British Journal of Nutrition (2005), **93**, 115–121
DOI: 10.1079/BJN20041290

The relation between alcohol intake and physical activity and the fatty acids 14 : 0, 15 : 0 and 17 : 0 in serum phospholipids and adipose tissue used as markers for dairy fat intake

M. Rosell[1]*†, G. Johansson[2], L. Berglund[3], B. Vessby[4], U. de Faire[1,5] and M.-L. Hellénius[5,6]

[1]*Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden*
[2]*Department of Food and Nutrition, University of Umeå, Umeå, Sweden*
[3]*UCR Uppsala Clinical Research Center, University of Uppsala, Uppsala, Sweden*
[4]*Unit for Clinical Nutrition Research, Department of Public Health and Caring Sciences, University of Uppsala, Uppsala, Sweden*
[5]*Center for Clinical Cardiovascular Research, Department of Cardiology, Karolinska Hospital, Stockholm, Sweden*
[6]*Department of Family Medicine, Karolinska Institutet, Stockholm, Sweden*

(Received 22 April 2004 – Revised 24 August 2004 – Accepted 1 September 2004)

The relative contents of the fatty acids 14 : 0, 15 : 0 and 17 : 0 in serum and adipose tissue may be used as biological markers of dairy fat intake. However, the determinants of these fatty acids are not fully understood. This study investigates the relationship between these fatty acids and the intake of macronutrients and physical activity in a cross-sectional study of 301 healthy men aged 61–64 years. Dietary intake was assessed using a pre-coded 7 d food record, and physical activity during the previous year was recorded in an interview. Under-reporters of energy intake were identified by the Goldberg cut-off. Fatty acid composition was determined in serum phospholipids (PL) and subcutaneous adipose tissue (AT) from the upper buttock. The relative content of each of 14 : 0, 15 : 0 and 17 : 0 in PL and AT was positively associated with the intake of dairy fat. In addition, all three fatty acids were inversely correlated with alcohol intake, R ranging from −0·28 to −0·53 ($P<0·001$). The results were not markedly affected when under-reporters (n 88) were excluded from the analyses. In both PL and AT, the relative content of the fatty acids was approximately 5% higher in a group of high physical activity compared with a group of low physical activity, although significant trends were only seen for 14 : 0 in PL and 17 : 0 in AT. The findings suggest that adjustments should be made for alcohol intake when the fatty acids 14 : 0, 15 : 0 and 17 : 0 are applied as markers for dairy fat intake.

Biological markers: Fatty acids: Dairy fat: Alcohol: Physical activity

Dairy products such as butter, cheese, milk, yoghurt and cream comprise a considerable part of the Western diet, but the health effects of these foods are unclear. Dairy fat is largely saturated, and a high intake of saturated fat is an established risk factor for CVD and type II diabetes (Mann, 2002). On the other hand, dairy products provide calcium and other nutrients that may produce beneficial health effects (Pfeuffer & Schrezenmeir, 2000).

Research in nutritional epidemiology is complicated by the difficulties that arise when people's habitual diet is to be measured. Large day-to-day variations in dietary intake, inaccurate reporting and changing food habits during the study period are factors that must be considered. In general, reported energy intake in dietary surveys is too low to represent a habitual diet (Black *et al.* 1993). The under-reporting of foods seems to be a differential phenomenon: foods consumed between meals and foods considered to be unhealthy have been shown to be under-reported to a higher degree than other foods (Bingham *et al.* 1995; Pryer *et al.* 1997; Poppitt *et al.* 1998; Goris *et al.* 2000; Lafay *et al.* 2000). It has been demonstrated that relationships between diet and disease variables can change depending on whether or not under-reporters (URs) are included (Macdiarmid *et al.* 1998; Rosell *et al.* 2003*a*).

An alternative to conventional dietary assessment methods is to use biological markers, i.e. variables that are related to dietary intake and are measured in blood or body tissues. There is no biological marker for the absolute amount of fat intake. However, the quality of the dietary fat is partly reflected in the fatty acid composition of the body tissues. It has previously been reported that the relative contents of myristic acid (14 : 0), pentadecanoic acid (15 : 0) and possibly heptadecanoic acid (17 : 0) in serum phospholipids (PL) and adipose tissue (AT) are related to the intake of dairy fat (Wolk *et al.* 1998, 2001). These fatty acids may therefore be valuable biological markers in dietary surveys. However, the metabolism of specific fatty acids is not fully understood, and other factors may also influence their concentration in body tissues (Arab, 2003). For example, it has been demonstrated that the fatty acid composition of muscle is affected by physical activity (Andersson *et al.* 1998). Information on determinants of

Abbreviations: AT, adipose tissue; PAL, physical activity level; PL, phospholipids; UR, under-reporter.

