can influence dietary intake in older people (52). To promote healthy eating in older people, other supporting factors and barriers need to be considered.

Eating behavior is complex and changeable over time. Therefore, to establish solid evidence on the association between oral health and nutrition in older adults, welldesigned and geographically diverse periodical studies are warranted. Studies that address mechanisms behind the relationships between oral health and frequently reported nutrients, such as vitamin A and C, would be worthwhile.

#### 4.7 Conclusion

Tooth loss, dry mouth, and poor chewing ability coincide with the lower intake of several micronutrients such as vitamin A,  $\beta$ -carotene, and folic acid. Oral health practitioners should pay attention to the nutritional intake of older people with these conditions.

# Appendix 1

Nutritional intake of 90-year-old Japanese people in 2018 (n = 83)

Variable	n (%)	Mean ±	- SD	Media	n (25%–75%)
Daily Energy Intake					
<2200 kcal/d (male) or <1750 kcal/d (female)	40 (47.6)	Total:	$2091.1 \pm 776.0 \text{ kcal/d}$	Total:	1954.3 (1641.2–2533.3) kcal/d
$\geq$ 2200 kcal/d (male) or $\geq$ 1750 kcal/d (female)	43 (51.2)	Male:	2325.2 ± 958.7 kcal/d	Male:	2087.3 (1664.2–2914.4) kcal/d <sup>b</sup>
		Female	$\pm 1923.5 \pm 493.0 \text{ kcal/d}$	Female	: 1880.6 (1577.3–2122.9) <sup>b</sup>
Protein					
<60 g/d (male) or <50 g/d (female)	13 (15.5)	Total:	44.6 ± 9.6 g/1000 kcal	Total:	43.3 (37.6–50.8) g/1000 kcal
$\geq 60 \text{ g/d} \text{ (male) or } \geq 50 \text{ g/d} \text{ (female)}$	70 (83.3)		$94.1 \pm 41.6 \text{ g/d}$		89.4 (63.7–113.0) g/d
Carbohydrate					
<60 % energy intake	73 (86.9)	Total:	$52.0 \pm 7.9$ %total energy	Total:	52.4 (47.2–56.1) %total energy
60–65 % energy intake	6 (7.1)		$268.4 \pm 90.6 \text{ g/d}$		254.6 (203.5–321.7) g/d
>65 % energy intake	4 (4.8)	Male:	292.2 ± 104.5 g/d °	Male:	266.5 (195.2–337.5) g/d
		Female	$\pm 248.3 \pm 72.0 \text{ g/d}^{\circ}$	Female	: 249.0 (196.7–301.4) g/d
Lipid					
<20 % energy intake	7 (8.3)	Total:	$27.5 \pm 5.3$ %total energy	Total:	27.2 (24.3-31.2) %total energy
20–30 % energy intake	49 (58.4)				
>30% energy intake	27 (32.1)				
Dietary Fiber					
<19 g/d (male) or <17 g/d (female)	34 (40.5)	Total:	$7.9 \pm 2.2$ g/1000 kcal	Total:	8.1 (6.0–9.0) g/1000 kcal
$\geq 19$ g/d (male) or $\geq 17$ g/d (female)	49 (58.3)		$16.3 \pm 6.3 \text{ g/d}$		16.1 (11.5–21.1) g/d
Calcium					
<700 mg/d (male) or <650 mg/d (female)	31 (36.9)	Total:	$379.8 \pm 119.9 \text{ mg}/1000 \text{ kcal}$	Total:	357.4 (300.5–432.9) mg/1000 kcal
$\geq$ 700 mg/d (male) or $\geq$ 650 mg/d (female)	52 (61.9)		$784.6 \pm 355.9 \text{ mg/d}$		777.8 (508.3–994.9) mg/d
Vitamin D					
<5.5 µg/d	6 (7.1)	Total:	$12.2 \pm 7.1 \ \mu g/1000 \ kcal$	Total:	11.1 (7.0–16.7) μg/1000 kcal
≥5.5 µg/d	77 (91.7)		$26.6\pm19.3~\mu\text{g/d}$		24.10 (10.3–36.0) µg/d
Vitamin C					
<100 mg/d	15 (17.8)	Total:	84.7 ± 32.1 mg/1000 kcal	Total:	83.3 (62.9–101.9) mg/1000 kcal
≥100 mg/d	68 (81.0)		$174.9 \pm 82.1 \text{ mg/d}$		170.3 (115.9–226.9) mg/d

