Calcium, vitamin D, casein and whey protein intakes and periodontitis among Danish adults

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Running Title: Calcium & vitamin D intakes and periodontitis

Abstract

Objective: To investigate whether intakes of calcium, vitamin D, casein and whey were associated with periodontitis, and to investigate the possibility of interactions between them.

Design, Setting, and Subjects: 3,292 adult participants from the Danish Health Examination Survey in 2007-2008 were included. An internet-based 267-items FFQ was used to assess dietary intake. Intakes of casein (32.0 g/day) and whey proteins (9.6 g/day) were classified as within *vs.* above the 50th percentile. Calcium intake was classified as within *vs.* below recommendations. Analyses were stratified by lower and higher (<5.8 *vs.* ≥5.8 µg/day) vitamin D intake. Periodontitis was defined as mean clinical attachment loss ≥ 4mm.

Results: Calcium intake within recommendations and whey intakes ≥ 9.6 g/day were associated with lower likelihood of periodontitis (OR 0.75, 95%CI 0.61-0.90 & OR 0.78, 95% CI 0.88-0.94, respectively) after adjustment for age, gender, education, smoking, sucrose intake, alcohol consumption, number of teeth, daily brushing, visit to the dentist and chronic illness. However, when the analyses were stratified by levels of vitamin D intake, associations remained significant only in the group with higher vitamin D intake. Casein intake ≥ 32.0 g/day was not significantly associated with periodontitis regardless of levels of vitamin D intake.

Conclusion: Intakes of calcium and whey protein were inversely associated with periodontitis, among those with higher, but not lower, vitamin D intakes. Consumption of foods rich in calcium (e.g. dairy foods) and providing vitamin D at least 5.8µg/day or more should be promoted, as they may contribute to the prevention of periodontitis.

Key-words: calcium; casein; oral health; periodontitis; vitamin D; whey

Introduction

Periodontitis is a bacterially induced chronic inflammation affecting the supporting structures of teeth and characterized by periodontal attachment loss and bone loss which can subsequently lead to tooth loss[1]. The pathogenesis of periodontitis involves an ecological shift in the biofilm leading to bacterial activation of immune-inflammatory mechanisms, of which several have adverse effects on bone and connective tissue remodelling[2].

Research points towards potential risk factors for periodontal diseases that lie well beyond the mouth, including hormonal changes, diabetes, stress, genetic susceptibility, smoking, alcohol consumption and other lifestyle factors[3-5]. Eating habits and a low intake of specific nutrients have also been found to be related to periodontal diseases[6, 7].

Dietary factors may impact periodontitis risk and overall oral health via systemic or local effects. Diet can have systemic effects, when absorbed nutrients and minerals affect tooth development and growth, or in relation to the individual's resistance to periodontitis and caries[8]. In addition, diet may affect the virulence of certain microorganisms[8]. Local effects include the influence of diet on dental plaque, which is the primary etiologic factor for the initiation of periodontal disease, and the amount, composition, and acidity of the saliva[9, 10], factors that also affect periodontitis risk.

Although healthy eating habits seem to be protective against periodontal diseases[11], original research on nutritional determinants of periodontitis is still scarce and, to date, few studies have investigated the importance of interactions between dietary nutrients in the development and progression of periodontal diseases and tooth loss[12]. Most studies have investigated the effect of single nutrients on periodontal disease. However, minerals, vitamins and other nutrients have interactions between them that affect their functionality, availability, or absorption[13].

We have suggested earlier that ingestion of dairy products (that are a good source of calcium, vitamin D, casein, and whey protein) confers protection against periodontitis[14]. High concentrations of calcium and phosphate in saliva may inhibit bacterial biofilm formation[15], and high calcium intakes may be associated with [16] prevention of alveolar bone loss and, consequently, better retention of natural dentition[17]. Vitamin D is essential for calcium absorption[13], and promotes oral health through its effects on bone metabolism and innate immunity[1]. Whey protein enhances immune system function and inhibits plaque formation[18]. Indeed, we found earlier that higher dairy calcium, but not non-dairy calcium, was protective against tooth loss and periodontitis and was associated with lower plaque scores;

furthermore, our findings suggested that sufficient vitamin D in the diet was of importance for the appearance of the significant benefits of higher calcium intakes[19]. However, our previous results were based on analyses of data on small population subsets, with insufficient power to test whether or not periodontitis risks were related to interactions between dairy nutrients such as calcium, vitamin D, or the dairy protein casein, or those in whey. This study, therefore, aimed to investigate whether intakes of calcium, vitamin D, casein, and whey were associated with periodontitis, and to investigate the possibility of interactions between them in relation to periodontitis.

