

Analytical, Nutritional and Clinical Methods Section

Results of analysis of the 1994 Dutch duplicate 24-hour diet samples: fatty acids

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Abstract

In spring and fall 1994, 123 respondents participated in a duplicate 24-h diet study. Each respondent collected one duplicate of the food and drinks, including drinking water he/she consumed in a continuous 24-h period. Lyophilised subsamples were analysed for fatty acid content by means of an in-house developed and validated gas chromatographic method with flame ionisation detection. From these data the fatty acid intake per person per day and the fatty acid profile were calculated. From the measured fatty acid content, the calculated intake of all participants was on average: for fat 79.9 g, for fatty acids 62.9 g, for saturated fatty acids 29.0 g, for monounsaturated fatty acids 22.8 g, for polyunsaturated fatty acids 9.7 g and for trans fatty acids 3.8 g. The intake expressed as percentage of the total energy intake (en%) of all participants was on average: for saturated fatty acids 12.7 en%, for monounsaturated fatty acids 10.0 en%, for polyunsaturated fatty acids 4.3 en% and for trans fatty acids 1.7 en%. The average intake on fat was 35.0 en% and the average fatty acids intake was 27.6 en%. No differences were found between both sampling periods nor between men and women. The average intake of fat equals and of saturated fatty acids was above the recommendation of the Dutch Food and Nutrition Council (Voedingsraad) of a maximum of 30–35 en% of fat and of a maximum of 10 en% of saturated fatty acids. The fatty acid profile found in this study corresponds well with the profile found in the 1984/1985 duplicate diet study. © 2000 Elsevier Science Ltd All rights reserved.

Keywords: Fatty acids; 24-h diets; Intake

1. Introduction

In continuation of previous duplicate diet studies carried out at our Institute (Stephany & Schuller, 1980; Vaessen, Van de Kamp & Van Ooik, 1988), a new study was undertaken in 1994 (Vaessen et al., 1995). In this study each respondent collected one duplicate of the food and drinks, including drinking water he/she consumed in a continuous 24-h period. Subsamples (lyophilised or deep frozen) of the collected material were used for (future) analysis of, amongst other, pesticides, some vitamins, PCBs, elements (Van Loon, Van Ooik & Ritsema, 1996, 1997a,b and c, 1998; Van Loon, De Joode, Van Ooik & Ritsema, 1998; Van Ooik, Van den Burg-van Essen, Ritsema, Van Loon & Vaessen, 1996), sterols (Schothorst

& Jekel, 1999), nitrate and nitrite (Vaessen & Schothorst, 1999) and fatty acids (Schothorst & Jekel, 1998).

A previous duplicate diet study was carried out in The Netherlands in 1984/1985 (Vaessen et al., 1988). In this study fatty acids were determined after extraction with a mixture of chloroform and methanol by gaschromatographic determination of the fatty acid methylesters. The trans fatty acid content was determined by infrared spectrophotometry (Van Dokkum & Kistemaker, 1987; Van Dokkum, Kistemaker & Hilwig, 1989).

For the present study a capillary gas chromatographic method with flame ionisation detection (FID) for determining the individual fatty acids in lyophilised portions of the duplicate 24-hour diets samples, has been developed and validated (Schothorst & Jekel, 1998).

The dietary intake of fatty acids and the fatty acid profile presented here are the results from the 1994 duplicate 24-h diet study.

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2. Study design

The design and sampling procedure of this 1994 duplicate 24-hour diet study has been described in detail by Vaessen and Schothorst (1999) and is summarised below.

Selection, recruiting and coaching the participants during this study were delegated to a marketing research institute experienced in nation-wide 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, the weekend included, 8 or 9 participants collected a duplicate portion of their food and drink.

In Table 1 information is given about the participants in both study periods and about the study as a whole.

3. Samples

All participants collected a duplicate portion of everything they consumed in the 24-h sampling period. The homogenised wet material was split into three portions,

which two were stored deep-frozen. The third portion was lyophilised, and the dried product obtained was re-homogenised and refrigerated until analysis.

