

## PAPER

# Associations between the intake of dairy fat and calcium and abdominal obesity

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**OBJECTIVE:** This study evaluates the association between abdominal obesity and the intake of dairy fat and calcium using information from dietary data and the relative content of the fatty acids 14:0, 15:0, and 17:0 in serum phospholipids (PL) and adipose tissue (AT), which are suggested biological markers for dairy fat intake. This study also explores how the associations were affected when under-reporters (URs) were separated from the analyses.

**DESIGN:** Cross-sectional study.

**SUBJECTS:** In all, 301 healthy 63-y-old men with different degrees of fasting-insulin concentrations.

**METHODS:** Sagittal abdominal obesity (SAD), dietary intake assessed by a 7-day food registration, and the fatty acid composition in serum PL and AT were measured. URs ( $n=88$ ) and non-under-reporters (non-URs,  $n=213$ ) were identified by Goldberg's equation, which compares energy intake with energy expenditure, both expressed as multiples of the basal metabolic rate.

**RESULTS:** The intake of dairy fat, expressed as g/100 g fat, was inversely correlated with SAD; however, this association was only observed in the URs ( $r=-0.36$ ,  $P=0.001$ ) and not in the non-URs ( $r=-0.04$ ,  $P=0.59$ ). The intake of calcium was inversely correlated with SAD in both groups, although the association was weaker in the non-URs. The intake of dairy fat was related to the relative content of the fatty acids 14:0, 15:0, and 17:0 in serum PL and AT ( $r$  ranging between 0.32 and 0.55). When these fatty acids were correlated to SAD, inverse associations were seen except for 14:0 in PL ( $r$  ranging between  $-0.17$  and  $-0.29$ ).

**CONCLUSION:** If there is a true inverse association between the intake of dairy fat and SAD, it remains to explain why this association was not seen in the non-URs. The data gave some indications of an inverse association between SAD and the intake of calcium. The diverse findings observed when the URs and non-URs were separated highlight the question of how to use and interpret dietary data in URs when diet-disease relationships are investigated.

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## Introduction

The increasing prevalence of obesity world-wide and the development of related disorders such as type 2 diabetes and cardiovascular diseases are a serious threat to public health.<sup>1</sup> There is a growing interest in the metabolic syndrome, a condition defined when several metabolic disturbances occur simultaneously such as abdominal obesity, insulin resistance, hypertension, and dyslipoproteinemia with high

levels of triglycerides and low HDL cholesterol.<sup>2</sup> The most important target of the prevention of obesity and the metabolic syndrome is to achieve lifestyle changes. Although it is agreed that the food habits play a significant role, the importance of different dietary components is not fully understood.

Dairy products like butter, cheese, milk products, and cream comprise a considerable part of the western diet. They represent a major source for the intake of saturated fat, which is regarded as a risk factor for cardiovascular disease and type 2 diabetes.<sup>3</sup> On the other hand, they also contribute to the intake of calcium and other nutrients that may mediate beneficial health effects.<sup>4</sup> Some cross-sectional studies report inverse relationships between the intake of

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dairy fat or dairy products and the metabolic syndrome or single parameters of the syndrome.<sup>5-7</sup> In a 10-y follow-up study, a decreased cumulative incidence of the metabolic syndrome was found in subjects with a high intake of dairy products compared with subjects with a low intake.<sup>8</sup> However, this inverse relationship was seen only in subjects with a body mass index  $\geq 25$ . In a 10-y follow-up of girls during the adolescent period, no association was seen between BMI and the intake of dairy products or calcium.<sup>9</sup>

It is well known that it is difficult to retrieve dietary data that represent what people usually eat.<sup>10</sup> Under-reporting is a common phenomenon that seems to occur nonrandomly in populations<sup>11-13</sup> and affect different kind of foods selectively.<sup>14,15</sup> Since errors in dietary data might be differential, uncertainties in dietary data may not ensure that an obtained association between diet and disease variables reflects a true relation. Biological markers for dietary intake are attractive alternatives, since they provide estimates of the dietary intake independent of information from the individuals of the dietary intake. Wolk *et al*<sup>16,17</sup> have suggested that the relative content of myristic acid (14:0), pentadecanoic acid (15:0), and possible heptadecanoic acid (17:0) in serum phospholipids (PL) and adipose tissue (AT) can be used as markers for long-term intake of dairy fat. In a case-control study, the proportion of these fatty acids in AT was lower among cases of myocardial infarction than controls,<sup>18</sup> which indicates that the intake of milk could have a protective effect.