Methods

Study design and population

The Danish Health Examination Survey (DANHES 2007-2008) is a cross-sectional study carried out in 13 of 98 municipalities in Denmark. The focus of the survey was on diet, smoking, alcohol, and physical activity[20].

In total, 538,497 adults, 18 years or older, were invited to complete a basic internet-based questionnaire concerning social factors, lifestyle, and general health. A random subsample of these individuals (n = 180,103) was also invited to participate in a general health examination and fill in a supplementary internet-based food frequency questionnaire (FFQ). 76,484 (out of 538,497) individuals completed the basic questionnaire, 48,065 (out of 180,103) individuals fully or partially completed the supplementary FFQ and 18,065 participated in the general health examination[20].

As a part of DANHES 2007-2008 an oral health study based on clinical examination, and a separate questionnaire, was carried out in 2008-2009. Participants were consecutively enrolled on a first come, first served basis. Among 18,065 participants, 4,402 individuals volunteered to participate in the present oral health study[21] (Figure 1). Participants from this subset (n = 4,402) and those participating in the general study (n = 18,065) did not differ with respect to age and gender. However participants reported higher income and more years of education than non-participants in the oral health study group.

The study was conducted in accordance with the Helsinki Declaration and approved by the Ethical Committees for the Region of Copenhagen. Written informed consent was obtained from all participants.

Dietary assessment

An internet-based 267 item food frequency questionnaire (FFQ) was used to assess dietary intake based on intake over the previous year[20]. The dietary questionnaire used had previously been validated and used in many Danish population studies[22]. In this survey, the FFQ was extended with 39 portion size items placed at the end of the questionnaire[23]. Each item referred to 4 or 6 size classes presented in a picture series developed by the Danish Veterinary and Food Administration.

Calculation of total energy and dietary calcium intake (mg/day) and the distribution of intake of fat, carbohydrate and protein were performed using the program FoodCalc® and Danish official food composition tables[24] Due to limited information on calcium contents of the supplements used, 800 mg was added to the total calcium intake, but only for those who reported daily intake of calcium supplements over the previous year. Total dietary and supplemental calcium intakes were classified as: below *vs*. within recommendations[25]. An intake of 1000 mg of calcium per day is recommended for female adults aged < 50 years and males aged < 70 years; an intake of 1,200 mg of calcium per day is recommended for female adults aged > 50 years and males aged > 70 years.

Total dietary and supplemental intakes of vitamin D2 and D3 (µg/day) were estimated. For daily supplemental vitamin D or for the use of multivitamins, intakes of 10 µg or 5 µg of vitamin D were added, respectively. Only 7.8% of participants reported vitamin D intakes within current recommendations (15 µg/day for adults aged 51–70 years and 20 µg/day for those aged ≥ 71 years)[25]. Therefore, vitamin D intake was classified as within *vs.* above the 50th percentile (5.8 µg/day).

Dairy protein intake was calculated from all dairy foods, including dairy ingredients used in preparing various foods, for example, cheese in pizza and milk in mashed potatoes. For each dairy food the content of whey and casein proteins was estimated. Whey proteins make up about 20% of the protein content of milk, yogurt, ice-cream, butter, and cream, the rest of the protein content being in the form of casein fractions (~80%). For most cheeses, the protein content is 100% casein, as whey proteins are eliminated after the curdling process from hard cheeses, but some are left in cottage cheese [26]. Intakes of casein (32.0 g/day) and whey proteins (9.6 g/day) were classified as within *vs.* above the 50th percentile.

Oral Examination

The participants filled in a questionnaire that asked validated questions about self-reported oral health status, oral health problems, utilization of dental care, and oral hygiene habits.

The oral health study focused upon dental status, dental caries and erosion, the quantity and type of restorative treatments, periodontal disease, salivary flow rates, self-assessment of oral disease, oral health care habits and oral-health-related quality of life.