4. Method of analysis

For the determination of individual fatty acids in the lyophilised portions of the duplicate 24-h diets samples, a capillary gas chromatographic method with flame ionisation detection (FID) was developed and validated. In this procedure a 500 mg test portion of the lyophilised material is mixed with the internal standard (nondecanoic acid, C19:0). The fatty acids are (trans)esterified to the corresponding methylesters in the presence of BF₃ in methanol. The formed fatty acid methylesters are extracted with *n*-hexane and washed with water. The fatty acid methylesters in the *n*-hexane extract are determined by capillary gas chromatography with flame ionisation detection (FID). Injection of standard solutions is used for peak identification. Results are expressed as fatty acids. The sample preparation procedure for determining the individual fatty acids in duplicate diets is shown schematically in Fig. 1. The final extract is analysed for individual fatty acids under the conditions specified in Table 2.

The performance characteristics of the method were established by recovery experiments and blank determinations. Also certified reference materials (BCR CRMs 162, 163 and 164) were analysed. The findings of these experiments are summarised in Tables 3 and 4 and illustrate the suitability of the method. For fatty acids with more than 8 carbon atoms the accuracy of the method is above 90%.

The method described was used to determine the fatty acid 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 duplicate 24-h diet study) was also incorporated in each series of analyses. The outcome of these quality assurance

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 26–73	45 23–73	45 23–73	73 54–110	82 61–108	77 54–110
September 19–25	32	29	61	44 18–73	43 17–74	44 18–74	69 49–90	75 65–103	72 49–103
Both periods	63	60	123	44 18–73	44 19–74	44 18–74	71 49–110	79 61–108	75 49–110

experiments complies with the performance characteristics established for the method (Schothorst & Jekel, 1998).

Total fat determinations were done by the IGB-Maastricht, The Netherlands by the method of Weibull-Stoldt (Vaessen et al., 1988).

Esterification
Weigh 500 mg lyophilised duplicate 24-hour diet
Add 1 ml internal standard solution.
Add 2 ml diisopropyl ether (DIPE) and mix
Blow N ₂ above the solution for 2 min.
Add 6 ml 14% boron trifluoride in methanol
Esterify at 70 °C for 2 hour.
Cool down to room temperature.
Extraction
Add: - 20 ml n-hexane and mix - 10 ml water and mix.
Shake for 5 min, then centrifuge
Clean-up
Transfer 0.4 ml water and 0.8 ml n-hexane to an autosampler vial; add 1.5 µl n-hexane extract.
Vortex at maximum speed for 30 s, then centrifuge.
Inject 1 µl from the top layer (n-hexane phase).

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

Table 2

Test conditions for the determination of fatty acids in duplicate 24-h diets

<i>Gas chromatograph, Fisons 8160 with Fisons AS 800 autosampler</i>	
Column type	: CP-Sil 88 (Chrompack), 100 m x 0.25 mm fused silica with 2 m x 0.53 mm retention gap, 0.2 µm film thickness.
Carrier gas	: Hydrogen at 200 kPa
Internal standard	: Nondecyclic acid (C19:0)
Injector	: Cold on-column
Injection volume	: 1 µl
Detection	: Flame ionisation detector (FID) at 240°C
Calculation	: Internal standard
Column temperature programme	: 5 minutes at 55°C 20°C/min to 174°C 20 min at 174°C 2.5°C/min to 240°C

5. Results and discussion

From the analysis results obtained for the lyophilised subsamples, the daily dietary fatty acid intake of each of the participants in the 24-h duplicate diet study was calculated. The main results of these intake data are summarised in Table 5. The mean, S.D. and median intake for a large number of fatty acids could not be

Table 3

Performance characteristics of the GC-FID determination of fatty acids in lyophilised duplicate 24-h diets. Number of recovery experiments: 7

