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Oral sterol intake in The Netherlands: evaluation of the results obtained by GC analysis of duplicate 24-h diet samples collected in 1994

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Abstract

In the spring and autumn of 1994, a total diet study, in which 123 participants collected duplicates of their 24-h diets, was carried out. One of the goals of this study was to determine the amount of sterols in the duplicates of 24-h diets so that we could establish the oral daily intake of these analytes. Lyophilised samples were analysed for cholesterol, coprosterol, brassicasterol, campesterol, stigmasterol and β-sitosterol content. The mean intake of all participants was: for cholesterol, 202 mg person^{-1} day^{-1}; for campesterol, 27 mg person^{-1} day^{-1}; for stigmasterol, 15 mg person^{-1} day^{-1}; and for β-sitosterol, 102 mg person^{-1} day^{-1}. The median intake of all participants was: for cholesterol, 160 mg person^{-1} day^{-1}; for campesterol, 26 mg person^{-1} day^{-1}; for stigmasterol, 14 mg person^{-1} day^{-1}; and for β-sitosterol, 98 mg person^{-1} day^{-1}. The mean and median intake for brassicasterol could not be calculated because too many values were below the limit of determination. The coprosterol intake was below the limit of determination for all samples. The mean intake for the plant sterols was 146 mg person^{-1} day^{-1}, and the mean total sterol intake was 348 mg person^{-1} day^{-1}. The median intake for the plant sterols was 138 mg person^{-1} day^{-1} and the median total sterol intake was 313 mg person^{-1} day^{-1}. On the day of sampling the cholesterol intake of 32 participants was above the recommendation of the Dutch Food and Nutrition Council (Voedingsraad) of a maximum of 33 mg MJ^{-1}. The overall mean intake of cholesterol of 25 mg MJ^{-1}, however, is below this recommendation. The total sterol intake found in this study corresponds well with the results found in the 1984/1985 duplicate diet study. The sterol intake in mg MJ^{-1} found in this study corresponds well with the results from the Dutch National Food Consumption Survey and the Market Basket study.

1. Introduction

Besides age, smoking habits and high blood pressure (among others), high levels of serum cholesterol have been identified as a risk factor in the development of atherosclerosis and coronary heart disease (CHD). Atherosclerosis and CHD caused 39% of all human death in The Netherlands in 1994. Although most cholesterol found in the blood is of endogenic origin, dietary intake of cholesterol is one factor influencing serum cholesterol levels and therefore the risk of atherosclerosis and CHD. Besides cholesterol, which is the main sterol of animal origin (zoösterols), a number of sterols of vegetable origin (phytosterols), such as campesterol, stigmasterol and β-sitosterol, are present in the diet. Dietary intake of phytosterols can influence serum cholesterol levels (Speijers & Vaessen, 1990).

As the Dutch government needs figures on the dietary intake of individual sterols in order to establish their policy about taking measures and giving advice, methods for the determination of the dietary intake of the individual sterols are important. Three approaches are in general available for collecting this information: biological monitoring programmes in which the compounds concerned are measured in human body tissues or fluids; programmes in which the data of a food analysis monitoring programme are linked to those of a food consumption survey, and total diet studies (Vaessen & Schothorst, 1997).

A duplicate portion, 24-h diet study is one of the approaches for carrying out a total diet study and collecting information on oral intake. In this study, a duplicate of all the food and drink, including drinking water, a participant consumes in a 24-h period is collected and analysed. The principal advantages and drawbacks of this duplicate diet approach are summarised by Vaessen & Schothorst (1997).

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A duplicate diet study was carried out in The Netherlands in 1984/1985 (Vaessen et al., 1988). In this study, an enzymatic method for determining sterols was used (Van Dokkum & Kistemaker, 1987). This method, however, did not differentiate between cholesterol and other sterols (Boehringer, 1980). In continuation of the 1984/1985 study, a new study was undertaken in 1994 (Vaessen et al., 1995). A capillary gas chromatographic (GC) method without derivatisation for determining individual sterols in duplicate 24-h diets has been developed and validated (Jekel et al., 1998).

The dietary intake of individual sterols (cholesterol, coprosterol, brassicasterol, campesterol, stigmasterol and β-sitosterol) presented here are the results from the 1994 duplicate 24-h diet study.

2. Study design

The design and sampling procedure of this 1994 duplicate 24-h diet study has been described in detail by Vaessen & Schothorst (1997) and is summarized below. Selection, recruiting and coaching the participants during this study were delegated to a marketing research institute experienced in nationwide surveys. From its data bank, this institute recruited 123 participants, representing the 18–74-year-old male and female segment of the Dutch population. This group, a cross section of all social classes, was selected within a distance of no more than 30 km from Bilthoven, the location of the National Institute of Public Health and the Environment. The Bilthoven region includes small villages, a larger city and small and medium-sized towns.