* **Corresponding author:** Dr Magdalena Rosell, fax +44 (0) 1865 310 545, email magdalena.rosell@imm.ki.se
† Present address: Cancer Research UK Epidemiology Unit, University of Oxford, Gibson Building, Radcliffe Infirmary, Oxford OX2 6HE, UK.

the relative content of 14 : 0, 15 : 0 and 17 : 0 in serum and AT increases the usefulness of these fatty acids as biological markers since possible relationships between these fatty acids and outcome variables can then be adjusted for factors that may influence the relationships. We report the relationship between the relative content of 14 : 0, 15 : 0 and 17 : 0 in PL and AT, and the intake of macronutrients and physical activity in a sample of 301 healthy men aged 61–64 years. Under-reporting was taken into consideration by identifying URs and analysing the data with and without them.

Methods

Subjects

The subjects were recruited from a cohort of men and women who had participated in a health screening study in 1997–9. Every third 60-year-old person in Stockholm County was invited, and 78 % (2198 women and 2093 men) agreed to take part. The healthy men in the cohort who fulfilled the criteria for inclusion in the current study (born in Sweden, no diagnosis of CVD, no pharmacological treatment of diabetes, hypertension or hypercholesterolaemia, a BMI between 20 and 35 and no other serious disease; *n* 995) were divided into three groups by the tertiles of their fasting insulin concentration. Requests to participate in a study concerning diet and the metabolic syndrome were repeatedly sent until the number of positive responders reached approximately 100 in each group. This classification was used in order to recruit subjects with a wide range of insulin levels and it was not used in the analyses presented here. The study was conducted between March 2000 and October 2001. Two men did not complete the 7 d food record and were therefore excluded, leaving 301 men aged 61–64 years for this study. The ethical committee at the Karolinska Institute approved the study. All the participants gave written, informed consent.

Clinical procedure

The participants underwent a medical examination including anthropometric measurements and blood sampling in the fasting state in the morning. An anaesthetic cream (Emla 5 % (Astra), containing 2·5 % lidocaine and 2·5 % prilocaine) was applied to the skin on the left upper buttock for 20–30 min. Thereafter, a biopsy of subcutaneous AT was taken (Beyen & Katan, 1985), using a 1·2 × 50 mm needle. Information about medications, smoking and physical activity was recorded during a structured interview. Written and oral instructions for how to complete a 7 d food record and collect a 24 h urine sample were given individually. The participants were told not to change their eating habits during the study. After approximately 1 week, the participant returned with the completed food record and a urine sample. The food record was examined, and ambiguities were resolved.

Dietary assessment

The dietary assessment method has been described in detail elsewhere (Rosell *et al.* 2003*b*). The subjects completed a 7 d food record, which was a preprinted, optically readable version of a questionnaire used by the Swedish National Food Administration and Statistics Sweden in a national dietary survey in 1989 (Becker, 1994). The record book contained preprinted options for foods and dishes commonly eaten and also space for recording foods and snacks not included in the preprinted list. The intake of foods and nutrients was calculated using the food-composition database of the Swedish National Food Administration, version 1/99 (PC Kost version 1/99, Swedish National Food Administration, Uppsala, Sweden) and the software program SAS (SAS Institute Inc, Cary, NC, USA). The intake of dairy fat was calculated as the sum of the amount of fat from milk, yoghurt, cream, cheese, ice-cream and butter.

Physical activity

Physical activity during the previous year was recorded in the interview. The subjects were categorised into four levels of physical activity at work – very light (e.g. working at a computer most of the day), light (e.g. light industrial work, sales clerk, office work that comprises light activities), moderate (e.g. cleaners, kitchen staff, mail carriers who walk or bicycle) and heavy (heavy industrial work, building workers, farmers) – and five levels of physical activity during leisure time: very light (almost no activity at all), light (sporadically walking, non-strenuous cycling, garden work), moderate (regular activity at least once a week, e.g. walking, cycling, gardening work, or walking to work 10-30 min per day), active (regular activities more than once a week, e.g. intensive walking or cycling, sports activities) and very active (strenuous activities several times a week). Based on this, the physical activity level (PAL, the ratio of energy expenditure to BMR) was systematically estimated for each individual on a scale ranging from 1·4 to 2·3 (Rosell *et al.* 2003*a*). The subjects were divided into approximate thirds of physical activity level: low (PAL $<$ 1·7), moderate (PAL $=$ 1·7) and high (PAL $>$ 1·7).

