論文内容の要旨

論文題目　Nutritional epidemiology of *trans* fatty acids among Japanese population

和訳　日本人におけるトランス型脂肪酸の栄養疫学研究

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INTRODUCTION

Prevalence of cardiovascular diseases and type 2 diabetes has been alarmingly increasing worldwide. Cardiovascular diseases are the leading cause of global death, representing 30% of total death. Among estimated 17.5 million deaths from cardiovascular diseases in 2005, 7.6 million and 5.7 million were attributed to coronary heart disease and cerebrovascular disease, respectively. Also, 171 millions are affected with diabetes in 2000 and the number is projected to rise to 366 million by 2030. It has been suggested that up to 80% of cases of coronary heart disease and up to 90% of cases of type 2 diabetes could potentially be avoided through changing lifestyle factors. Diet is one of the most important modifiable lifestyle factors associated with these metabolic disorders.

Trans fatty acids are unsaturated fatty acids formed during the partial hydrogenation of commercial liquid vegetable oils to semi-solid fats. They are often found in margarine, shortening, and frying fats. Over the past two decades, *trans* fatty acid intake among free-living populations has been unfavorably associated with metabolic diseases including coronary heart disease and type 2 diabetes, and with metabolic risk factors including blood lipid concentrations, systemic inflammation, glucose concentrations, waist circumference, and body weight, although not all results have been consistent. Moreover, some studies have suggested different effects on metabolic risk factors between hydrogenated *trans* fatty acids, produced by partial hydrogenation of commercial liquid vegetable oils, and natural *trans* fatty acid, found naturally in ruminants as a results of bio-hydrogenation of polyunsaturated fatty acids.

Experimental studies also suggested the unfavorable effects of *trans* fatty acid intake on several metabolic risk factors. In these experiments, however, intake levels were generally higher (3.8%-20% energy) than those in free-living populations (0.87%-4.30% energy), hampering their direct extrapolation to free-living populations. Moreover, despite the preventive value of early identification of modifiable dietary factors among healthy and young populations and the possible onset of chronic diseases associated with each of these metabolic risk factors in the young, most of these observational studies were conducted in middle-aged and elderly populations (mean subject age across studies: >30 years). Further, these observational studies examining the association of *trans* fatty acid intake and metabolic risk factors were largely conducted in Western populations. Information from Asian countries, including Japan, where dietary fat intake is relatively low is limited. Given these facts, research examining the association of *trans* fatty acid intake and metabolic risk factors among Japanese population is needed.

To investigate the effects of *trans* fatty acids in specific population, obtaining data of estimated *trans* fatty acid intake among the population is a prerequisite step. However, few data are available on mean intake among individuals which were estimated from *trans* fatty acid food composition tables covering foods high in *trans* fatty acids in Asian countries, including Japan. Several estimates among Japanese populations have been published, but their usefulness is limited by problems with databases, dietary assessment methodologies, or sample sizes.

Therefore, the aim of the present doctoral thesis was 1) to develop a comprehensive *trans* fatty acid database covering broader categories of foods, 2) to estimate *trans* fatty acid intake among a Japanese population based on the database, and 3) to examine associations of total, hydrogenated, and natural *trans* fatty acid intake with metabolic risk factors, including body mass index (BMI), waist circumference, and serum cholesterol levels, triacylglycerol, glucose, and glycated hemoglobin (Hb A_{1c}) among free-living young Japanese populations based on the database.

The present doctoral thesis is divided into three chapters. The first chapter includes overview of fatty acids and *trans* fatty acids and the latter two chapters include my doctoral research.

CHAPTER I

Chapter I contains two parts: the first part describing the basic biochemical knowledge of fatty acids and the second part describing overview of *trans* fatty acids. In the first part, subjects of fatty acids include definition, nomenclature, chemistry, classification (degree of saturation, chain length, *cis* versus *trans* configuration, and essential fatty acids), major dietary sources, physiology (digestion, absorption, transport, metabolism, and excretion). In the second part, subjects of *trans* fatty acids include definition, history of *trans* fatty acids, process of producing *trans* fatty acids, chemistry, dietary sources, physiology (digestion, absorption, transport, metabolism, and excretion), estimated intake of trans fatty acids around the world, and current evidence of *trans* fatty acid intake on metabolic risk factors, cardiovascular diseases, and type 2 diabetes.