Recovery			
Fatty acid	Mean (%)	Range (%)	Coefficient of variation (%)
C4:0	62	59–64	2.4
C6:0	82	78–86	3.4
C8:0	93	91–96	1.7
C10:0	99	97–101	1.4
C20:0	99	92–104	3.6
C22:0	98	92–101	3.0
C14:1c9	92	89–94	1.6
C16:1c9	96	93–99	2.1
C20:1c11	98	90–103	4.3
C18:2t9t12	93	87–96	3.1
C18:3c9c12c15	95	91–98	2.2
Limit of determination, 0.15 g/person/day			

Table 4

Certified fatty acid content in CRM 162 (soya/maize oil blend), CRM 163 (beef/pig fat blend) and CRM 164 (milk fat), measured mean content and accuracy towards the certified value ($n=2$)

Fatty acid	Certified value	Measured mean	Accuracy (%)
CRM 162			
C16:0	10.7	10.3	96.3
C18:0	2.9	2.9	99.4
C18:1c9	24.1	23.5	97.3
C18:2c9c12	56.7	57.6	101.6
C18:3c9c12c15	4.7	4.6	98.6
CRM 163			
C14:0	2.3	2.2	95.1
C16:0	26.0	27.0	104.2
C16:1c9	2.6	2.7	106.4
C18:0	18.3	18.9	103.1
C18:1c9	38.3	39.1	102.0
C18:2c9c12	7.1	6.7	95.5
C18:3c9c12c15	0.9	0.9	100.7
CRM 164			
C6:0	2.4	1.7	72.5
C8:0	1.4	1.2	88.2
C10:0	2.9	2.8	96.2
C12:0	4.0	4.1	100.6
C14:0	10.8	11.4	105.9
C16:0	26.9	29.1	108.0
C18:0	10.5	11.2	106.5
C18:1c9	2.8	26.2	105.7
C18:3c9c12c15	0.5	0.5	98.2

Table 5

Daily dietary intake data for the fatty acids calculated from the results of the duplicate 24-h diet samples collected in 1994 in g/person/day