This study evaluates the association between abdominal obesity and the intake of dairy fat and calcium in a sample of 301 healthy 63-y-old men, using information from both dietary data and the relative content of fatty acids in S-PL and AT. We also intended to explore how under-reporting might influence the results.

## Methods

### Study sample

The subjects were recruited from a cohort of men and women who had attended a health screening study in 1997-1999. Every third 60-y-old person in Stockholm County was invited and 78% ( $n = 4232$ ) agreed to take part. The healthy men in the cohort who fulfilled the criteria for the current study (born in Sweden, no diagnoses of cardiovascular disease, and no pharmacological treatment of diabetes, hypertension or hypercholesterolemia, a body mass index between 20 and 35, and no other serious disease,  $n = 995$ ) were divided into three groups by the tertiles of their fasting-insulin concentrations. Requests to participate in a study concerning diet and the metabolic syndrome were continually sent until the number of positive responders reached approximately 100 in each group. This classification was used only in order to recruit subjects with a wide range of insulin levels and not used for the analyses in this paper. The study was conducted between March 2000 and October 2001

and the participation rate was 71% (77, 73 and 64% in the first, second, and third tertile, respectively). Two men did not complete the 7-day food record and were therefore excluded, leaving 301 men aged 63 y (range 61.8-64.2 y) for this study. The ethical committee at Karolinska Institutet approved the study.

### Clinical procedures

The participants visited the Karolinska Hospital in the fasting state in the morning. A research nurse measured the body weight to the nearest 0.1 kg and height to the nearest 0.5 cm. BMI was computed by dividing weight (kg) with the square of height ( $m^2$ ). The sagittal abdominal diameter (SAD) was determined to the nearest 0.1 cm using a ruler and a water level, with the participant lying horizontally with legs straight. A needle biopsy of subcutaneous AT was taken from the left upper buttock,<sup>19</sup> using a  $1.2 \times 50 \text{ mm}^2$  needle. An anesthetic cream (EMLA 5% (Astra), containing 2.5% lidocain and 2.5% trilocain) was applied on the skin for 20-30 min before the biopsy was taken. Information about medications, smoking, and physical activity was recorded during a structured interview. Written and oral instructions of how to fill in a 7-day food record and how to collect a 24-h urine sample were given individually by a nutritionist (MR). The participants were told not to change their eating habits during the study. After approximately 1 week, the participant returned to the hospital 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.<sup>20</sup> The 7-day food record was an optically readable version of a questionnaire used by the Swedish National Food Administration and Statistics, Sweden in a national dietary survey in 1989.<sup>21</sup> The booklet contained preprinted alternatives for commonly eaten food items and meals. The participant estimated the amount of food eaten using household measures (eg servings, glassfuls, cupfuls, and spoonfuls). Eight photos were used to estimate portion sizes of cooked food and the amount of fat spread on bread. The record also contained space for recording foods and snacks other than those included in the printed list. For the present study, the food record was slightly modified: we added two extra pages for recording in-between meals, resulting in a total of three pages for in-between meals placed after the respective sections for breakfast, lunch, and dinner. Originally, the preprinted food items for in-between eating were coffee, tea, sugar, and water. In the new version, we also added buns and biscuits. Small adjustments were also made in order to match the codes of the preprinted food items with a later version of the food-composition database and the predefined portion sizes allocated in the data analyses were altered according to a validation study.<sup>22</sup>

The food quantities recorded in the space for free text were converted to grams by using a weight guide.<sup>23</sup> The intake of food and nutrients was calculated by using the food-composition database of the Swedish National Food Administration, version 1/99,<sup>24</sup> 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 products, cream, cheese, ice cream, and butter.