Classification of under-reporters

The classification of URs and the validation of the food record have been reported in detail elsewhere (Rosell *et al.* 2003*a*). We identified URs according to the Goldberg cut-off (Black, 2000*a*,*b*), which compares the reported energy intake with the energy expenditure, both expressed as multiples of the BMR. BMR was estimated from equations based on age, sex and body weight (Department of Health, 1991). Individually estimated PAL values were used in the equation.

Fatty acids in serum phospholipids and adipose tissue

Serum and AT samples were stored up to one year at −70 °C until the analyses. A 1 ml sample of serum was prepared for the analyses: the serum lipids were extracted by adding 5 ml methanol, 10 ml chloroform containing 0·005 % butylated hydroxytoluene as an antioxidant, and 15 ml sodium dihydrogen phosphate (NaH_2PO_4, 0·2 mmol/l). The sample was mixed and left at +4°C overnight. The lower layer, consisting of chloroform and lipids, was removed and evaporated to dryness under nitrogen. The lipids were separated by TLC. The fatty acids in the PL were methylised by adding 2 ml 5 % H_2SO_4-methanol and left at +60°C overnight. The remaining methanol was removed by adding 1·5 ml distilled water and 3 ml petroleum ether containing 0·005 % butylated hydroxytoluene. The sample was centrifuged for 10 min; the ether phase was pipetted off and evaporated under nitrogen. The fatty acids were redissolved in 0·5 ml hexane and then analysed by GLC.

The samples of AT were prepared as described above except that no TLC was performed.

The fatty acid compositions were analysed by GLC on a 25 m wall-coated open tubular glass capillary column coated with SLP OV-351 (Quadrex, New, CT, USA), using He as a carrier gas. A Hewlett-Packard system (Avondale, PA, USA) consisting of GLC 5890, integrator 3396 and auto-sampler 7671A was used. The temperature was programmed to 100–210°C. The fatty acids were identified using standards from Nu Check Prep (Elysian, MN, USA). The amounts of fatty acids were expressed as the percentage of the sum of the fatty acids that were analysed.

Statistics

Data are presented as means and standard deviations, or, for skewed distributions, as medians and 25th and 75th percentiles. Tests for differences between URs and non-URs were performed using the Student's *t* test or, for skewed distributions, the Mann–Whitney U test. Associations between the relative contents of the fatty acids in PL and AT and dairy fat intake and other factors were examined using Spearman correlation and linear regression. The differences between the physical activity groups regarding the relative content of the fatty acids in PL and AT and the intake of dairy fat are presented as 95 % confidence intervals, using the low physical activity group as a reference. Trend analyses were performed using Spearman correlation. Statistical significance was defined as $P<0{\cdot}05$. The analyses were carried out using SPSS software (release 10.0.5, SPSS Inc. Chicago, IL, USA) and SAS (release 8.01, SAS Institute Inc. Cary, NC, USA).

Results

Thirty-nine per cent (*n* 88) of the subjects were classified as URs. The URs had significantly lower intakes of energy, dairy fat (g/100 g fat intake) and total fat (percentage of energy), and a higher percentage of energy from protein and alcohol and intake of fibre, than the non-URs (Table 1). A larger proportion of URs than non-URs were classified as having high physical activity.

The relative content of the fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT were each significantly correlated with the intake of dairy fat (g/100 g fat intake), the correlation coefficients ranging from 0·23 to 0·52 (Table 2). In addition, all fatty acids except for 14 : 0 in PL were inversely related to the intake of alcohol (expressed as percentage of energy), the correlation coefficients ranging from −0·25 to −0·52. When the absolute amount of alcohol intake (g/d) was used instead, almost identical correlation coefficients were obtained (data not shown). There were no associations between the fatty acids and the percentage of energy from protein and carbohydrates except for 17 : 0 in PL, where positive correlations were seen. The intake of fibre was positively correlated with 17 : 0 in PL, and with 15 : 0 in PL and in AT. The exclusion of URs did not markedly affect the results; generally, the correlations were similar or somewhat stronger in the non-URs compared with the whole group. Since 14 : 0, 15 : 0 and 17 : 0 may also be present in fish and meat products, we adjusted the results for intake of these foods; this did not, however, noticeably affect the correlations presented in Table 2.