CHAPTER II

Chapter II is divided in to two parts: the first part reporting on the development of a comprehensive *trans* fatty acid database, and the second part estimating *trans* fatty acid intake among a Japanese population using 16-day diet records (DRs).

1) Development of a *trans* fatty acid database

I developed a *trans* fatty acid database for 1995 foods [1976 foods appearing in the Standard Tables of Food Composition in Japan (STFCJ) and 19 foods added in the present study]. Among the 1995 foods, 1469 were determined to contain no *trans* fatty acids since they contained no or only a trace amount of fat and no industrially-produced hydrogenated oils or ruminants.

The primary data source for *trans* fatty acid values of the remaining 526 foods was direct chemical analyses. For this, I searched the PubMed, CiNii, and Medical Online Library databases for the English or Japanese literature reporting analyses of the *trans* fatty acid content of foods conducted in Japan. From these articles and their reference lists, I selected articles which assessed *trans* fatty acid content in foods by gas chromatography only. Additionally, I referenced the literature of other countries reporting foods with a high *trans* fatty acid content and selected those which were not included in the STFCJ.

Determination was done in a 4-step process, as follows:

Step 1: Assigning analytic values reported in the literature

Trans fatty acid values in the analytic data were converted to grams per 100 g of food, adjusting for total fat content in the STFCJ. For any reference which reported the *trans* fatty acid value for a specific food as % fat without indicating the fat content (g/100 g) of the food, I used the fat content (g/100 g) provided in the STFCJ to calculate the *trans* fatty acid value of the food.

I then considered a strategy to determine the *trans* fatty acid content of individual foods. Several articles analyzed the same type of food using the same method but provided different mean values. The disagreements arose from variations in *trans* fatty acid content among food products. Also, most articles provided mean, minimum, and maximum values of analyzed products. In these cases, *trans* fatty acid content in individual foods might have been determined by choosing the highest or lowest mean value of multiple reports, or by choosing the minimum or maximum value of the reports. To deal with such complexities, the following guideline was applied.

1) When only one article existed and this article analyzed the *trans* fatty acid content in a single example of a food only, this value was selected (n = 13).

2) When only one article existed and this article analyzed the *trans* fatty acid content of several samples and reported minimum, maximum, and/or mean values, I selected the mean value for the food (n = 71).

3) When multiple articles existed but reported different mean *trans* fatty acid values for a specific food, I calculated the mean value by weighting the number of foods analyzed in each article (n = 59).

Step 2: Assigning analytic values of similar foods

2-1A: When the *trans* fatty acid value for a specific food (except meat cuts) could not be obtained using Step 1 but an analytic value was available for a similar food within the same food category of the same food group by Step 1, that value (*trans* fatty acid % of total fat) was assigned after comparison with nutrient content (total energy and macronutrients) in the STFCJ (n = 102).

2-1B: When the *trans* fatty acid value for a specific food (except meat cuts) could not be obtained using Step 1 but an analytic value was available for a similar food within the same food group by Step 1, that value (*trans* fatty acid % of total fat) was assigned (n = 78).

2-2A: When the analytic *trans* fatty acid value of a specific meat cut was unavailable (Step 1) but an analytic value for the same part but with a different nutrient composition was available, that value was assigned (n = 22).

2-2B: When the analytic *trans* fatty acid value of a specific meat cut was unavailable (Step 1) but an analytic value of a similar part having a similar nutrient composition was available, that value was assigned (n = 17).

2-2C: When the analytic *trans* fatty acid value of a specific type of animal was unavailable (Step 1) but an analytic value of a similar type of animal having a similar nutrient composition and belonging to the same species was available, that value was assigned (n = 37).

2-2D: When the analytic *trans* fatty acid value of a specific type of animal was unavailable (Step 1) but an analytic value of a different type of animal belonging to a different species was available, that value was assigned (*n* = 7).

2-2E: When the analytic *trans* fatty acid value of a specific meat cut was unavailable (Step 1) but an analytic value of the same meat group was available, that value was assigned (*n* = 88).