#### Urine collection and biological markers of food intake

The participants collected one 24-h urine sample at the start of the food registration.<sup>25</sup> We used the PABA-check method to verify that the urine collection was carried out properly.<sup>26</sup> In the collections with a PABA recovery of 50–85% ( $n=46$ ), the contents of nitrogen, sodium, and potassium were adjusted according to Johansson *et al.*<sup>27</sup> Nitrogen content in 24-h urine was used as a biological marker for dietary intake of protein.<sup>28</sup> The nitrogen content in urine was converted to dietary protein by multiplying by 7.72. The sodium content in urine was used as a biological marker of dietary intake of sodium.<sup>29</sup> The biological marker for potassium intake was taken as the potassium content in urine divided by 0.77.<sup>29</sup>

#### Classification of under-reporters (URs)

The classification of the URs and non-under-reporters (non-URs) and the validation of the food record have been reported elsewhere.<sup>25</sup> Briefly, we identified the URs according to the Goldberg cutoff, which compares the reported energy intake (EI) with the energy expenditure (EE), both expressed as multiples of the basal metabolic rate (BMR).<sup>30–32</sup> The latter is referred to as the physical activity level (PAL). The Goldberg equation follows:

$$EI : BMR = PAL \times \exp[-2 \times (S/100)] / \sqrt{n}$$

where  $S = (CV_{WEI}^2/d + CV_{WB}^2 + CV_{TP}^2)^{1/2}$  and  $n$  are the number of subjects.

The  $S$  factor takes into account the number of assessments days ( $d=7$ ) and the variations in EI, BMR, and energy requirements. The coefficients of variation used were  $CV_{WEI}=23\%$ ,  $CV_{WB}=8.5\%$ , and  $CV_{TP}=15\%$ .<sup>31</sup> Physical activity during the latest year was recorded during the interview, where the subjects were categorized into four levels of physical activity at work and five levels of physical activity during leisure time. Based on this, the PAL was systematically estimated for each individual on a 0.1 scale<sup>25</sup> and used in the Goldberg equation. The URs were defined when  $FIL < \text{Goldberg cutoff}$  and the non-URs were defined when  $FIL \geq \text{Goldberg cutoff}$ .

#### Fatty acid composition in S-PL and AT

Serum and AT samples were stored up to 1 y in  $-70^\circ\text{C}$  until the analyses. A measure of 1 ml of serum was prepared for the

analyses as follows: the serum lipids were extracted by adding 5 ml of methanol, 10 ml chloroform containing 0.005% butylated hydroxytoluene as an antioxidant, and 15 ml sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ , 0.2 mmol/l). The sample was mixed and left at  $+4^\circ\text{C}$  overnight. The under layer, consisting of chloroform and lipids, was removed and evaporated to dryness under nitrogen. The different sorts of lipids were separated by thin-layer chromatography. The fatty acids in the PL were methylated by adding 2 ml 5%  $\text{H}_2\text{SO}_4$ -methanol and left at  $+60^\circ\text{C}$  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 at  $1500 \times g$  for 10 min; the ether phase was pipetted off and evaporated under nitrogen. The fatty acids were redissolved in 0.5 ml of hexane and then analyzed by gas liquid chromatography (GLC). The samples of AT were prepared as described above; however, no thin-layer chromatography was made.

The fatty acid compositions were analyzed by GLC on a 25-m WCOT (wall-coated open tubular) glass capillary column coated with SLP OV-351 (Quadrex, New, CT, USA), using helium as the carrier gas. A Hewlett-Packard system (Avondale, PA, USA) consisting of GLC 5890, integrator 3396, and autosampler 7671A was used. The temperature was programmed to  $100\text{--}210^\circ\text{C}$ , increasing by  $15^\circ\text{C}/\text{min}$ . The fatty acids were identified using standards from Nu Check Prep (Elysian, MN, USA). The amounts of fatty acids were given as the relative percentage of the sum of the fatty acids that were analyzed.