Fatty acid	FC ^b	March (n = 62)				September (n = 61)				Both periods (n = 123)			
		Mean	S.D. ^c	Median	Range	Mean	S.D. ^c	Median	Range	Mean	S.D. ^c	Median	Range
C4:0	S	0.4	0.3	0.3	<0.15–1.3	0.4	0.3	0.4	<0.15–1.2	0.4	0.3	0.3	<0.15–1.3
C6:0	S	0.5	0.4	0.4	<0.15–1.4	0.5	0.5	0.4	<0.15–2.7	0.5	0.4	0.4	<0.15–2.7
C8:0	S	0.4	0.3	0.3	<0.15–1.2	0.3	0.3	0.3	<0.15–1.2	0.3	0.3	0.3	<0.15–1.2
C10:0	S	0.6	0.4	0.6	<0.15–1.5	0.5	0.4	0.5	<0.15–1.4	0.6	0.4	0.5	<0.15–1.5
C11:0	S	^a	–	–	–	–	–	–	<0.15–0.3	–	–	–	<0.15–0.3
C12:0	S	1.9	1.7	1.3	<0.15–7.2	1.5	1.3	1.1	<0.15–6.8	1.7	1.5	1.2	<0.15–7.2
C13:0	S	–	–	–	–	–	–	–	<0.15–0.7	–	–	–	<0.15–0.7
C14:0	S	2.8	1.4	2.7	0.3–6.1	2.6	1.5	2.4	<0.15–6.7	2.7	1.5	2.4	<0.15–6.7
C14:1c9	M	–	–	–	<0.15–0.5	–	–	–	<0.15–0.5	–	–	–	<0.15–0.5
C15:0	S	0.2	0.2	0.2	<0.15–0.6	0.2	0.2	0.2	<0.15–0.7	0.2	0.2	0.2	<0.15–0.7
C15:1c10	M	–	–	–	<0.15–0.2	0.2	1.1	–	<0.15–8.6	–	–	–	<0.15–8.6
C16:0	S	16.1	6.7	16.2	2.1–36.6	15.1	7.3	13.2	<0.15–33.1	15.6	7.0	14.7	<0.15–36.6
C16:1 trans	M, t	0.4	0.3	0.3	<0.15–1.5	0.5	0.4	0.4	<0.15–1.4	0.4	0.4	0.4	<0.1–1.5
C16:1c9	M	0.7	0.4	0.6	<0.15–2.6	0.6	0.3	0.5	<0.15–1.6	0.6	0.4	0.5	<0.15–2.6
C16:1cis-other	M	–	–	–	<0.15–0.3	–	–	–	<0.15–	1.0	–	–	<0.15–1.0
C17:0	S	0.2	0.2	0.2	<0.15–0.5	0.2	0.2	0.2	<0.15–0.6	0.2	0.2	0.2	<0.15–0.6
C17:1c10	M	–	–	–	–	–	–	–	<0.15–0.2	–	–	–	<0.15–0.2
C18:0	S	6.6	2.9	6.4	0.9–15.9	6.5	3.0	5.8	0.6–14.4	6.6	2.9	6.0	0.6–15.9
C18:1trans	M, t	3.3	2.4	2.9	0.8–12.9	3.2	1.9	2.7	<0.15–8.1	3.3	2.1	2.8	<0.15–12.9
C18:1c9	M	16.8	9.3	15.5	3.4–42.8	16.6	11.4	14.4	3.6–71.4	16.7	10.4	14.8	3.4–71.4
C18:1cis-other	M	1.6	1.0	1.5	0.4–5.2	1.3	0.6	1.2	<0.15–2.9	1.5	0.8	1.3	<0.15–5.2
C18:2t9t12	P, t	–	–	–	–	–	–	–	–	–	–	–	–
C18:2c9t12	P, t	–	–	–	<0.15–0.4	–	–	–	<0.15–0.4	–	–	–	<0.15–0.4
C18:2t9c12	P, t	–	–	–	<0.15–0.2	–	–	–	<0.15–0.3	–	–	–	<0.15–0.3
C18:2c9c12	P	8.8	7.2	7.8	0.4–26.5	9.0	10.7	6.3	0.4–49.2	8.9	9.0	7.4	0.4–49.2
C20:0	S	–	–	–	<0.15–0.9	0.2	0.2	0.2	<0.15–1.2	–	–	–	<0.15–1.2
C18:3c6c9c12	P	–	–	–	–	–	–	–	–	–	–	–	–
C18:3t	P, t	–	–	–	<0.15–0.7	–	–	–	<0.15–1.2	–	–	–	<0.15–1.2
C20:1c11	M	–	–	–	<0.15–0.5	–	–	–	<0.15–0.8	–	–	–	<0.15–0.8
C18:3c9c12c15	P	0.7	0.7	0.6	<0.15–2.6	0.6	0.8	0.5	<0.15–3.8	0.7	0.7	0.6	<0.15–3.8
C21:0	S	–	–	–	–	–	–	–	<0.15–0.2	–	–	–	<0.15–0.2
C20:2c11c14	P	–	–	–	<0.15–0.2	–	–	–	–	–	–	–	<0.15–0.2
C22:0	S	–	–	–	<0.15–1.2	0.2	0.3	–	<0.15	–	–	–	<0.15–2.1
C20:3c8c11c14	P	–	–	–	–	–	–	–	–	–	–	–	–
C22:1c13	M	–	–	–	<0.15–0.3	–	–	–	<0.15–0.6	–	–	–	<0.15–0.6
C20:3c11c14c17	P	–	–	–	–	–	–	–	–	–	–	–	–
C20:4c5.8.11.14	P	–	–	–	<0.15–0.2	–	–	–	<0.15–0.2	–	–	–	<0.15–0.2
C23:0	S	–	–	–	–	–	–	–	–	–	–	–	–
C22:2c13c16	P	–	–	–	–	–	–	–	–	–	–	–	–
C20:5EPA	P	–	–	–	<0.15–0.3	–	–	–	<0.15–0.5	–	–	–	<0.15–0.5
C24:0	S	–	–	–	<0.15–0.5	–	–	–	<0.15–0.9	–	–	–	<0.15–0.9
C24:1c15	M	–	–	–	–	–	–	–	<0.15–0.4	–	–	–	<0.15–0.4
C22:6DHA	P	–	–	–	–	–	–	–	<0.15–0.8	–	–	–	<0.15–0.8
Not identified		1.2	1.6	0.7	<0.15–6.9	1.5	1.9	0.8	<0.15–9.0	1.4	1.7	0.7	<0.15–9.0
Total		63.7	27.8	62.3	12.1–131.1	62.0	33.4	53.5	8.9–174.7	62.9	30.6	57.4	8.9–174.7