Step 3: Assigning values obtained from the ESHA Food Processor

For food products whose *trans* fatty acid values were unavailable using Steps 1 and 2 but for which the manufacturer had a presence in both Japan and the United States (US), I compared the nutrient composition of the food in Japan, as shown on the website of the company, with that of the US, as provided in the ESHA Food Processor SQL, which covers >35000 foods including food products and fast foods sold in the US. The analytic nutrient values in ESHA databases were compiled from the latest United States Department of Agriculture Standard Reference database, selected items from the Continuing Survey of Food Intakes by Individuals survey database, manufacturer's data, data from fast food companies and data from literature sources. For foods whose nutrient compositions (total energy and macronutrients) were similar, I assigned the value obtained from the ESHA (*n* = 14).

Step 4: Assigning values estimated from recipes and nutrient compositions

When the *trans* fatty acid values for a specific food were unavailable using Steps 1-3, I then imputed values by referring to recipes and the nutrient composition (total energy and macronutrients) of foods (*n* = 16). Among 16 foods, 4 foods were determined to contain *trans* fatty acids.

2) Study population and study protocol

In the second part, the study population, study protocol and the results are explained. Briefly, the study was conducted between November 2002 and September 2003 in four areas in Japan (Osaka, Okinawa, Nagano, and Tottori. In each area, apparently healthy women aged 30-69 years who were living together with their husbands and willing to participate with their husbands without consideration to the husband's age were first recruited. The recruitment strategy was to obtain eight women for each 10-year age strata. Group orientations for the subjects were held prior to the study at which the study purpose and protocol were explained. Written informed consent was obtained from each subject. A total of 121 women and 121 men completed the study protocol. For analyses, a women whose body weight was mistyped in the database and men aged < 30 or > 69 years (n = 11) were excluded. Further, outliers of *trans* fatty acid intake [below or above mean ± 3 standard deviation (g/day or % total energy)] were excluded, leaving 119 women and 106 men aged 30-69 years in the analyses.

Subjects completed a 4-nonconsecutive-day semi-weighed DRs four times, one in each season, at intervals of approximately 3 months. Each set of four recording days consisted of one weekend day and three weekdays. During the orientation session, registered dietitians gave the subjects both written and verbal instructions on how to keep the DRs, provided recording sheets and a digital scale, and asked to record and weigh all foods and beverages consumed on each recording day. All the collected records were checked by trained registered dietitians in the respective local center and then again in the study center.

A total of 1320 food and beverage items appeared in the DRs. Intake of total energy and total fat were estimated based on the estimated intakes of all items and the STFCJ . *Trans* fatty acid intake was estimated based on the database created in the present study.

RESULTS AND DISCUSSION

Mean *trans* fatty acid intake was 1.7 g/day (0.8% total energy) for women and 1.7 g/day (0.7% total energy) for men. Major contributors to *trans* fatty acid intake were confectionaries, bakery, and fats and oils. Industrially-produced *trans* fatty acids contributed approximately 75%. Twenty-four percent of women (n = 29) and 6% of men (n = 6) showed a mean intake of more than 1% of total energy intake, the maximum recommended intake of the WHO; the frequency was particularly high in women living in urban areas and those aged 30-39 and 40-49 years.

Although mean *trans* fatty acid intake was below the maximum recommended intake of the WHO, intake of subgroups, especially women living in urban areas and those aged 30-39 and 40-49 years, was of concern. Further public health efforts to reduce *trans* fatty acid intake should be encouraged.

CHAPTER III

Using the newly developed *trans* fatty acid database, I investigated associations of total, hydrogenated, and natural *trans* fatty acid intake with metabolic risk factors, including BMI, waist circumference, and serum levels of cholesterol [total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)], triacylglycerol, glucose, and Hb A_{1c} based on data of a cross-sectional study among free-living young Japanese women whose fat intake is relatively low.

Study population

The study was based on a multi-center cross-sectional study conducted from February to March 2006 and from January to March 2007 among female dietetic students at 15 institutions in Japan. All measurements at each institution were carried out in accordance with the survey protocol. Briefly, staff at each institution provided an outline of the survey to potential subjects. Those who responded positively were provided detailed written and oral explanations of the survey's general purpose and procedure. A total of 1,176 Japanese women took part. For the present analyses, those aged <18 or >22 years (*n* = 22) were excluded since the majority of Japanese dietetic students are in the 18-22 years of age range. Also, subjects who provided incomplete survey questionnaires (*n* = 1); reported extremely low or high energy intake (<500 or >4,000 kcal/day; *n* = 2); were currently receiving dietary counseling from a doctor or dietitian (*n* = 13); had a history of a diagnosis of diabetes, hypertension, or cardiovascular disease (*n* = 1); or had no data for body height and weight (*n* = 2) were excluded. Those who provided non-fasting blood samples (*n* = 34) or who had missing information on any metabolic risk factor (*n* = 16) were excluded from the analyses of biochemical measurements. A total of 1,136 women were eligible for BMI and waist circumference analyses, and 1,087 for serum cholesterol (total, LDL, and HDL), triacylglycerol, glucose, and Hb A_{1c}. The study was approved by the Ethics Committee of the National Institute of Health and Nutrition. Written informed consent was obtained from all subjects and from a parent of subjects aged <20 years.