#### Statistics

The data are presented as means and standard deviations or, when skewed distributions, as medians and 25th and 75th percentiles. Tests for differences between URs and non-URs were performed using Student's  $t$ -test or, when skewed distributions, Mann-Whitney  $U$ -test. Associations between SAD and variables of dairy fat intake were examined using a Spearman correlation. Adjustment for possible confounders was performed using partial Spearman's correlation. To explore nonlinear relationships between dairy fat intake and SAD, we compared the Pearson's correlation coefficients using a nontransformed and a log-transformed variable of SAD, respectively. Differences between the correlations in the URs and non-URs were tested by applying Fisher's  $Z$  transformation on each coefficient and using a normal approximation to achieve the significance level. The analyses were carried out using SPSS software (release 10.0.5, SPSS Inc.) and SAS (release 8.01, SAS Institute Inc.).

#### Results

##### Characteristics of the study sample

The URs had higher weight, BMI, and SAD compared with the non-URs (Table 1). The reported intakes of total fat, dairy

**Table 1** Characteristics of the participants in the whole sample (All) and in subgroups of URs and non-URs<sup>a</sup>

	All	URs	Non-URs	P-value <sup>b</sup>
<i>n</i>	301		88	213
Body weight (kg)	82.3 (10.8)	84.6 (11.1)	81.4 (10.6)	0.021
BMI (kg/m <sup>2</sup> )	25.9 (3.1)	26.7 (3.0)	25.5 (3.0)	0.003
SAD (cm)	21.3 (2.3)	21.7 (2.3)	21.1 (2.2)	0.021
Energy intake (MJ/day)	9.5 (2.0)	7.5 (1.1)	10.3 (1.7)	<0.001
Total fat intake (g/day)	88 (28)	66 (14)	98 (27)	<0.001
Diary fat intake				
g/day	23 (15, 36)	16 (9.0, 24)	27 (18, 39)	<0.001
g/100 g fat intake	30 (14)	27 (15)	31 (14)	0.030
% of energy intake	9.7 (6.4, 13)	7.9 (4.6, 12)	10.2 (6.9, 14)	0.001
14:0 Myristic acid intake				
g/day	4.0 (3.1, 5.3)	3.0 (2.4, 3.7)	4.6 (3.6, 5.9)	<0.001
g/100 g fat intake	5.0 (1.0)	4.8 (1.0)	5.0 (0.9)	0.02
% of energy intake	1.7 (0.5)	1.5 (0.5)	1.8 (0.5)	<0.001
Calcium intake				
g/day	1.0 (0.8, 1.3)	0.8 (0.6, 1.0)	1.1 (0.9, 1.4)	<0.001
g/10 MJ	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	0.38
Fatty acids in PL (%)				
14:0	0.48 (0.09)	0.47 (0.09)	0.48 (0.09)	0.55
15:0	0.22 (0.05)	0.21 (0.04)	0.22 (0.06)	0.062
17:0	0.40 (0.06)	0.39 (0.07)	0.40 (0.06)	0.045
Fatty acids in AT (%)				
14:0	4.0 (0.8)	3.8 (0.7)	4.1 (0.8)	0.001
15:0	0.39 (0.09)	0.37 (0.07)	0.40 (0.09)	0.007
17:0	0.23 (0.04)	0.22 (0.04)	0.23 (0.04)	0.063

AT = adipose tissue; PL = phospholipids. <sup>a</sup>Data are presented as means (s.d.) or, when skewed distributions, as medians (25th, 75th percentiles). <sup>b</sup>P-value for difference between URs and non-URs based on Student's *t*-test or Mann-Whitney *U*-test.

fat, and myristic acid were lower in the URs, while the intake of calcium did not significantly differ after energy adjustment. The relative content of the fatty acids 17:0 in PL and 14:0 and 15:0 in AT were also lower in the URs compared to the non-URs.