Using a linear regression model for predicting the relative content of 14 : 0, 15 : 0 and 17 : 0 in PL and AT, the intakes of dairy fat and alcohol were independently related to all fatty acids except for 14 : 0 in PL, which was not associated with alcohol intake (Table 3). The R^2 values for the models ranged from 0·16 to 0·36. The R^2 values were similar or somewhat stronger when the URs were excluded from the analyses. As shown in Table 4, the relative content of the fatty acid 17 : 0 in PL was also associated with the intakes of fibre and protein. The R^2 value for the model increased from 0·42 to 0·52 when the URs were excluded.

The relative content of the fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT was approximately 5 % higher in the high physical activity group compared with the low physical activity group (Fig. 1). Statistically significant trends were, however, only seen for 14 : 0 in PL and 17 : 0 in AT. These associations were independent of the intakes of dairy fat and myristic acid, which did not differ between the physical activity groups regardless of whether the URs were excluded or not (data not shown). Physical activity was unrelated to the intake of alcohol (data not shown).

Discussion

The difficulties in obtaining reliable data on habitual diet are well known. The use of fatty acids in human fluids or tissues as

Table 1. Characteristics of the participants in the whole group (All) and in subgroups of under-reporters (URs) and non-under-reporters (non-URs)*

	All		URs		non-URs		*P* value†
n	301		88		213		
Dietary intake							
Energy (MJ/d)	9·5	(2·0)	7·5	(1·1)	10·3	(1·7)	<0·001
Dairy fat (g/100 g)	30	(14)	27	(15)	31	(14)	0·030
Fat (E%)	34	(5·5)	32	(5·0)	35	(5·5)	<0·001
Protein (E%)	16	(2·1)	17	(2·2)	16	(1·9)	<0·001
Carbohydrates (E%)	44	(6·1)	44	(6·2)	44	(6·0)	0·89
Fibre (g/10 MJ)	21·2	(5·9)	22·3	(6·5)	20·7	(5·6)	0·038
Alcohol (E%)	4·4	(2, 8)	4·9	(3, 10)	3·8	(2, 7)	0·025
Physical activity							
Low (%)	29	(*n* 88)	27	(*n* 24)	30	(*n* 64)	
Medium (%)	35	(*n* 106)	27	(*n* 24)	39	(*n* 82)	0·054‡
High (%)	36	(*n* 107)	45	(*n* 40)	32	(*n* 67)	

* Values are means (SD) except for alcohol intake, for which values are medians (25th and 75th percentiles). The proportions of subjects in the physical activity groups are presented as percentages (number of subjects).
† *P* values for difference between URs and non-URs were derived from the Student's *t* test or Mann–Whitney U test.
‡ Fisher's Exact test for difference in physical activity between URs and non-URs.
E% Percentage energy intake

Table 2. Correlations between the relative contents of 14 : 0, 15 : 0 and 17 : 0 in serum phospholipids (PL) and adipose tissue (AT) and the intake of fats, protein, carbohydrates, fibre and alcohol[†]

	Fatty acids in PL (%)			Fatty acids in AT (%)		
	14 : 0	15 : 0	17 : 0	14 : 0	15 : 0	17 : 0
All[‡]						
Dairy fat (g/100 g)	0·27***	0·43***	0·23***	0·47***	0·52***	0·30***
Fat (E%)	0·12*	0·23***	0·07	0·21***	0·21***	0·17*
Protein (E%)	0·00	0·09	0·21***	−0·01	0·07	0·13[§]
Carbohydrates (E%)	0·01	0·16**	0·29***	0·04	0·07	0·04
Fibre (g/10 MJ)	0·08	0·20**	0·27***	0·05	0·14*	0·05
Alcohol (E%)	−0·10	−0·44***	−0·52***	−0·26***	−0·29***	−0·25***
Non-URs[§]						
Dairy fat (g/100 g)	0·35***	0·51***	0·24***	0·45***	0·55***	0·32***
Fat (E%)	0·15**	0·30***	0·08	0·20**	0·23**	0·22**
Protein (E%)	0·01	0·11	0·27***	0·03	0·09	0·09
Carbohydrates (E%)	−0·06	0·09	0·26***	0·07	0·05	0·06
Fibre (g/10 MJ)	−0·01	0·17***	0·31***	0·09	0·14*	0·03
Alcohol (E%)	−0·09	−0·45***	−0·53***	−0·30***	−0·28***	−0·29***