Standard dental equipment was used for the clinical oral examination. The manual periodontal probe included pagination at 2, 4, 6, 8, 10, and 12 mm (Product: LM-Instruments, Finland, Model 8-520B). The examination protocol was designed to minimize measurement errors. Three dental hygienists were responsible for the oral examination, and they had been carefully trained by two experienced clinical examiners. At the beginning and at the end of the data collection the dental hygienists were revalidated. Inter- and intra-examiner agreement ranged between 60 and 80% for periodontal measurements[21].

The periodontal examination included half-mouth (one upper and one lower quadrant randomly selected) records, except for third molars. The assessments comprising pocket depths (PD), bleeding on probing (BOP), and gingival margin levels were registered at six sites of each tooth measured. Gingival recession was recorded as a negative value. Clinical attachment loss (CAL) was calculated as the sum of the PD and gingival level measurements.

Mean CAL was categorized as a binary outcome variable based on the 75th percentile (< 4 and ≥ 4 mm). For each individual, bleeding scores (as a continuous outcome) were determined as the percentage of sites with bleeding, amongst the total number of sites examined.

Covariates

These included age, gender, years of education (< 10 *vs*. ≥ 10 years), presence of any long-term and chronic illness (yes/no), regular visits to a dentist (at least once a year for a check-up during the last 5 years, yes/no), daily tooth brushing (yes/no), number of remaining teeth, current smoking (yes/no) and alcohol intake (above *vs*. within-recommendations from the Danish National Board of Health (maximum consumption of 14 units alcohol/ week for women and 21 units alcohol/ week for men))[27]. Sucrose consumption was classified as above 10% of the total energy intake (yes/no) as this value is in accordance with the suggested threshold that potentially increases caries rates and that is associated with lower consumption of vitamins and minerals[28].

Analysis

Characteristics of the study population were presented as “mean (SD)” or “percentage (*n*)”. Logistic regression, adjusted for potential confounders, was used to evaluate associations between intakes of calcium, vitamin D and casein, and whey proteins and mean CAL.

Since vitamin D is essential for calcium absorption[13], analyses regarding calcium intake were stratified by vitamin D intakes (within *vs.* above the 50th percentile). Furthermore, interactions between whey protein and vitamin D intake were explored via stratified analyses. Effect modification by gender was evaluated. As no significant interaction was found, data analyses were not stratified by gender. The level of significance was set at *P* value < 0.05. All statistical analyses were performed using STATA 9.2 (Stata Corp, TX, USA)

Results

Among 4,402 participants, 1,068 individuals were excluded due to incomplete dietary information and edentulism, leaving a final study population of 3,292 individuals with complete information on diet and oral health (Figure 1). Participants with complete information tended to be younger, more educated, less likely to smoke daily and have lower mean systolic blood pressure as compared to those excluded because of incomplete information (n = 1,110) (*P* value < 0.05) but included and excluded subjects did not differ with respect to gender, presence of chronic illness, diabetes or regular visits to the dentist (data not shown).

The final study population was composed of 38.7% men and 61.3% women and their ages ranged from 18 to 95 years (mean 53.5 y SD 13.9). CAL and percentage of sites with BOP ranged from 1 mm to 12.5 mm (mean 3.4 SD 1.3) and from 0% to 100% (mean 22% SD 21.4), respectively. 25.4% of the final study population had mean CAL ≥4 mm. Descriptive data according to periodontitis status are presented in Table 1. Individuals presenting mean CAL ≥ 4 mm were older, less educated, more likely to be male, to smoke and to have chronic illness, fewer teeth and more sites with BOP, compared to those with mean CAL < 4 mm. In addition, individuals presenting mean CAL ≥ 4 mm were more likely to report alcohol consumption above recommendations, calcium consumption below recommendations, whey intake below the median, and lower vitamin D intake and higher sucrose consumption compared to those with mean CAL < 4 mm.

Table 2 shows the associations between intakes of calcium, vitamin D, casein and whey (as continuous variables), and mean CAL ≥ 4mm. In the crude analysis none of the nutrients were significantly associated with periodontitis. However, when the analyses were adjusted for age, gender, education, smoking, sucrose intake, alcohol consumption, number of teeth, daily brushing, regular visit to the dentist and chronic illness a weak inverse association between high intakes of calcium (*P* = 0.048) and of vitamin D (*P* = 0.047) and periodontitis was observed. Whey and casein protein intakes (as continuous variable) remained non-significant after adjustments.