The validity of the dietary data with regard to the intake of energy, protein, sodium, and potassium was lower in the URs than the non-URs and has been reported in detail elsewhere. The ratio of EI:basal metabolic rate was 1.05 (0.14) in the URs and 1.47 (0.24) in the non-URs. The ratios of protein<sub>diet</sub>:protein<sub>urine</sub>, sodium<sub>diet</sub>:sodium<sub>urine</sub>, and potassium<sub>diet</sub>:potassium<sub>urine</sub> were 0.77 (0.06), 0.83 (0.30), and 0.73 (0.18) in the URs, and 0.95 (0.21), 0.97 (0.31), and 0.89 (0.22) in the non-URs (*P*-value for difference between the URs and non-URs was <0.001 in all cases).<sup>25</sup>

#### Relation between dairy fat intake and fatty acids in PL and AT

The intake of dairy fat (g/100 g fat intake) was related to the relative contents of 14:0, 15:0, and 17:0 in PL and AT

**Table 2** Correlations between dairy fat intake and the relative content of the fatty acids 14:0, 15:0, and 17:0 in S-PL and AT<sup>a</sup>

Fatty acid (%)	Spearman's correlation to dairy fat intake (g/100 g fat)					
	All <sup>b</sup>		URs <sup>c</sup>		Non-URs <sup>d</sup>	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
14:0						
PL	0.27	<0.001	0.07	0.54	0.35	<0.001
AT	0.47	<0.001	0.47	<0.001	0.45	<0.001
15:0						
PL	0.43	<0.001	0.23	0.030	0.51	<0.001
AT	0.52	<0.001	0.38	<0.001	0.55	<0.001
17:0						
PL	0.23	<0.001	0.16	0.15	0.24	<0.001
AT	0.30	<0.001	0.22	0.043	0.32	<0.001

<sup>a</sup>Data are presented as Spearman's correlation coefficients (*r*) and *P*-values. <sup>b</sup>The whole group, *n* = 299 (PL) and *n* = 297 (AT). <sup>c</sup>URs, *n* = 88 (PL) and *n* = 87 (AT). <sup>d</sup>Non-URs, *n* = 211 (PL) and *n* = 210 (AT).

(Table 2). The correlation in 15:0 was stronger compared with 17:0, *P*-value for difference being <0.001 in both PL and AT. All correlations, except for 14:0 in AT, were strengthened when the URs group was excluded. Significantly different correlations between URs and non-URs were seen in 14:0 in PL (*P* = 0.022) and 15:0 in PL (*P* = 0.011). In the non-URs, the correlations were not affected when they were controlled for SAD (data not shown). In the URs, the correlations were weakened when controlling for SAD (data not shown).

#### Relation between dairy fat and calcium intake and SAD

When SAD was correlated to variables of reported dairy fat intake, inverse associations were seen in the URs but not in the non-URs (Table 3). Similar results were seen in the adjusted intakes of myristic acid; however, the correlations were weaker compared with dairy fat intake. When SAD was correlated to the energy-adjusted intake of calcium, inverse correlations were seen in both URs and non-URs. When the relation between SAD and dairy fat (g/100 g fat) was controlled for calcium intake (by using Spearman's partial correlation), the correlation coefficients were -0.06 (*P* = 0.28), -0.24 (*P* = 0.025), and 0.04 (*P* = 0.60) in All, URs and non-URs, respectively. When the relation between SAD and calcium (g/10 MJ) was controlled for dairy fat intake (g/100 g fat), the corresponding correlation coefficients were -0.18 (*P* = 0.002), -0.16 (*P* = 0.15), and -0.17 (*P* = 0.011).