$^{*}P<0·05$, $^{**}P<0·01$, $^{***}P<0·001$.
[†] Data are presented as Spearman correlation coefficients
[‡] The whole group; *n* 299 (PL) and *n* 297 (AT).
[§] Non URs, non-under-reporters; *n* 211 (PL) and *n* 210 (AT).
E% Percentage energy intake.

Table 3. Linear regression models predicting the relative contents of 14 : 0, 15 : 0 and 17 : 0 in serum phospholipids (PL) and adipose tissue (AT)

		All[*]			Non-URs[†]		
Dependent variable	Predictors	Beta[‡]	*P*	R^2	Beta[§]	*P*	R^2
14 : 0 in PL	Dairy fat intake (g/100 g)	0·29	<0·001	0·09	0·35	<0·001	0·12
	Alcohol intake (E%)	−0·09	0·12		−0·02	0·81	
15 : 0 in PL	Dairy fat intake (g/100 g)	0·32	<0·001	0·28	0·32	<0·001	0·29
	Alcohol intake (E%)	−0·41	<0·001		−0·39	<0·001	
17 : 0 in PL	Dairy fat intake (g/100 g)	0·16	0·001	0·36	0·14	0·009	0·44
	Alcohol intake (E%)	−0·58	<0·001		−0·63	<0·001	
14 : 0 in AT	Dairy fat intake (g/100 g)	0·42	<0·001	0·23	0·39	<0·001	0·23
	Alcohol intake (E%)	−0·22	<0·001		−0·23	<0·001	
15 : 0 in AT	Dairy fat intake (g/100 g)	0·47	<0·001	0·31	0·48	<0·001	0·32
	Alcohol intake (E%)	−0·27	<0·001		−0·24	<0·001	
17 : 0 in AT	Dairy fat intake (g/100 g)	0·28	<0·001	0·16	0·29	<0·001	0·20
	Alcohol intake (E%)	−0·27	<0·001		−0·31	<0·001	

[*] The whole group; *n* 299 (PL) and *n* 297 (AT).
[†] Non-URs, non-under-reporters; *n* 211 (PL) and *n* 210 (AT).
[‡] Standardized beta coefficients.
[§] R^2 values for the models with both predictors included.
E% Percentage energy intake.

biological markers for dietary intake may therefore be a valuable complement or alternative to conventional dietary assessments in epidemiological studies. Myristic acid (14 : 0), pentadecanoic acid (15 : 0) and heptadecanoic acid (17 : 0) are characteristic of dairy fat, and the relative content of these fatty acids in serum lipids and AT has previously been shown to be correlated with dairy fat intake in both men and women (Wolk *et al.* 1998, 2001). The fatty acids 15 : 0 and 17 : 0 contain an uneven number of carbon atoms and cannot be synthesised in the human body, which further supports their possible use as biological markers. In the current study, we investigated other determinants of these fatty acids and found that the relative contents of the fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT were, besides being associated with the intake of dairy fat, also associated with alcohol intake and physical activity. We attempted to take errors derived from under-reporting into consideration by identifying URs, and the data were analysed with and without them. When the URs were excluded from the analyses, the results were similar or somewhat more pronounced compared with the results for the whole group, which strengthens the conclusions of this study.

Biological markers of protein, Na and K in 24 h urine sampling verified a lower validity of the dietary data in the URs compared with the non-URs (Rosell *et al.* 2003*a*). The different proportions of energy from macronutrients in the URs and non-URs is in agreement with findings in other studies (Pryer *et al.* 1997; Goris *et al.* 2000; Lafay *et al.* 2000) and indicates that the under-reporting is selective and affects different kinds of foods. Although the URs tended to be classified as being more physically active than the non-URs, there was a fairly large and evenly distributed number of participants in the three physical activity groups in the non-URs, suggesting

Table 4. Linear regression models predicting the relative content of 17 : 0 in serum phospholipids (PL)