**Appendix.** Nutritional intake of 90-year-old Japanese people in 2018 ( $n = 83^{a}$ ).

** • • •	(0/)				
variable	$n(\%)$ Mean $\pm$ SD		± SD	Media	n (25%)-/5%)
Zinc <9 mg/d (male) or <7 mg/d (female) $\geq$ 9 mg/d (male) or $\geq$ 7 mg/d (female)	22 (26.2) 61 (72.6)	Total:	5.0 ± 0.8 mg/1000 kcal 10.4 ± 4.1 mg/d	Total:	5.0 (4.6–5.4) mg/1000 kcal 9.8 (7.7–12.5) mg/d
<b>Folic Acid</b> <240 µg/d ≥240 µg/d	13 (15.5) 70 (83.3)	Total:	$\begin{array}{l} 227.3 \pm 70.5 \; \mu g / 1000 \; kcal \\ 474.4 \pm 231.3 \; \mu g / d \end{array}$	Total:	222.4 (184.3–271.9) μg/1000 kcal 430.9 (321.2–606.8) μg/d
Iron <7 mg/d (male) or <6 mg/d (female) ≥7 mg/d (male) or ≥6 mg/d (female)	16 (19.0) 67 (79.8)	Total:	5.1 ± 1.2 mg/1000 kcal 10.8 ± 4.9 mg/d	Total:	5.0 (4.3–5.9) μg/1000 kcal 9.8 (7.3–13.7) mg/d
Vitamin A <800 µgRAE <sup>d</sup> /d (male) or <650 µgRAE <sup>d</sup> /d (female) ≥800 µgRAE <sup>d</sup> /d (male) or ≥650 µgRAE <sup>d</sup> /d (female)	27 (32.1) 56 (66.7)	Total:	$525.6 \pm 347.7 \ \mu gRAE^a/1000 \ kcal \\ 1158.4 \pm 1274.4 \ \mu gRAE^a/d$	Total: kcal	436.5 (330.2–605.3) μgRAE <sup>a</sup> /1000 848.7 (621.2–1238.1) μgRAE <sup>a</sup> /d
<b>Vitamin B1</b> <1.2 mg/d (male) or <0.9 mg/d (female) ≥1.2 mg/d (male) or ≥0.9 mg/d (female)	40 (47.6) 43 (51.2)	Total:	0.5 ± 0.1 mg/1000 kcal 1.0 ± 0.4 mg/d	Total:	0.5 (0.4–0.6) mg/1000 kcal 1.0 (0.7–1.3) mg/d
Vitamin B2 <1.3 mg/d (male) or <1.1 mg/d (female) ≥1.3 mg/d (male) or ≥1.1 mg/d (female)	18 (21.4) 65 (77.4)	Total:	0.8 ± 0.2 mg/1000 kcal 1.8 ± 0.8 mg/d	Total:	0.8 (0.7–0.9) mg/1000 kcal 1.7 (1.2–2.2) mg/d
Vitamin B3 (Niacin) <13 mg/d (male) or <10 mg/d (female) $\geq$ 13 mg/d (male) or $\geq$ 10 mg/d (female)	14 (16.7) 69 (82.1)	Total:	10.7 ± 3.3 mg/1000 kcal 22.8 ± 11.6 mg/d	Total:	10.4 (8.0–12.8) mg/1000 kcal 21.7 (13.4–31.1) mg/d
Vitamin B5 (Pantothenic acid) <5 mg/d ≥5 mg/d	12 (14.3) 71 (84.5)	Total:	4.1 ± 0.8 mg/1000 kcal 8.5 ± 3.4 mg/d	Total:	4.1 (3.6–4.6) mg/1000 kcal 8.2 (5.9–10.9) mg/d
<b>Vitamin B6</b> <1.4 mg/d (male) or <1.2 mg/d (female) ≥1.4 mg/d (male) or ≥1.2 mg/d (female)	12 (14.3) 71 (84.5)	Total:	0.9 ± 0.2 mg/1000 kcal 1.8 ± 0.8 mg/d	Total:	0.9 (0.7–1.0) mg/1000 kcal 1.7 (1.2–2.3) mg/d