^a Below limit of determination (0.15 g/person/day).^b FC, fatty acids cluster; S, saturated fatty acid; M, monounsaturated fatty acid; P, polyunsaturated fatty acid; t, trans fatty acid.^c S.D., standard deviation.

calculated because too many values were below the limit of determination. (With a test portion of 500 mg lyophilised duplicate 24-h diet, the limit of determination is about 0.15 g/person/day at a signal to noise value of 3.)

Only C18:0, C18:1c9 and C18:2c9c12 were found in all 123 duplicate diets. The C18:2t9t12, C18:3c6c9c12, C20:3c8c11c14, C20:3c11c14c17, C22:2c13c16 and C23:0 content was below the limit of determination for all

samples. For all these compounds it can be concluded that they do not contribute significantly to the total intake.

The mean fatty acid profile found in duplicate diets for both sampling periods is presented in Fig. 2 for men and for women in Fig. 3. Results are expressed as percentage of the total fatty acid intake including the not-identified fraction of the fatty acids.

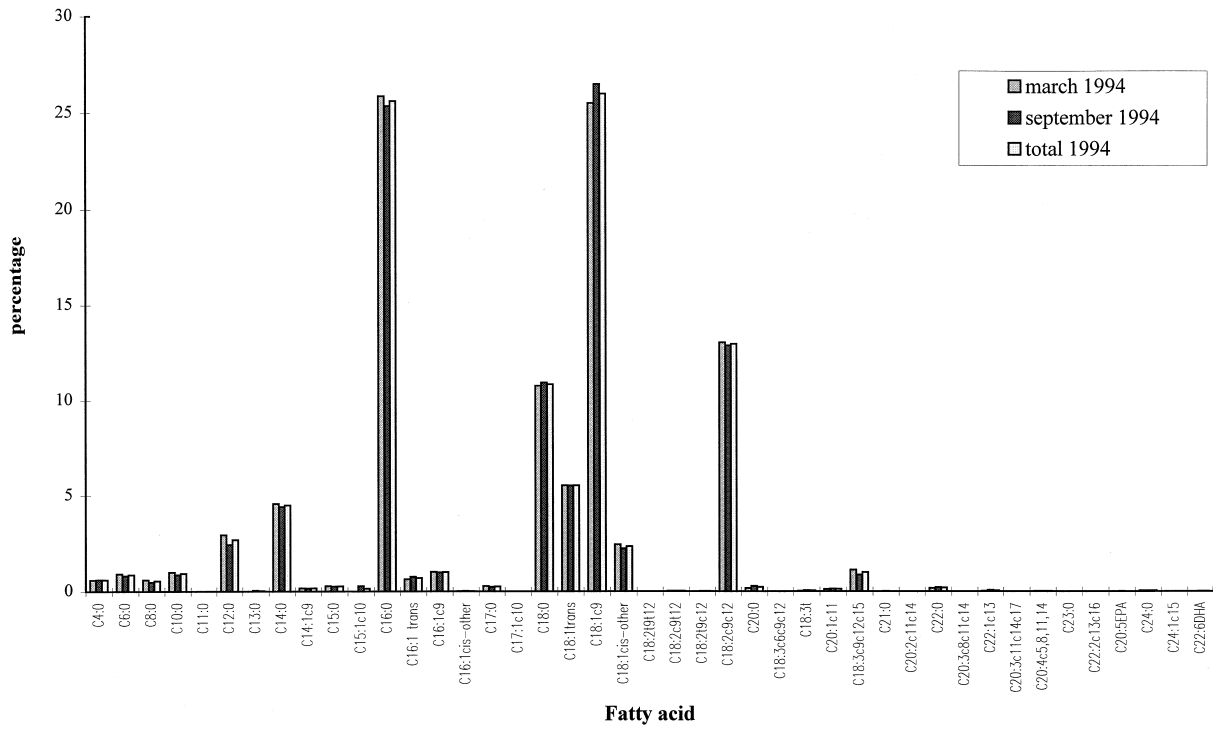


Fig. 2. The mean fatty acid profile found in duplicate diets in March, September and total 1994. Results are expressed as percentage of the total fatty acid intake including the not-identified fraction of the fatty acids.

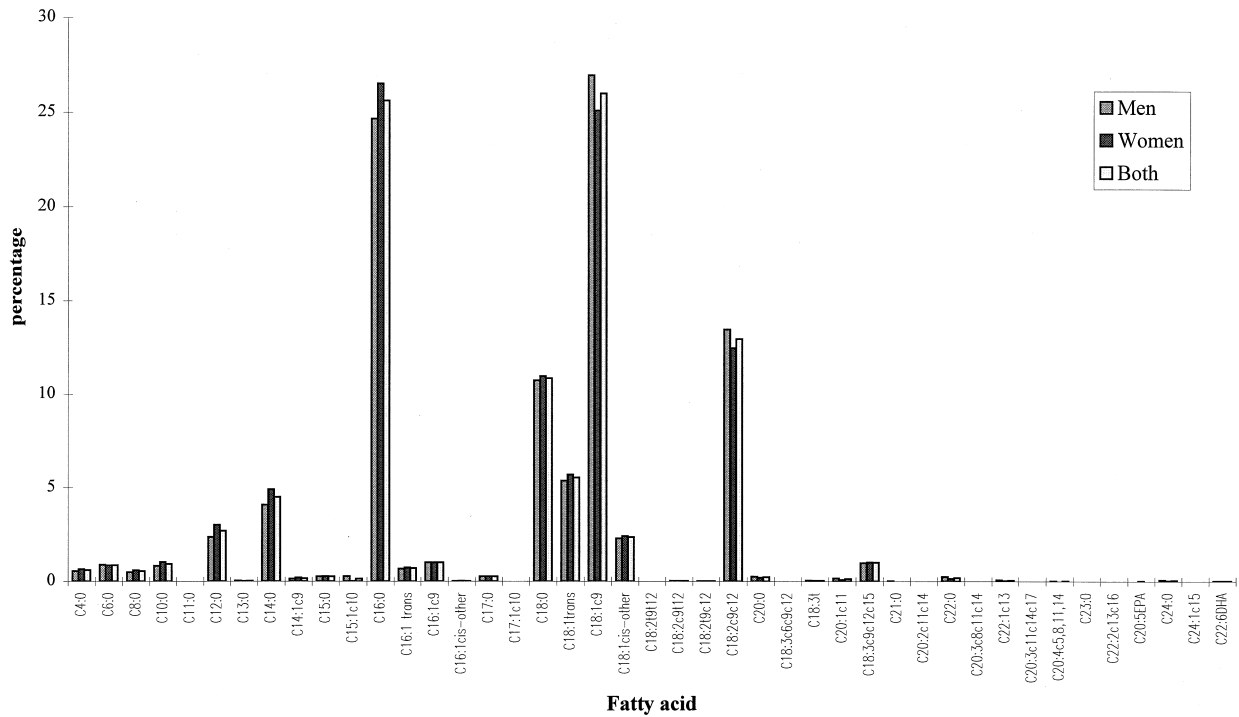


Fig. 3. The mean fatty acid profile found in duplicate diets for men, women and both. Results are expressed as percentage of the total fatty acid intake including the not-identified fraction of the fatty acids.