		All*			Non-URs†		
Dependent variable	Predictors	Beta‡	*P*	R^2	Beta§	*P*	R^2
17 : 0 in PL	Dairy fat intake (g/100 g)	0·23	< 0·001	0·42	0·19	0·001	0·52
	Alcohol intake (E%)	−0·46	< 0·001		−0·54	< 0·001	
	Carbohydrates (E%)	0·07	0·28		0·02	0·74	
	Protein intake (E%)	0·16	0·001		0·19	< 0·001	
	Fibre intake (g/10 MJ)	0·17	0·003		0·20	0·001	

* The whole group; *n* 299 (PL) and *n* 297 (AT).
† Non-URs, non-under-reporters; *n* 211 (PL) and *n* 210 (AT).
‡ Standardised beta coefficients.
§ R^2-values for the models with all predictors included.
E% Percentage energy intake.

that possible associations between dietary intake and physical activity would be detected even if the URs were excluded.

The correlations between the intake of dairy fat and the relative contents of the fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT seen in our study were similar to the findings reported by Wolk *et al.* (1998, 2001). These results have been presented and discussed in detail elsewhere (Rosell *et al.* 2004). The objective of the current study was to explore other determinants of these fatty acids. We found that all three fatty acids in PL and AT, except for 14 : 0 in PL, were inversely correlated with the intake of alcohol, independently of dairy fat intake, these two nutrients explaining between 20 % and 44 % of the variance in the proportions of the fatty acids in PL and AT. The fatty acid 17 : 0 in PL was also associated with the intakes of protein and fibre. The fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT may also be present in fish and meat, but intakes of these foods did not affect the results in our study. In Sweden, the intake of milk products is high and the intake of fish relatively low. In another Swedish study, the correlation between dairy fat intake and the fatty acids in serum did not change when the intake of ruminant meat was taken into account (Wolk *et al.* 1998).

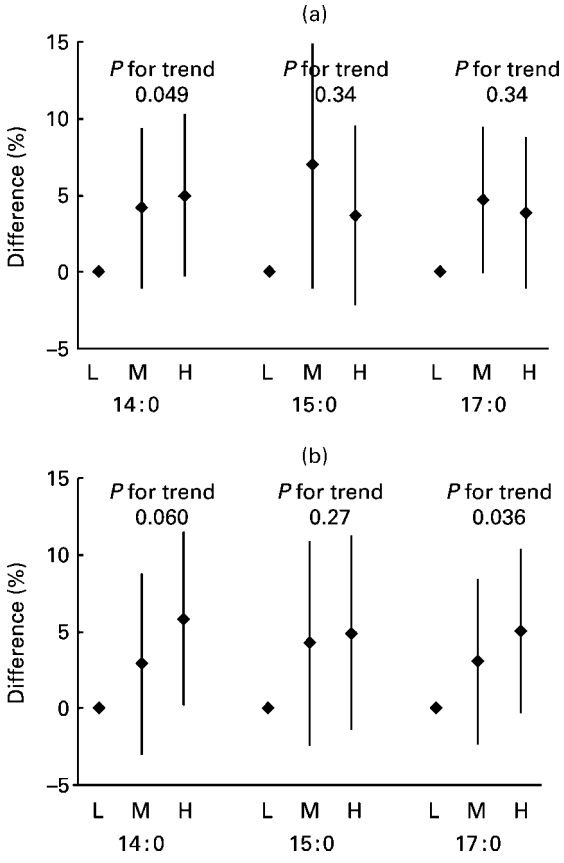

Fig. 1. Differences in the proportions of fatty acids 14 : 0, 15 : 0 and 17 : 0 in serum phospholipids (PL;a) and adipose tissue (AT;b) between the low (L), medium (M) and high (H) physical activity groups. Data are presented as percentage differences with 95% CI, using the low physical activity group as a reference. The analyses are based on the whole group; *n* 299 (PL) and *n* 297 (AT).

The biological mechanisms underlying the associations between alcohol and the other dietary factors and the fatty acids in PL and AT are unclear. The consumption of alcohol, as well as fibre, may be related to lifestyle factors that may influence fatty acid composition. These factors may differ between populations, and the findings in our study should therefore be generalised with caution. However, alcohol intake has a marked influence on the metabolism of lipoproteins, and its HDL-cholesterol-raising effect is well known. Alcohol intake interferes with enzymes that are involved in the metabolism of fatty acids (Hannuksela *et al.* 2002), which may be part of the explanation for its association with fatty acid composition in body tissues.