Variable	n (%)	Mean ± SD		Median (25%-75%)	
Vitamin B12 <2.4 μg/d ≥2.4 μg/d	1 (1.2) 82 (97.6)	Total:	8.0 ± 4.3 μg/1000 kcal 17.5 ± 13.6 μg/d	Total:	7.7 (4.4–10.6) μg/1000 kcal 14.4 (7.5–23.9) μg/d
Sodium <600 mg/d ≥600 mg/d ≥2000 mg/d	0 (0) 83 (98.8) 1 (1.2) 82 (97.6)	Total:	2560.6 ± 547.4 mg/1000 kcal 5283.3 ± 1939.3 mg/d	Total:	2416.0 (2196.5–2807.7) mg/1000 kcal 5010.6 (3860.7–6571.0) mg/d
Potassium <2500 mg/d (male) or <2000 mg/d (female) ≥2500 mg/d (male) or ≥2000 mg/d (female)	18 (21.4) 65 (77.4)	Total:	1708.5 ± 420.8 mg/1000 kcal 3533.7 ± 1390.5 mg/d	Total:	1675.7 (1444.9–2004.5) mg/1000 kcal 3368.1 (2543.0–4430.3) mg/d
Magnesium <320 mg/d (male) or <270 mg/d (female) ≥320 mg/d (male) or ≥270 mg/d (female)	32 (38.1) 51 (60.7)	Total:	159.8 ± 34.3 mg/1000 kcal 333.0 ± 133.2 mg/d	Total:	153.5 (137.6–184.7) mg/1000 kcal 330.7 (232.2–417.6) mg/d
<b>Vitamin K</b> <150 µg/d ≥150 µg/d	8 (9.5) 75 (89.3)	Total:	195.9 ± 80.5 μg/1000 kcal 409.8 ± 219.0 μg/d	Total:	192.4 (140.0–250.8) μg/1000 kcal 381.0 (240.6–534.5) μg/d
<b>n-3 fatty acids</b> <2.2 g/d (male) or <1.9 g/d (female) ≥2.2 g/d (male) or ≥1.9 g/d (female)	12 (14.3) 71 (84.5)	Total:	$1.6 \pm 0.6$ % total energy $3.9 \pm 2.5$ g/d	Total:	1.6 (1.2–2.0) %total energy 3.2 (2.3–5.0) g/d
<b>n-6 fatty acids</b> <8 g/d (male) or <7 g/d (female) $\geq$ 8 g/d (male) or $\geq$ 7 g/d (female)	14 (16.7) 69 (82.1)	Total:	$5.0 \pm 1.1$ % total energy $11.7 \pm 5.5$ g/d	Total:	5.0 (4.3–5.8) %total energy 11.0 (8.4–13.8) g/d

<sup>a</sup> One participant (1.2%) did not complete the questionnaire for nutritional intake because of low cognitive function. <sup>b</sup> *Mann–Whitney U* test showed a significant difference between sexes (p = 0.036). <sup>c</sup> Independent *t*-test showed a significant difference between sexes (p = 0.032). <sup>d</sup> Retinol activity equivalent ( $\mu$ gRAE) = retinol ( $\mu$ g) +  $\beta$ -carotene ( $\mu$ g) × 1/12 +  $\alpha$ -carotene ( $\mu$ g) × 1/24 +  $\beta$ -cryptoxanthin ( $\mu$ g) × 1/24 + other provitamin A carotenoids ( $\mu$ g) × 1/24.

# Appendix 2

Plots of the unstandardized correlation coefficients (B) and compatibility intervals (CI) of the results from multivariable linear regression analyses



Masticatory Performance



#### Lower masticatory performance







Number of teeth

#### Acknowledgments

The authors appreciate the assistance of colleagues from the Division of Preventive Dentistry and the Division of Oral Rehabilitation, Faculty of Dentistry, Niigata University, in data collection as part of the Niigata cohort study. We sincerely thank the Assistant Professor Dr. Natchalee Srimaneekarn for her helpful advice regarding statistical analysis. This work can never be done without the critical comments from Dr. Piyada Gaewkhiew and Dr. Nuntaporn Karawekpanyawong.