Assuming that there was a true inverse association and that the validity of the reported intake of dairy fat was similar in the URs and the non-URs, one hypothesis that could explain why the inverse correlation was seen only in the URs and not in the non-URs is that the relation between dairy fat and SAD is present only in obese people but not in lean people (since the URs were more obese than the

**Table 3** Correlations between the dairy fat and calcium intake and SAD<sup>a</sup>

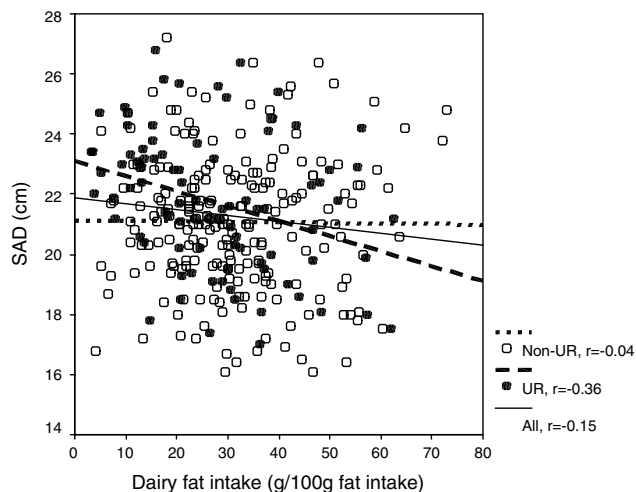
	Spearman's correlation to SAD					
	All <sup>b</sup>		URs <sup>c</sup>		Non-URs <sup>d</sup>	
Variables of dairy fat intake	r	P-value	r	P-value	r	P-value
Dairy fat intake						
g/day	-0.07	0.22	-0.21	0.054	0.04	0.55
g/100 g fat intake	-0.15	0.008	-0.36	0.001	-0.04	0.59
% of energy intake	-0.11	0.056	-0.30	0.005	0.00	0.99
14:0 Myristic acid intake						
g/day	-0.02	0.73	0.01	0.90	0.08	0.23
g/100 g fat intake	-0.17	0.003	-0.32	0.003	-0.07	0.32
% of energy intake	-0.07	0.25	-0.13	0.21	0.02	0.77
Calcium intake						
g/day	-0.11	0.053	-0.13	0.23	-0.04	0.54
g/10MJ	-0.22	<0.001	-0.31	0.003	-0.18	0.011

<sup>a</sup>Data are presented as Spearman's correlation coefficient (*r*) and *P*-values. <sup>b</sup>The whole group, *n* = 301. <sup>c</sup>URs, *n* = 88. <sup>d</sup>Non-URs, *n* = 213.

non-URs), that is, that SAD was an effect modifier and that the relation between SAD and the intake of dairy fat therefore is nonlinear. Using Pearson's correlation (which calculates a correlation coefficient for a linear relationship), the correlation coefficients between SAD and the intake of dairy fat (g/100 g fat intake) did not change when the logarithm of SAD was used compared to when the nontransformed SAD was used (data not shown). If there was a nonlinear relationship, the correlation would become stronger when SAD was transformed. Therefore, this test indicates that the difference between URs and non-URs was not explained by a nonlinear relationship. To test if the inverse correlations in the URs was related to the degree of under-reporting of energy, we controlled a variable computed as PAL minus the ratio EI:BMR. However, this did not affect the correlations (data not shown). Although SAD on average was somewhat higher in the URs, and the intake of dairy fat was lower, the plot of the relationship between SAD and the intake of dairy fat illustrates that the URs were still widely distributed in the sample (Figure 1). We also tested to split the study sample into two groups of BMI < 25 and ≥ 25 kg/m<sup>2</sup>. An inverse association between dairy fat intake (g/100 g fat intake) and SAD was seen in the lean subjects (*r* = -0.21, *P* = 0.016, *n* = 130), whereas no association was seen in the obese subjects (*r* = 0.05, *P* = 0.54, *n* = 171); however, *P*-value for difference in correlations between the two groups was 0.15.