It should also be remembered that the fatty acids are expressed as relative amounts (as proportions of the total amount of fatty acids). As a result, if the relative content of one fatty acid increases, the relative content of one or several other fatty acids will decrease. Levels of many of the fatty acids are highly correlated with each other, and the effects of the intake of alcohol, protein and fibre on fatty acids 14 : 0, 15 : 0 and 17 : 0 may therefore be mediated by alterations in other fatty acids in PL and AT.

Although this cross-sectional study did not allow us to explore the mechanisms behind the associations between the dietary factors and the fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT, the findings are still relevant for studies using these fatty acids as markers of dairy fat intake. The results indicate that the fatty acids reflect the intake not only of dairy fat but also of alcohol and, for 17 : 0 in PL, protein and fibre. This information may improve the use and interpretation of these fatty acids since possible relationships between these fatty acids and outcome variables can be adjusted for possible confounders.

The other, non-dietary, determinant of the relative contents of 14 : 0, 15 : 0 and 17 : 0 in PL and AT that we investigated was physical activity. Although each fatty acid was positively associated with physical activity, independent of the intake of dairy fat and alcohol, significant trends were only seen for 14 : 0 in PL and

17 : 0 in AT. Few studies have investigated the associations between physical activity and fatty acid compositions in PL and AT, and, to our knowledge, no study has examined the fatty acids 15 : 0 and 17 : 0. In one study including 20 men, the relative content of the fatty acid 14 : 0 in subcutaneous gluteal fat was found to increase after four months of physical training (Sutherland *et al.* 1981). There are also reports demonstrating that the release of fatty acids from AT is related to the length and number of double bonds in the molecular structure of the fatty acid (Halliwell *et al.* 1996; Raclot *et al.* 1997; Yli-Jama *et al.* 2001). This is a mechanism that could mediate the effects of physical activity on fatty acid composition in AT. Again, since the fatty acids are expressed in relative amounts, the findings in our study could be mediated by alterations in other fatty acids in PL and AT.

Our study is limited by the cross-sectional design and by the relatively crude classification of physical activity groups based on self-reported data. To investigate the effects of physical activity (type, amount and frequency) and the mechanisms behind these possible associations controlled intervention studies are needed. The influence of physical activity on the composition of fatty acids in AT and serum may also be of interest in studying possible mechanisms behind the beneficial health effects of physical activity. For example, fatty acid composition in serum has been found to be related to insulin sensitivity (Vessby, 2000).

In this study, the male participants were of similar age. However, age, sex and changes in body weight may affect fatty acid composition as well and should therefore be considered when fatty acids in body tissues are used as markers for dietary intake in epidemiological studies (Nikkari *et al.* 1995; Bolton-Smith *et al.* 1997; Arab, 2003). Even though some fatty acids can only be obtained via the diet, these fatty acids can still be converted and metabolised in the body, and since they also are expressed as relative amounts, the fatty acids are not simply markers for dietary intake. As long as we do not fully understand the determinants of the relative content of the fatty acids in body tissues, they should be interpreted with caution as biological markers for dietary intake. Our study indicates that the relative contents of the fatty acids 14 : 0, 15 : 0 and 17 : 0 in PL and AT, which may be used as biological markers for dairy fat intake, were also associated with alcohol intake and possibly with physical activity independent of the intake of dairy fat. The findings suggest that adjustments should be made for alcohol intake when these fatty acids are used as markers of dairy fat intake.

Acknowledgements

We thank Merja Heinonen for clinical assistance, Siv Tengblad for performing the fatty acid analyses in serum and adipose tissue, and Paul Appleby for reading and checking the manuscript. This study was supported by grants from the Swedish Council for Working Life and Social Research, the Swedish Heart–Lung Foundation, the Swedish Society for Medicine, the Swedish Research Council, the Swedish Nutrition Foundation and the Swedish Dairy Association.

References

Andersson A, Sjodin A, Olsson R & Vessby B (1998) Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. *Am J Physiol* **274**, E432–E438.

Arab L (2003) Biomarkers of fat and fatty acid intake. *J Nutr* **133**, S925–S932.

Becker W (1994) *Food habits and nutrient intake in Sweden 1989* [in Swedish]. Uppsala, Sweden: National Food Administration.

Beyen AC & Katan MB (1985) Rapid sampling and long-term storage of subcutaneous adipose tissue biopsies for determination of fatty acid composition. *Am J Clin Nutr* **42**, 317–322.