#### **Statement of Ethics**

This study was conducted according to the Declaration of Helsinki, and all procedures involving human individuals have been approved by the Ethics Committee of the Faculty of Dentistry, Niigata University (12-R1-4-21).

#### **Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

#### **Funding Sources**

This work was supported by JSPS KAKENHI grant 16K11884. Financial support for statistical software, editing service, and publication fees was provided by a Mahidol scholarship (Mahidol University, Nakhon Pathom Province, Thailand) awarded to Raksanan Karawekpanyawong.

#### **Author Contributions**

KN and HO prepared and submitted the proposal for research grant and ethical approval. All authors participated in the data collection and revision of the manuscript. RK performed a literature review, analyzed the data, and wrote and edited the manuscript. KN and YK supervised this work, and HO was responsible for manuscript submission.

#### Data Availability Statement

The data that support the findings of this study are not publicly available because the data are owned by various faculties from Niigata University, Japan, and their permissions are needed. However, the data are accessible through the corresponding author (Hiroshi Ogawa) upon reasonable requests.

# References

1. Tada A, Miura H. Systematic review of the association of mastication with food and nutrient intake in the independent elderly. Arch Gerontol Geriatr. 2014;59(3):497–505.

2. Dormenval V, Mojon P, Budtz-Jorgensen E. Associations between self-assessed masticatory ability, nutritional status, prosthetic status and salivary flow rate in hospitalized elders. Oral Dis. 1999;5(1):32–8.

3. Batisse C, Bonnet G, Eschevins C, Hennequin M, Nicolas E. The influence of oral health on patients' food perception: a systematic review. J Oral Rehabil. 2017;44(12):996–1003.

4. Iinuma T, Arai Y, Takayama M, Takayama M, Abe Y, Osawa Y, et al. Satisfaction with dietary life affects oral health-related quality of life and subjective well-being in very elderly people. J Oral Sci. 2017;59(2):207–13.

5. Bernstein M. Nutritional needs of the older adult. Phys Med Rehabil Clin N Am. 2017;28(4):747–66.

6. Lorenzo-Lopez L, Maseda A, de Labra C, Regueiro-Folgueira L, Rodriguez-Villamil JL, Millan-Calenti JC. Nutritional determinants of frailty in older adults: a systematic review. BMC Geriatr. 2017;17(1):108.

7. Henshaw MM, Calabrese JM. Oral health and nutrition in the elderly. Nutr Clin Care. 2001;4(1):34–42.

8. Tada A, Miura H. The relationship between vitamin C and periodontal diseases: a systematic review. Int J Environ Res Public Health. 2019;16(14):2472.

9. Yu YH, Kuo HK, Lai YL. The association between serum folate levels and periodontal disease in older adults: data from the National Health and Nutrition Examination Survey 2001/02. J Am Geriatr Soc. 2007;55(1):108–13.

10. Watson S, McGowan L, McCrum LA, Cardwell CR, McGuinness B, Moore C, et al. The impact of dental status on perceived ability to eat certain foods and nutrient intakes in older adults: cross-sectional analysis of the UK National Diet and Nutrition Survey 2008-2014. Int J Behav Nutr Phys Act. 2019;16(1):43.

11. Samnieng P, Ueno M, Shinada K, Zaitsu T, Wright FA, Kawaguchi Y. Association of hyposalivation with oral function, nutrition and oral health in community-dwelling elderly Thai. Community Dent Health. 2012;29(1):117–23.

12. Sahyoun NR, Lin CL, Krall E. Nutritional status of the older adult is associated with dentition status. J Am Diet Assoc. 2003;103(1):61–6.

13. Wakai K, Naito M, Naito T, Kojima M, Nakagaki H, Umemura O, et al. Tooth loss and intakes of nutrients and foods: a nationwide survey of Japanese dentists. Community Dent Oral Epidemiol. 2010;38(1):43–9.

14. Marcenes W, Steele JG, Sheiham A, Walls AW. The relationship between dental status, food selection, nutrient intake, nutritional status, and body mass index in older people. Cad Saude Publica. 2003;19(3):809–16.