**Relation between fatty acids in PL and AT and SAD**

When SAD was correlated to the relative content of 14:0, 15:0, and 17:0 in PL and AT, respectively, inverse associations were seen except in 14:0 in PL, where the relation was positive after adjustments (Table 4). The unadjusted correlation in 15:0 was weaker compared with 17:0, *P*-value for difference was 0.04 and 0.06 in PL and AT, respectively. In



**Figure 1** Plot of the intake of dairy fat (g/100 g fat) and SAD.

**Table 4** Correlations between the relative content of the fatty acids 14:0, 15:0, and 17:0 in S-PL and AT and SAD<sup>a</sup>

Fatty acid (%)	Spearman's correlation to SAD					
	Crude		Adjusted <sup>b</sup>		Adjusted <sup>c</sup>	
	r	P-value	r	P-value	r	P-value
14:0						
PL	0.09	0.13	0.13	0.026	0.18	0.002
AT	-0.30	<0.001	-0.27	<0.001	-0.22	<0.001
15:0						
PL	-0.22	<0.001	-0.19	0.001	-0.15	0.012
AT	-0.20	0.001	-0.17	0.004	-0.11	0.056
17:0						
PL	-0.30	<0.001	-0.29	<0.001	-0.27	<0.001
AT	-0.31	<0.001	-0.27	<0.001	-0.25	<0.001

<sup>a</sup>Data are presented as Spearman's correlation coefficient (*r*) and *P*-values, *n* = 299 (PL) and *n* = 297 (AT). <sup>b</sup>Adjusted for alcohol intake and physical activity. <sup>c</sup>Adjusted for alcohol intake, physical activity, and calcium intake.

the non-URs, the correlations remained unchanged when they were controlled for the intake of dairy fat (data not shown).

**Discussion**

This study explores the association between abdominal obesity and intake of dairy fat and calcium in a sample of 301 healthy 63-y-old men. As a measure of abdominal obesity, we used the SAD. Compared with other measures of abdominal obesity such as waist or waist:hip ratio, the SAD has been shown to have highest reliability in both overweight and lean persons,<sup>33</sup> and also to correlate better to

cardiovascular risk,<sup>34</sup> We also intended to explore how under-reporting may influence the results. Under-reporting of EI is a common phenomenon in dietary surveys.<sup>35</sup> This error does not necessarily attenuate when studies are repeated. It has been demonstrated that subjects classified as under-reporters at one occasion also tended to be classified as under-reporters when the measurement was repeated regardless of whether different assessment methods were used.<sup>36</sup> Still, there are few studies that have investigated the implications of this when diet–disease relationships are investigated.

The participants in this study were selected to be free of disease, but to have different degree of fasting-insulin levels. Insulin can be considered as a marker of insulin resistance and is related to the metabolic syndrome as well as to SAD. A wide range of SAD is favorable when the aim is to study how SAD is related to diet. It is also an advantage that the men were free of disease, since the dietary intake was supposed to reflect a habitual diet and a diagnosis of a disease may affect the subject's food habits. It would have been difficult to perform the analyses on a sample that represents the total population, since many uncontrolled factors would distort the associations. However, a consequence of the selection procedure is that participants do not represent the general population. The lower participation rate among those with high insulin levels, compared with those with low levels indicate that there was a biased selection also among those men who did fulfil the criteria for participating in the study. Although the men were recruited to have different degrees of insulin, it is likely that the participants comprised a relatively homogenous group, which may contribute to that it could be more difficult to detect associations between diet and SAD. Still, we did find associations between diet and SAD, and the relatively large number of subjects in this study reduces the risk that the associations were caused by chance.

According to Black's evaluation, where the Goldberg cutoff was compared with direct comparison of energy expenditure measured by doubly labeled water,<sup>32</sup> sensitivity for detecting under-reporters was 0.74 when the subjects were categorised into three different levels of physical activity and the specificity for detecting non-URs was 0.97. In our study, the sensitivity and specificity should be at least in the same order as reported in Black's study, since our study has several categories of PAL. In other words, there should be a few non-URs in the URs group, whereas the non-URs group probably is more mixed. In both groups, the mean EI was too low to be due to chance. However, the ratio of EI:basal metabolic rate was much lower in the URs compared with the non-URs and the biological markers of protein, sodium, and potassium measured in 24-h urine collections verified a significantly lower validity of the dietary data in the URs compared with the non-URs.<sup>25</sup>