No differences were found between both sampling periods nor between men and women. From the fat content of the duplicate diet samples, measured by the IGB-Maastricht, the daily dietary fat intake of each of the participants in the study was calculated.

The mean daily dietary intake, the standard deviation and the range for fat, fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, trans fatty acids and not-identified fatty acids are presented in Table 6. Results are expressed in g/person/day and as percentage of the mean daily energy intake (en%) of the total diet. The overall mean intake of fat of 35 en% equals the recommendation of the Dutch Food and Nutrition Council (Voedingsraad, 1991) of a maximum fat intake of 30–35 en%. The overall mean intake of saturated fatty acids of 12.7 en% was above the recommendation of a maximum intake of 10 en%.

The intake figures from recent Dutch studies are reviewed in Table 7.

- 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 & De Vos, 1987).
- CIVO-TNO The Market Basket study 1984–1986 (Van Dokkum, De Vos, Dukel & Hilwig, 1990).
- RIVM duplicate 24-h diet study 1984/1985 (Van Dokkum & Kistemaker, 1987 and Van Dokkum et al., 1989).
- 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

Table 6

Daily dietary intake data for fat, fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, trans fatty acids and not identified fatty acids calculated from the results of the duplicate 24-h diet samples collected in 1994 ($n = 123$)

	Intake in g/person/day				Intake in en%
	Mean	S.D. ^a	Median	Range	Mean
Fat ^b	79.9	34.2	75.1	11.6–192.9	35.0
Fatty acids	62.9	30.6	57.4	8.9–174.7	27.6
Saturated fatty acids	29.0	13.0	27.8	3.3–62.2	12.7
Monounsaturated fatty acids	22.8	12.3	20.7	3.6–78.7	10.0
Polyunsaturated fatty acids	9.7	9.8	8.3	0.4–54.3	4.3
Trans fatty acids	3.8	2.3	3.5	<0.15–13.4	1.7
Not identified fatty acids	1.4	1.7	0.7	<0.15–9.0	0.6

^a S.D., standard deviation.

^b Data provided by the IGB-Maastricht.

Table 7

Mean dietary fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and trans fatty acids intake information from recent Dutch studies. Results expressed in g/person/day and as percentage of the mean daily energy intake (en%) of the total diet

	Study population		Fat		Saturated fatty acids		Monounsaturated fatty acids		Polyunsaturated fatty acids		Trans fatty acids	
	Age (year)	Sex	g/person/day	en%	g/person/day	en%	g/person/day	en%	g/person/day	en%	g/person/day	en%
1st Dutch National Food Consumption Survey 1987/1988	1–74	Female + Male	104.9	39.8	43	16.4	40.4	15.3	18.2	6.8	– ^a	–
2nd Dutch National Food Consumption Survey 1992	1–74	Female + Male	92	36.9	35	14.1	34	13.6	17	6.8	–	–
CIVO-TNO The Market Basket Study 1976–1978	16–18	Male	140	40.5	–	–	–	–	20.4 ^b	5.9	–	–
CIVO-TNO The Market Basket Study 1984–1986	18	Male	124	35.4	48.9	14	41.4	11.8	16.1	4.6	–	–
RIVM duplicate 24-hour diet study 1984/1985	18–74	Female + Male	74	36	30.2	14.7	25.7	12.5	9.9	4.8	–	5
RIVM duplicate 24-hour diet study 1994 (this study)	18–74	Female + Male	79.9	35.0	29.0	12.7	22.8	10.0	9.7	4.3	3.8	1.7

^a No data available.

^b Linoleic + linolenic acid.

men in the age categories of 16–18 years and of 18 years. The duplicate 24-hour diet studies concentrated on Dutch men and women in the age category of 18–74 years.

The mean daily dietary intake, for fat, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids found in the present study corresponds well with the results found in the 1984/1985 duplicate diet study.

Although the variation in mean absolute dietary fat and fatty acid intake between the different studies is large, the fat and fatty acid intake in en% 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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