15. Samnieng P, Ueno M, Shinada K, Zaitsu T, Wright FA, Kawaguchi Y. Oral health status and chewing ability is related to mini-nutritional assessment results in an older adult population in Thailand. J Nutr Gerontol Geriatr. 2011;30(3):291–304.

16. Tsugane S. Why has Japan become the world's most long-lived country: insights from a food and nutrition perspective. Eur J Clin Nutr. 2020.

17. Gaewkhiew P, Sabbah W, Bernabé E. Does tooth loss affect dietary intake and nutritional status? A systematic review of longitudinal studies. J Dent. 2017;67:1–8.

18. Ando Y, Yoshinara A, Seida Y, Hirotomi T, Ogawa H, Kaneko N, et al. The Study of Sampling Bias in an Oral Health Survey of Elderly: Comparison of Oral and General Health Condition between Respondents and Non-Respondents to a Questionnaire and between Participants and Non-Participants in an Examination. J Dent Hlth. 2000;50(3):322-33.

19. Koyano W, Shibata H, Nakazato K, Haga H, Suyama Y. Measurement of competence: reliability and validity of the TMIG Index of Competence. Arch Gerontol Geriatr. 1991;13(2):103–16.

20. Sasaki S. Development and evaluation of dietary assessment methods using biomarkers and diet history questionnaires for individuals. Research for evaluation methods of nutrition and dietary lifestyle programs held on Healthy Japan 21 Summary report: Ministry of Health, Welfare, and Labour; 2004. p. 10–44.

21. Sasaki S. Development and evaluation of dietary assessment methods using biomarkers and diet history questionnaires for individuals. Research for evaluation methods of nutrition and dietary lifestyle programs held on Healthy Japan 21 Summary report. 2004:10-44.

22. Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, et al. Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr. 2011;14(7):1200-11.

23. Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, Hirota N, et al. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol. 2012;22(2):151-9.

24. World Health Organization. Oral health surveys: basic methods. 5th ed ed. Geneva: World Health Organization; 2013. vii, 125 pages p.

25. Shiga H, Kobayashi Y, Arakawa I, Yokoyama M, Unno M. Validation of a portable blood glucose testing device in measuring masticatory performance. Prosthodont Res Prac. 2006;5(1):15–20.

26. Hirotomi T, Yoshihara A, Ogawa H, Ito K, Igarashi A, Miyazaki H. A preliminary study on the relationship between stimulated saliva and periodontal conditions in community-dwelling elderly people. J Dent. 2006;34(9):692–8.

27. Ministry of Health, Labour, and Welfare, Japan. National Health and Nutrition Survey. Tokyo, Japan: Ministry of Health Labour and Welfare; 2018.

28. Navazesh M, Kumar SKS. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc. 2008;139:35S-40S.

29. Storey JD. A direct approach to false discovery rates. J R Stat Soc Series B Stat Methodol. 2002;64(3):479–98.

30. Moynihan P, Varghese R. Impact of Wearing Dentures on Dietary Intake, Nutritional Status, and Eating: A Systematic Review. JDR Clin Trans Res. 2021:23800844211026608.

31. Lingstrom P, Moynihan P. Nutrition, saliva, and oral health. Nutrition. 2003;19(6):567–9.

32. Kobayashi S, Yuan X, Sasaki S, Osawa Y, Hirata T, Abe Y, et al. Relative validity of brief-type

self-administered diet history questionnaire among very old Japanese aged 80 years or older. Public health nutrition. 2019;22(2):212-22.

33. Ervin RB, Dye BA. Number of natural and prosthetic teeth impact nutrient intakes of older adults in the United States. Gerodontology. 2012;29(2):e693–702.

34. Nowjack-Raymer RE, Sheiham A. Numbers of natural teeth, diet, and nutritional status in US adults. J Dent Res. 2007;86(12):1171–5.

35. Zelig R, Jones VM, Touger-Decker R, Hoskin ER, Singer SR, Byham-Gray L, et al. The eating experience: adaptive and maladaptive strategies of older adults with tooth loss. JDR Clin Trans Res. 2019;4(3):217–28.

36. Luo PP, Xu HS, Chen YW, Wu SP. Periodontal disease severity is associated with micronutrient intake. Aust Dent J. 2018;63(2):193–201.