The participants were briefed at the beginning and coached during the study by employees of the marketing research institute. The briefing included detailed information on the goals and the background of the study and instructions how best to collect the duplicate portion of the diet and how to complete the supplied questionnaire. This 12-page document contained questions on age, sex, weight and details about the type and quantity of food and drinks consumed in the sampling period.

Duplicate 24-h food and drink portions, including drinking water, were collected during two study periods of one week each to include seasonal fluctuations in food composition and consumption. The first period was in March 1994 and the second in September 1994. On each day of these two weeks, including the weekend, 8 or 9 participants collected a duplicate portion of their food and drink. Information is given about the participants in both study periods and about the study as a whole (Table 1).

3. Samples

All participants collected a duplicate portion of everything they consumed in the 24-h sampling period. The homogenized wet material was split into three portions, of which two were stored deep frozen. The third portion was freeze dried, and the dried product obtained was re-homogenized and refrigerated until analysis.

4. Method of analysis

For the determination of individual sterols in duplicate 24-h diets, a capillary gas chromatographic method without derivatisation was developed and validated (Jekel et al., 1998). In this method the test portion with the internal standard (5α-cholestane and 5,7-dimethyltocol) is hydrolysed. The sterols are then extracted with cyclohexane. The sterols in the extract are determined by capillary gas chromatography with flame ionisation detection (FID). The sample preparation procedure for determining the individual sterols in duplicate diets is shown schematically in Fig. 1. The final extract is analysed for individual sterols under the conditions specified in Table 2.

The method described was used to determine the sterol content of the collected 24-h duplicate diets. During the analytical sessions, duplicate determinations, blanks (determination without test portion) and recovery experiments were performed at regular intervals. A control sample (a lyophilised sample of the 1984/1985

Table 1
Data on the study population of the 24-h duplicate portion study 1994

Period	Number of participants			Age (mean and range, years)			Weight (mean and range, kg)		
	Female	Male	All	Female	Male	All	Female	Male	All
March 14–20	31	31	62	45	45	45	73	82	77
				26–73	23–73	23–73	54–110	61–108	54–110
September 19–25	32	29	61	44	45	44	69	75	72
				18–73	19–74	18–74	49–90	65–103	49–103
Both periods	63	60	123	44	44	44	71	79	75
				18–73	19–74	18–74	49–110	61–108	49–110

duplicate 24-h diet study) was also incorporated in each series of analyses. The outcome of these quality assurance experiments complies with the performance characteristics established for the method (Jekel et al., 1998).

Saponification

Weigh: - 500 mg lyophilised duplicate 24-hour diet
- 1 ml internal standard solution.

Blow N_2 above the solution for 2 min.

Add: - 8 ml 3% pyrogallol solution in ethanol
- 0.5 ml saturated KOH solution in water.

Saponify at 80 °C for 30 min.

Cool down to room temperature.

Extraction

Add: - 20 ml cyclohexane and mix
- 12 ml water and mix.

Shake for 5 min.

Let the layers separate (15 min).

Clean-up

Transfer 0.4 ml 3% pyrogallol solution in water to an autosampler vial; add 0.8 ml cyclohexane extract.

Vortex at maximum speed for 30 s, then centrifuge.

Inject 1 µl from the top layer (cyclohexane phase).

Fig. 1. Sample preparation procedure for the determination of sterols in duplicate 24-h diets.

Table 2
Test conditions for the determination of the individual sterols in duplicate 24-h diets

Gas chromatograph, Fisons 8160 with Fisons AS 800 autosampler	
Column type	SAC-5 (Supelco), 30 m×0.25 mm fused silica with 2 m ×0.53 mm retention gap, 0.25 µm SAC-5 coating
Carrier gas	Hydrogen at 125 kPa
Internal standard	5α-cholestane and 5,7-dimethyltocol
Injector	Cold on-column
Injection volume	1 µl
Detection	Flame ionisation detector (FID) at 300°C
Calculation	Internal standard
Column temperature programme	3 minutes at 105°C 20°C min^{-1} to 275°C 15 minutes at 275°C

5. Results and discussion

Cholesterol, campesterol and β-sitosterol were found in all 123 duplicate diets; stigmasterol, in 121 duplicate diets; and brassicasterol, in only 17 diets. The coprosterol content was below the limit of determination for all samples. The daily dietary sterol intake of each of the participants in the study was calculated from the results. The main results of these intake data are summarised in Table 3. For all participants the mean intake was: for cholesterol, 202 mg person^{-1} day^{-1}; for campesterol, 27 mg person^{-1} day^{-1}; for stigmasterol, 15 mg person^{-1} day^{-1}; and for β–sitosterol, 102 mg person^{-1} day^{-1}. The mean intake for brassicasterol could not be calculated because too many values were below the limit of determination. The mean intake for the plant sterols was 146 mg person^{-1} day^{-1}, and the mean total sterol