Bingham SA, Cassidy A, Cole TJ, *et al.* (1995) Validation of weighed food records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *Br J Nutr* **73**, 531–550.

Black AE (2000*a*) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes* **24**, 1119–1130.

Black AE (2000*b*) The sensitivity and specificity of the Goldberg cut-off for EI:BMR for identifying diet reports of poor validity. *Eur J Clin Nutr* **54**, 395–404.

Black AE, Prentice AM, Goldberg GR, Jebb SA, Bingham SA, Livingstone MBE & Coward WA (1993) Measurements for total energy expenditure provide insights into the validity of dietary measurements of energy intake. *J Am Diet Assoc* **93**, 572–579.

Bolton-Smith C, Woodward M & Tavendale R (1997) Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. *Eur J Clin Nutr* **51**, 619–624.

Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects*. London: HSMO.

Goris AHC, Westerterp-Plantenga MS & Westerterp KR (2000) Undereating and underrecording of habitual food intake in obese men: selective under-reporting of fat intake. *Am J Clin Nutr* **71**, 130–134.

Halliwell KJ, Fielding BA, Samra JS & Humphreys SM (1996) Release of individual fatty acids from human adipose tissue in vivo after an overnight fast. *J Lipid Res* **37**, 1842–1848.

Hannuksela ML, Liisanantt MK & Savolainen MJ (2002) Effect of alcohol on lipids and lipoproteins in relation to atherosclerosis. *Crit Rev Clin Lab Sci* **39**, 225–283.

Lafay L, Mennen L, Basdevant A, Charles MA, Borys JM, Eschwege E & Romon M (2000) Does energy intake under-reporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Sante (FLVS) study. *Int J Obes* **24**, 1500–1506.

Macdiarmid JI, Vail A, Cade JE & Blundell JE (1998) The sugar-fat relationship revised: difference in consumption between men and women of varying BMI. *Int J Obes* **22**, 1053–1061.

Mann JI (2002) Diet and risk of coronary heart disease and type 2 diabetes. *Lancet* **360**, 783–789.

Nikkari T, Luukkainen P, Pietinen P & Puska P (1995) Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. *Ann Med* **27**, 491–498.

Pfeuffer M & Schrezenmeir J (2000) Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. *Br J Nutr* **85**, Suppl. 1, S155–S159.

Poppitt SD, Swann D, Black AE & Prentice AM (1998) Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Int J Obes* **22**, 303–331.

Pryer AJ, Vrijheid M, Nochols R, Kiggins M & Elliott P (1997) Who are the 'low engery reporters' in the dietary and nuritional survey of British adults? *Int J Epidemiol* **26**, 146–154.

Raclot T, Langin D, Lafontan M & Groscolas R (1997) Selective release of human adipocyte fatty acids according to molecular structure. *Biochem J* **324**, 911–915.

Rosell M, Hellenius M-L, de Faire U, Berglund L, Gustafsson I-B & Johansson G (2003*b*) The contribution of a manually coded part in a optically readable, precoded 7-day food record to the intakes of energy, nutrients and foods. *Scand J Nutr* **47**, 123–131.

Rosell MS, Hellenius M-LB, de Faire U & Johansson GK (2003*a*) The associations between diet and the metabolic syndrome vary with the validity of the dietary data. *Am J Clin Nutr* **78**, 84–90.

Rosell M, Johansson G, Berglund L, Vessby B, de Faire U &

Hellenius M-L (2004) Associations between the intake of dairy fat and calcium and abdominal obesity. *Int J Obes* (in the press).
Sutherland W, Woodhouse S & Heyworth M (1981) Physical training and adipose tissue fatty acid composition in men. *Metabolism* **30**, 839–844.
Vessby B (2000) Dietary fat and insulin action in humans. *Br J Nutr* **83**, Suppl. 1, S91–S96.
Wolk A, Furuheim M & Vessby B (2001) Fatty acid composition of adipose tissue and serum lipids are valid biological markers of dairy fat intake in men. *J Nutr* **131**, 828–833.
Wolk A, Vessby B, Ljung H & Barrefors P (1998) Evaluation of a biological marker of dairy fat intake. *Am J Clin Nutr* **68**, 291–295.
Yli-Jama P, Haugen TS, Rebnord HM, Ringstad J & Pedersen JI (2001) Selective mobilisation of fatty acids from human adipose tissue. *Eur J Intern Med* **12**, 107–115.