37. Horn VJ, Redman RS, Ambudkar IS. Response of rat salivary glands to mastication of pelleted vitamin A-deficient diet. Arch Oral Biol. 1996;41(8):769–77.

38. Huang Z, Liu Y, Qi G, Brand D, Zheng SG. Role of vitamin A in the immune system. J Clin Med. 2018;7(9):258.

39. Gutierrez Gossweiler A, Martinez-Mier EA. Chapter 6: Vitamins and Oral Health. Monogr Oral Sci. 2020;28:59–67.

40. de Andrade FB, Caldas Junior Ade F, Kitoko PM, Zandonade E. The relationship between nutrient intake, dental status and family cohesion among older Brazilians. Cad Saude Publica. 2011;27(1):113–22.

Sheiham A, Steele JG, Marcenes W, Lowe C, Finch S, Bates CJ, et al. The relationship among dental status, nutrient intake, and nutritional status in older people. J Dent Res. 2001;80(2):408-13.
 Turin TC, Okuda N, Miura K, Nakamura Y, Rumana N, Kadota A, et al. Iron intake and associated factors in general Japanese population: NIPPON DATA80, NIPPON DATA90 and national nutrition monitoring. J Epidemiol. 2010;20 Suppl 3(Suppl 3):S557-66.

43. Dormenval V, Budtz-Jørgensen E, Mojon P, Bruyère A, Rapin CH. Nutrition, general health status and oral health status in hospitalised elders. Gerodontology. 1995;12(12):73–80.

44. Pedersen A, Sørensen C, Proctor G, Carpenter G. Salivary functions in mastication, taste and textural perception, swallowing and initial digestion. Oral Diseases. 2018;24(8):1399-416.

45. Rogus-Pulia NM, Logemann JA. Effects of reduced saliva production on swallowing in patients with Sjogren's syndrome. Dysphagia. 2011;26(3):295-303.

46. Jiang Q, Christen S, Shigenaga MK, Ames BN. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr. 2001;74(6):714-22.

47. Morr Verenzuela CS, Davis MDP, Bruce AJ, Torgerson RR. Burning mouth syndrome: results of screening tests for vitamin and mineral deficiencies, thyroid hormone, and glucose levels-experience at Mayo Clinic over a decade. Int J Dermatol. 2017;56(9):952-6.

48. Blanck HM, Bowman BA, Serdula MK, Khan LK, Kohn W, Woodruff BA. Angular stomatitis and riboflavin status among adolescent Bhutanese refugees living in southeastern Nepal. Am J Clin Nutr. 2002;76(2):430-5.

49. Castrejón-Morales CY, Granados-Portillo O, Cruz-Bautista I, Ruiz-Quintero N, Manjarrez I, Lima G, et al. Omega-3 and omega-6 fatty acids in primary Sjögren's syndrome: clinical meaning and association with inflammation. Clin Exp Rheumatol. 2020;38 Suppl 126(4):34-9.

50. Yoshihara A, Watanabe R, Nishimuta M, Hanada N, Miyazaki H. The relationship between

dietary intake and the number of teeth in elderly Japanese subjects. Gerodontology. 2005;22(4):211–8.

51. Osterberg T, Tsuga K, Rothenberg E, Carlsson GE, Steen B. Masticatory ability in 80-year-old subjects and its relation to intake of energy, nutrients and food items. Gerodontology. 2002;19(2):95–101.

52. Nieuwenhuizen WF, Weenen H, Rigby P, Hetherington MM. Older adults and patients in need of nutritional support: review of current treatment options and factors influencing nutritional intake. Clin Nutr. 2010;29(2):160-9.

#### **List of Tables**

- 1.1 **Table 1.** Characteristics of the sample (2018) (n = 84).
- 1.2 **Table 2.** Characteristics of the sample at baseline (1998) and the dropout group.
- 1.3 **Table 3.** Results of univariable linear regression analysis for intake of each nutrient as the dependent variable against each oral health-related factor as the independent variable (n = 84).
- 1.4 **Table 4**. Results of multivariable linear regression analysis for intake of each nutrient as the dependent variable against each oral health-related factor as the independent variable (n = 84).
- 1.5 **Table 5.** Summary of the main findings

#### **List of Figures**

Figure. 1. Flow chart of the participant selection