Calcium intake, above the current recommendations, and whey protein intake ≥ 9.6 g/day were significantly associated with lower risk of periodontitis (Table 3). However, when the analyses were stratified by levels of vitamin D intake, associations remained significant only in the group with higher vitamin D intake and associations were not significant amongst those with lower vitamin D intakes. Casein intake ≥ 32.0 g/day was not significantly associated with periodontitis regardless of levels of vitamin D intakes.

Discussion

Our findings showed that both higher intakes of calcium (i.e. within current recommendations) and of whey protein were individually associated with a lower occurrence of periodontitis in a subgroup of participants in whom dental health was examined, when vitamin D intakes were ≥ 5.8 μg/day, but not when intakes were <5.8 μg/day, after adjustments for age, gender, education, smoking, sucrose intake, alcohol consumption, number of teeth, daily brushing, regular visit to the dentist, and chronic illness. Casein intake was not associated with periodontitis regardless of levels of vitamin D intakes.

To our knowledge, this is the first observational study examining associations between intakes of casein and whey protein and periodontitis and, therefore, it cannot be compared to other data. Previous studies have shown significant inverse associations between dietary calcium intake and periodontitis[7, 14], but those analyses did not take into account levels of vitamin D intake.

Activated vitamin D (1,25(OH)2D) plays a role in maintaining oral health through its effects on calcium and bone metabolism and innate immunity. Although cholecalciferol [vitamin D3] is produced endogenously, upon ultraviolet B sunlight irradiation of the skin, some individuals have a greater need for dietary and supplemental sources[13], such as elderly and Nordic populations, who are less exposed to sun light or less efficient in generating endogenous vitamin D[29].

The present study showed that vitamin D intake alone, had a weak, but significant, association with periodontitis. Furthermore, level of vitamin D intake was an important effect modifier in the associations between calcium and whey intakes and periodontitis risk. These results are consistent with our previous findings showing that intakes of calcium and dairy-servings within-recommendations were inversely associated with oral plaque score among those with higher, but not lower, vitamin D intakes[19]. This might be explained by the fact that vitamin D status is an important factor influencing intestinal calcium absorption[13]. While we did not measure serum concentrations of 25-hydroxyvitamin D (25(OH)D, or vitamin D status), our findings are generally consistent with other observational studies which found that higher serum 25(OH)D concentrations correlated with less CAL and a lower risk of periodontitis[30].

Vitamin-D deficiency is a risk factor for several disorders with inflammatory components and one randomized clinical trial showed reductions in inflammatory markers (matrix metalloproteinases (MMP) 9 and 2 and c-reactive protein) with higher vitamin D status at baseline and after supplementation[31].

Strengths of our study include the use of a large population including both men and women in a wide age range (18-95 years) with detailed information in respect of both medical and dental outcomes and risk factors. Therefore, our results might be generalisable to other Western populations. However, limitations of the study design exist. The major limitation of this study was the lack of detailed information on calcium and vitamin D supplementation resulting in possible underestimation of calcium and vitamin D intakes and consequently, underestimation of our findings. In addition, this study was hampered by exclusion of participants due to insufficient information on dietary intake, clinical periodontal examination or other covariates (in 3,292 participants with complete data out of 18,065 participants). However, when responders and non-responders were compared no significant differences in sex ratio or age were observed. Responders were more likely to report higher income and more years of education so that the presence of selection bias, or 'healthy participant effect,' cannot be entirely ruled out and, if responders tended to belong to a low-risk group this might also have led to an underestimation of our findings. Furthermore, it was not possible to explore the role of ethnicity in these associations as approximately 95% of the sample was born in Denmark and 92% of the parents were Danish.

In the present study, periodontal examinations were performed in one randomly selected upper and lower quadrant. In total, 25.4% of the present study population (18 - 95 years) had mean CAL ≥4 mm. Although full mouth examination is preferable, time limitations for the clinical examinations resulted in the decision to use partial recording. It has been estimated that a partial recording protocol similar to that used in the present study, with random sampling of one maxillary and one mandibular quadrant evaluating CAL at 6 sites per tooth, reveals 5% fewer individuals with maximum CAL ≥4 mm compared to full mouth registrations[32].This might indicate that associations presented in this study could have been somewhat underestimated.