Diet Assessment

A previously validated, self-administered, comprehensive diet history questionnaire (DHQ) was used. The DHQ is a 16-page structured questionnaire which asks about dietary habits during the previous month. Responses to the DHQ and to an accompanying lifestyle questionnaire were checked at least twice for completeness. Estimated dietary intake for a total of 150 food and beverage items, energy, and selected nutrients (except *trans* fatty acids) were calculated using an ad hoc computer algorithm for the DHQ, which was based on the STFCJ. Estimated total, hydrogenated, and natural *trans* fatty acid intake was calculated based on a food composition database developed for the present study. To minimize the influence of dietary misreporting, a known problem of self-reported dietary assessment methods, nutrient intake was energy-adjusted using the density method.

The relative validity of *trans* fatty acid intake estimated from the DHQ was examined against that from the 16-day weighed DRs among 92 women 31-69 years of age. Subjects completed a 4-nonconsecutive-day (one weekend day and three weekdays) semi-weighed DRs four times, at intervals of approximately 3 months (from November 2002 to September 2003). The diet record procedure is discussed in previous chapter.

Development of a *trans* fatty acid database for DHQ

I developed a *trans* fatty acid database for the DHQ. Since a comprehensive *trans* fatty acid food composition tables is not available in Japan, at first, I developed a *trans* fatty acid database for

1,976 foods appearing in the STFCJ. Among 1,976 foods, 1,469 were determined to contain no *trans* fatty acids since they contained no or only a trace amount of fat , no hydrogenated oils, or no ruminants. *Trans* fatty acid content was determined in a 4-step process as explained in the Chapter II. Using the *trans* fatty acid database (*n* =1,976), I matched food codes in the database and those in the DHQ. A total of 48 foods in the DHQ were determined to contain *trans* fatty acids. Among them, *trans* fatty acid values for 37 foods were determined by direct matching from published data. *Trans* fatty acid values for the rest of 11 foods were determined as follows: direct matching from unpublished data (*n* = 1); direct matching from both published and unpublished data (*n* = 2); assignment of a similar food value whose *trans* fatty acid value was determined by direct matching from published data (*n* = 3); assignment of a similar food value whose *trans* fatty acid value was determined by direct matching from both published and unpublished data (*n* = 1); direct matching obtained from EHSA Food Processor (*n* = 2); and direct matching of ingredients in a recipe with published data (*n* = 2).

Validation of *trans* fatty acid intake

Intake of energy and fatty acids were estimated based on the estimated intakes of all items and the STFCJ. Total, hydrogenated, and natural *trans* fatty acid intake was estimated based on the database created in the present study. Pearson's correlation coefficients between the DHQ and 16-day weighed DRs were 0.58 for total fat, 0.67 for saturated fatty acids, 0.54 for monounsaturated fatty acids, 0.34 for polyunsaturated fatty acids, 0.63 for total *trans* fatty acids, 0.58 for hydrogenated *trans* fatty acids, and 0.39 for natural *trans* fatty acids.

Assessment of metabolic risk factors

Metabolic risk factors were assessed 1-3 days after completion of the questionnaires. Body weight and body height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index was calculated as body weight (kg) divided by the square of body height (m^2). Waist circumference was measured at the level of the umbilicus to the nearest 0.1 cm. Overnight fasting blood samples were collected with vacuum tubes which contained no preservative, allowed to clot, and centrifuged at 3,000 g for 10 minutes at room temperature to separate the serum. Serum concentrations of total, LDL, and HDL cholesterol, triacylglycerol, and glucose were measured by enzymatic assay methods. Glycated hemoglobin was measured by latex agglutination-turbidimetric immunoassay. In-house quality-control procedures for all assays were conducted at the respective laboratories.