Table 3
Daily dietary intake data for the sterols calculated from the results of the duplicate 24-h diet samples collected in 1994 in mg person^{-1} day^{-1}

Analyte	March (n=62)	September (n=61)	Both periods (n=123)
Cholesterol			
Mean	200	204	202
SD	125	159	132
Median	166	155	160
Range	19–674	38–533	19–674
Brassicasterol			
Mean	—	—	—
SD	—	—	—
Median	—	—	—
Range	<4–19	<4–12	<4–19
Campesterol			
Mean	29	25	27
SD	13	12	13
Median	26	25	26
Range	10–81	5–71	5–81
Stigmasterol			
Mean	16	15	15
SD	9	9	9
Median	14	12	14
Range	<4–40	<4–44	<4–44
β-Sitosterol			
Mean	106	98	102
SD	40	44	42
Median	104	96	98
Range	46–289	29–215	29–289
Total sterol			
Mean	352	343	348
SD	150	159	154
Median	322	304	313
Range	106–962	119–784	106–962

The mean, SD and median intake for brassicasterol could not be calculated because too many values were below the limit of determination. Coprosterol was not detected in the samples.
—, below limit of determination (4 mg person^{-1} day^{-1}).
SD, standard deviation.

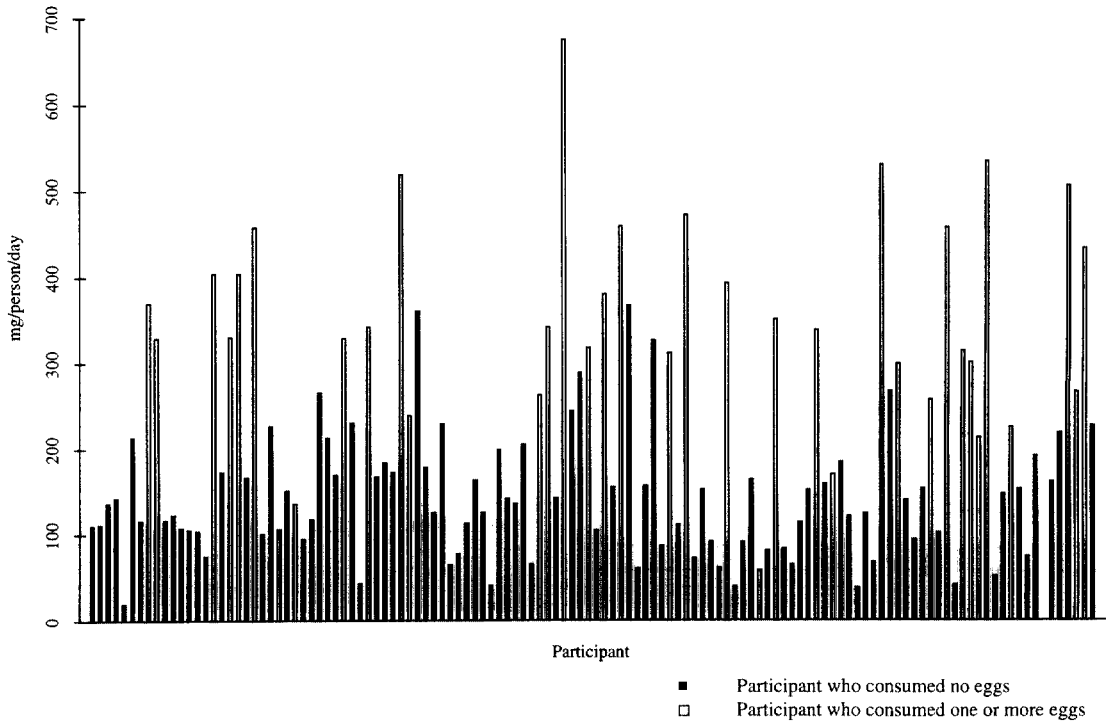

Fig. 2. Cholesterol intake for all participants in the 1994 duplicate portion total diet study.

intake was 348 mg person^{-1} day^{-1}. Although there is only a small difference in mean intake in both seasons, the range of intakes is larger in the spring than in the autumn.

The median intake for cholesterol is substantially below the mean intake. Clearly, the distribution of cholesterol intake is strongly influenced by the consumption of eggs. It became evident from the evaluation of the completed questionnaires that 30% of the participants consumed one or more eggs on the sampling day. The sum of the cholesterol intake of the egg consumers represents 52% of the sum of the cholesterol intake for all participants. This is also shown by Fig. 2 where the cholesterol intake is given for all participants. The figure shows whether each participant consumed one or more eggs on the sampling day. We can see that participants who consumed one or more eggs on the sampling day generally had a cholesterol intake above the mean intake of 202 mg person^{-1} day^{-1}.