Low vitamin D status is prevalent in Western countries and according to the National Board of Health approximately half of the Danish adult population have vitamin D insufficiency (serum 25(OH)D < 25 nmol/L). In this study population, only 33.1% and 7.8% of participants reported calcium and vitamin D intakes within current recommendations, respectively. In 2011, Denmark started voluntary fortification of milk with vitamin D, but this did not affect the estimations of dietary vitamin D intake in this study as it was carried out prior to implementation of this fortification policy.

In conclusion, our cross-sectional study findings corroborate the hypothesis that higher calcium and whey intakes are associated with lower periodontitis risk amongst those with higher, but not lower, vitamin D intakes, though it is not possible to infer causality from these cross-sectional associations. However, adherence to the suggested recommendations for vitamin D and calcium intakes may not only have a beneficial impact on bone health but also on oral health and other chronic diseases. Therefore, consumption of foods rich in calcium (e.g. dairy foods) and providing vitamin D intakes of 5.8 μg/day or more should be promoted, as this could contribute to prevention of periodontitis.

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Table 1. General characteristics of the study population according to clinical attachment loss status.

	Mean Clinical Attachment Loss [a]			
Characteristics	**Total study group % (n)**	**< 4 mm % (n)**	**≥ 4 mm % (n)**	***P***
Age(years)[b]	52.7 (13.4)	49.7 (13.2)	61.7 (9.4)	< 0.0001
< 10 y of education	8.78 (289)	6.7 (165)	14.8 (124)	< 0.0001
Males	38.7 (1619)	35.3 (1085)	48 (534)	< 0.0001
Daily smoking	8.8 (290)	6.1 (151)	16.6 (139)	< 0.0001
Vitamin D intake < 5.8 µg/d	49.9 (1644)	47.9 (1176)	55.9 (468)	< 0.0001
Vitamin D intake (µg/d) [b]	7.2 (5.0)	7.6 (4.8)	7.1 (5.1)	0.03
Calcium intake below recommendations	66.8 (2,198)	63.4 (541)	67.9 (1686)	0.018
Calcium intake (mg/d) [b]	1084.5 (567.1)	1086(555.8)	1078.2 (599)	0.7
Whey protein intake < 9.6 g/d	50.4 (1659)	49.3 (1210)	53.6 (449)	0.029
Whey protein intake (g/d) [b]	10.0 (4.2)	10.1 (4.1)	9.8 (4.4)	0.107
Casein intake < 32.0 g/day	49.9 (1663)	49.4 (1225)	51.1 (438)	0.4
Casein intake (g/d) [b]	34.2 (15.0)	34.3 (14.8)	34.0 (15.5)	0.5
> 10% energy from sucrose	3.2 (105)	2.5 (61)	5.2 (44)	< 0.0001
Alcohol > recommendations	11.6 (383)	9.8 (241)	17 (142)	< 0.0001
Chronic illness	36.4 (1200)	34.2 (840)	43.0 (3)	< 0.0001
Regular visit to the dentist	85.8 (2,825)	86.1 (2113)	85.1 (677)	0.472
Daily tooth brushing	81.7 (2,690)	82 (2,013)	80.9 (677)	0.5

Number of teeth [b]	27.0 (3.9)	27.7 (3.0)	24.9 (5.2)	< 0.0001
Sites with bleeding on probing[b]	22.0 (21.4)	18.3 (18.1)	32.9 (25.9)	< 0.0001

[a]Data presented as % (n) unless otherwise stated. [b]Data presented as mean (SD)

Analysis performed with 3,292 observations (n = 837 for clinical attachment loss ≥ 4 mm) .

Table 2. Associations between intakes of calcium, vitamin D and whey with mean clinical attachment loss ≥ 4 mm

	Crude		Adjusted[b]	
	OR[a] (95% CI)	***p***	**OR[a] (95% CI)**	***p***
Calcium (mg/day)	0.99 (0.99 – 1.0)	0.86	0.99 (0.98 – 0.99)	0.048
Vitamin D (µg/day)	1.0 (0.99 – 1.03)	0.07	0.98 (0.96 – 0.99)	0.047
Whey (g/day)	0.98 (0.97 – 1.00)	0.13	0.98 (0.96 – 1.00)	0.12
Casein (g/day)	0.99 (0.99 – 1.00)	0.70	0.88 (0.73 – 1.06)	0.18

[a]Odds ratio is for one unit increase in calcium, vitamin D and whey protein intakes. Analysis performed with 3,292 observations.