All statistical analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc, Cary, NC). Linear regression models were used (in PROC GLM) to assess the associations of total, hydrogenated, and natural *trans* fatty acid intake with eight metabolic risk factors. For the analyses, subjects were divided into quintiles according to total, hydrogenated, and natural *trans* fatty acid intake. The mean ± standard error for metabolic risk factor values were calculated by quintiles of total, hydrogenated, and natural *trans* fatty acid intake after multivariate adjustments for potential confounding variables. Covariates included residential block, size of residential area, survey year, current smoking, current alcohol drinking, rate of eating, physical activity (continuous), BMI (continuous; except for the analysis of BMI itself), waist circumference (continuous; except for the analysis of waist circumference itself), and intake of energy (continuous), total fat (continuous), and saturated fatty acids (continuous; model 1). Monounsaturated fatty acids (continuous; model 2) or polyunsaturated fatty acids (continuous; model 3) were added in place of saturated fatty acids. Linear trends with increasing levels of total, hydrogenated, and natural *trans* fatty acid intake were tested by assigning each subject the median value for the category and modeling this value as a continuous variable. All reported *p* values are two-tailed, and a p value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Mean (standard deviation) BMI and waist circumference were 21.3 (2.7) kg/m^2 and 72.9 (7.1) cm, respectively. Intake of total, hydrogenated, and natural *trans* fatty acid intake ranged from 0.26% to 2.25%; 0.09 to 2.18%; and 0.01 to 0.64% of energy, respectively. Hydrogenated *trans* fatty acids contributed 77% of total *trans* fatty acid intake. Major food groups contributing to total *trans* fatty acid intake were fat and oil (24.3%), bakery (20.2%), and confections (19.5%). After adjustment for potential confounding factors, total *trans* fatty acid intake was positively associated with waist circumference, triacylglycerol, and Hb A_{1c} (p for trend ≤ 0.046; models 1-3), except for the analysis of triacylglycerol with adjustment for monounsaturated fatty acids (model 2). No associations were found for total *trans* fatty acid intake with other metabolic risk factors. Additionally, hydrogenated *trans* fatty acid intake was positively associated with waist circumference and Hb A_{1c} (p for trend ≤ 0.03; models 1-3), but not with other metabolic risk factors. Conversely, natural *trans* fatty acid was not associated with any of the metabolic risk factors.

To my knowledge, this is the first study to examine associations of total, hydrogenated, and natural *trans* fatty acid intake with metabolic risk factors in a free-living young Asian population.

Mean total *trans* fatty acid intake (0.90% energy) in our Japanese subjects was comparable to that in one Western study (women: 0.95% energy; men: 0.87% energy), but less than those in 20 Western studies (e.g., 1.3% energy-4.3% energy). This variation in intake among studies is likely due to different dietary habits among the populations. The National Health and Nutrition Survey in Japan shows that Japanese population has a high intake of rice and fish and low intake of meat and confectionaries, and consequently a low fat intake.

In the present study, I found that *trans* fatty acid intake was positively associated with several metabolic risk factors among free-living young Japanese women with relatively low intake. The associations seemed to be largely explained by hydrogenated *trans* fatty acid intake, since they accounted for 77% of total intake.

CONCLUSIONS

Trans fatty acids are unsaturated fatty acids formed during the partial hydrogenation of commercial liquid vegetable oils to semi-solid fats. They are often found in margarine, shortening, and frying fats. Over the past two decades, *trans* fatty acid intake among free-living populations has been unfavorably associated with metabolic diseases including coronary heart disease and type 2 diabetes, and with metabolic risk factors, although not all results have been consistent.

In the present study, although mean *trans* fatty acid intake was below the maximum recommended intake of the WHO, intake of subgroups, especially women living in urban areas and those aged 30-39 and 40-49 years, was of concern. Further public health efforts to reduce *trans* fatty acid intake should be encouraged. Moreover, *trans* fatty acid intake was positively associated with several metabolic risk factors among free-living young Japanese women with relatively low intake. The associations seemed to be largely explained by hydrogenated *trans* fatty acid intake, since they accounted for 77% of total intake. Since the cross-sectional nature of the study precludes causal inferences, further observational studies to clarify the relationship between *trans* fatty acid intake and metabolic risk factors are required.