The mean intake data for men and women are presented in Table 4. Results are expressed in mg person^{-1}

Table 4
Mean daily dietary sterol intake and standard deviation calculated from the results of the duplicate 24-h diet samples collected in 1994. Results are expressed in mg person day and in energy units (mg MJ^{-1}) 24-h duplicate diet

Analyt	Intake (mg person^{-1} day^{-1}) March 1994				Intake (mg person^{-1} day^{-1}) September 1994				Intake (mg person^{-1} day^{-1}) Both periods 1994				Intake (mg MJ^{-1}) Both periods 1994			
	M (n=31)		F (n=31)		M (n=29)		F (n=32)		M (n=60)		F (n=63)		M (n=60)		F (n=63)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholesterol	208	125	192	127	225	158	185	119	216	141	188	122	24.5	15.3	26.0	16.9
Brassicasterol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Campesterol	31	15	27	12	30	13	21	10	31	14	24	11	3.4	1.5	3.3	1.5
Stigmasterol	18	10	13	6	17	9	13	8	17	10	13	7	1.9	1.1	1.8	1.0
β-Sitosterol	119	46	94	28	115	47	82	34	117	46	88	32	12.7	5.0	12.3	4.4
Total sterol	377	152	327	146	389	169	302	140	383	159	314	143	42.6	17.3	43.5	19.9

The mean intake and standard deviation for brassicasterol could not be calculated because too many values were below the limit of determination.
–, below limit of determination (4 mg person^{-1} day^{-1}).
SD, standard deviation.

Table 5
Comparison of the dietary sterol intake information from recent Dutch studies. Results expressed in mg person^{-1} day^{-1} and in energy units (mg MJ^{-1}) diet

	Study population		Cholesterol		Plant sterols		Total sterols	
	Age (year)	Sex	mg person^{-1} day^{-1}	mg MJ^{-1}	mg person^{-1} day^{-1}	mg MJ^{-1}	mg person^{-1} day^{-1}	mg MJ^{-1}
First Dutch National Food Consumption Survey 1987/1988	1–74	Female + Male	297	30.5	–	–	–	–
Second Dutch National Food Consumption Survey 1992	1–74	Female + Male	246	26.8	–	–	–	–
CIVO-TNO The Market Basket Study 1976–1978	16–18	Male	374	29	–	–	–	–
CIVO-TNO The Market Basket Study 1984–1986	18	Male	330	25	190	14	520	39
RIVM duplicate 24-h diet study 1984/1985[a]	18–74	Female + Male	–	–	–	–	353	45
RIVM duplicate 24-h diet study 1994 (this study)	18–74	Female + Male	202	25	146	18	348	43

—, No data available.
[a] Results are reported as cholesterol. The analysis method used did not differentiate between cholesterol and the other sterols; the results are therefore expressed as total sterols.

day^{-1} and in energy units (mg MJ^{-1}) 24-h duplicate diet. For both sampling periods the individual and total sterol intake for men is higher than for women. However, when these results are expressed in mg MJ^{-1}, they correspond much better.

On the day of sampling, the cholesterol intake of 32 (26%) participants was above the recommendation of the Dutch Food and Nutrition Council (Voedingsraad, 1990) of a maximum of 33 mg MJ^{-1}. The overall mean intake of cholesterol of 25 mg MJ^{-1}, however, is below this recommendation. No recommendations are available for the plant sterols.

The intake figures from recent Dutch studies are reviewed in Table 5:

- First Dutch National Food Consumption Survey 1987/1988 (Ministerie van Welzijn, Volksgezondheid en Cultuur and Ministerie van Landbouw en Visserij, 1988).
- Second Dutch National Food Consumption Survey 1992 (Voorlichtingsbureau voor de Voeding, 1993).
- CIVO-TNO The Market Basket study 1976–1978 (Van Dokkum and De Vos, 1987).
- CIVO-TNO The Market Basket study 1984–1986 (Van Dokkum et al., 1990).
- RIVM duplicate 24-h diet study 1984/1985 (Van Dokkum and Kistemaker, 1987).
- RIVM duplicate 24-h diet study 1994 (this study).

In the first and second Dutch National Food Consumption Surveys the focus was on the whole population and in the Market Basket studies on young Dutch men in the age categories of 16–18 years and of 18 years. The duplicate 24-h diet studies concentrated on Dutch men and women in the age category of 18–74 years.

Clearly, the total sterol intake found in the present study corresponds well with the results found in the 1984/1985 duplicate diet study. Although the variation in absolute oral sterol intake between subjects is large, the sterol intake in mg MJ^{-1} found in this study corresponds well with the results from the two Dutch National Food Consumption Surveys and the two Market Basket studies.

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