[b]Models adjusted for age, gender, education, smoking, sucrose intake, alcohol consumption, number of teeth, daily brushing, regular visit to the dentist and chronic illness.

Table 3. Associations between intakes of calcium and whey and clinical attachment loss ≥ 4 mm stratified by vitamin D intake.

	Calcium within recommendations		**Whey ≥ 9.6 g/day**		**Casein ≥ 32.0 g/day**	
	OR[a] (95% CI)	***p***	**OR[a] (95% CI)**	***p***	**OR[a] (95% CI)**	***p***
Overall	0.75 (0.61 – 0.90)	0.003	0.78 (0.65 – 0.94)	0.009	0.88 (0.73 – 1.06)	0.18
Vitamin D Intake						
< **5.8 µg/d**	0.78 (0.56 - 1.09)	0.152	0.85 (0.64 – 1.25)	0.26	0.93 (0.71 – 1.21)	0.60
≥ 5.8 µg/d	0.69 (0.54 - 0.89)	0.005	0.72 (0.56 – 0.92)	0.008	0.83 (0.64 – 1.07)	0.15

[a]Models adjusted for age, gender, education, smoking, sucrose intake, alcohol consumption, number of teeth, daily brushing, regular visit to the dentist and chronic illness. Analysis performed with 3,292 observations.

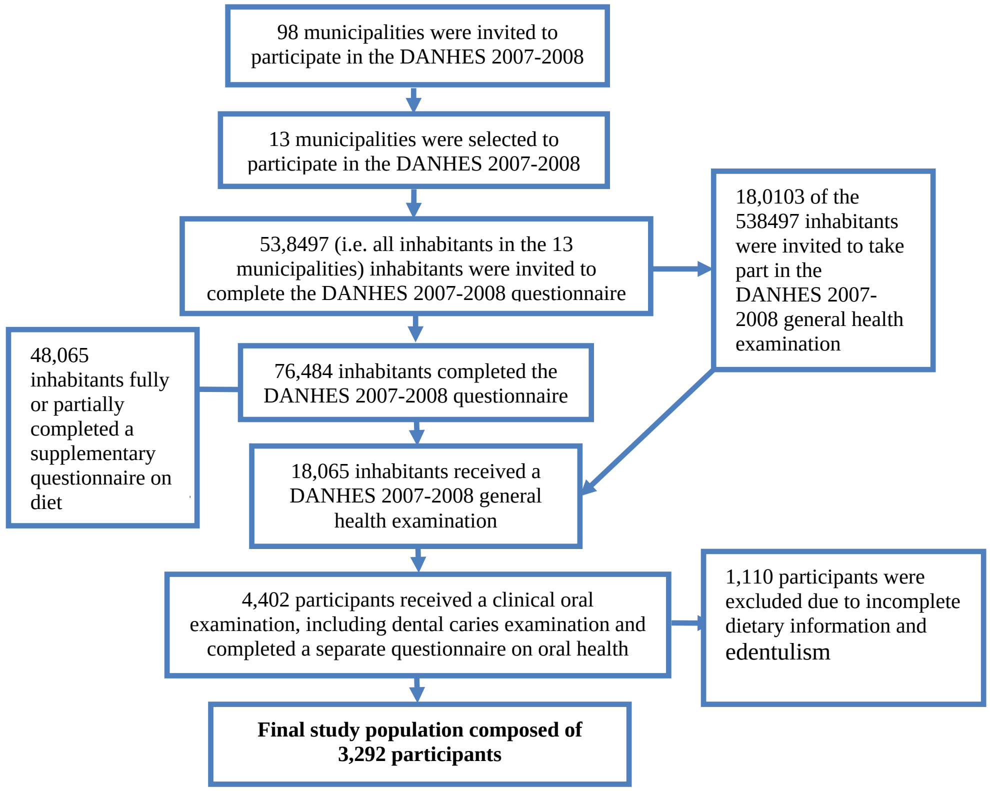

Figure 1. Flow chart for participants in the Danish Health Examination Survey (DANHES 2007-2008)