The higher BMI and SAD in the URs indicate that this group might be selected to have poorer health compared to the non-URs (Table 1). As presented elsewhere, the metabolic syndrome was also more prevalent among the URs.<sup>25</sup> The

suspicion of a biased selection of the URs is supported by the fact that overweight has been related to under-reporting also in other studies.<sup>11–13</sup> Excluding the URs from the analyses may therefore affect the possibility to detect associations between the SAD and dietary intake, a potential drawback that will be considered further. Although under-reporting occurred in both groups, a larger quantity of the EI was not reported in the URs compared with the non-URs. Several studies indicate that under-reporting affects foods selectively and specifically concerns foods considered as unhealthy or foods eaten in-between meals.<sup>12,14,15,25,37–39</sup> In this study, the reported intake of dairy fat and myristic acid was lower in the URs compared with the non-URs, regardless of whether the intake was expressed as absolute intake, percent of total fat intake, or percent of EI (Table 1). The intake of calcium also differed between the URs and non-URs. However, this difference was not statistically significant when the intake was adjusted for energy intake. The relative contents of the fatty acids 17:0 in PL and 15:0 and 17:0 in AT were also lower in the URs compared with the non-URs. Although these differences were small, this might indicate that the dairy fat intake was in fact lower in the URs.

The relative content of the fatty acids 14:0, 15:0, and 17:0 in PL and AT was associated with the intake of dairy fat in our study (Table 2). Generally, the correlations were lower in the URs. Overall, the results are comparable with the correlations reported by Wolk *et al.*<sup>16,17</sup> That the fatty acids 14:0, 15:0, and 17:0 in PL are affected by diet is also demonstrated by intervention studies. In the KANWU study, the effect of a 3-month diet containing a high proportion of saturated fatty acids from dairy foods was compared with a diet containing a high proportion of monounsaturated fatty acids. In the first case, the relative contents of 14:0, 15:0 and 17:0 in PL increased significantly, and in the second case they decreased.<sup>40</sup>

Unexpectedly, when SAD was correlated to the reported intake of dairy fat, inverse correlations were seen in the URs but not in the non-URs even if the intake was expressed as absolute intake, percent of total fat, or percent of energy intake (Table 3). If a true association between dairy fat intake and SAD existed, a similar association would reasonably be expected also in the non-URs. Since the URs were more obese than the non-URs, they may have an altered metabolism and one possible explanation to our finding could be that the relationship between SAD and dairy fat was nonlinear and that there is an inverse relationship among the obese subjects and not among the lean. However, according to our test described in the results section, this was not the case. The lack of an association in the non-URs was therefore not caused by a selection bias due to the exclusion of the URs. Furthermore, when the study sample was split into groups of BMI < 25 and BMI ≥ 25 instead of groups of URs and non-URs, an inverse association was seen in the lean subjects and not in the obese. Although the difference was not significant, these results do not support the above hypothesis. Although the inverse correlation in the URs was not

explained by the degree of under-reporting of energy, this does not exclude the possibility that a differential error, correlated to SAD, could cause the inverse correlation between dairy fat intake and SAD in the URs. Few studies have investigated the impact of dietary data of low validity on diet-disease relationships by excluding URs. However, Macdiarmid *et al*<sup>41</sup> found that 'low-energy reporters', defined as having a ratio of EI:BMR < 1.2, can both create and remove associations between BMI and the intake of sugar and fat in a study on 1853 individuals. Nevertheless, the explanation for the different correlations in the URs and the non-URs in our study remains unclear.

When SAD was correlated to the relative content of the fatty acids 14:0, 15:0, and 17:0 in PL and AT, inverse associations were seen except for 14:0 in PL, where the correlation was positive after adjustment (Table 4). Since the proportion of these fatty acids were found to be related to the intake of alcohol and physical activity independent of dairy fat intake (Rosell *et al*, submitted manuscript), we adjusted for these factors by using the Spearman's partial correlation. Overall, when the fatty acids in PL and AT are interpreted as markers for the intake of dairy fat, the results from these fatty acids (with the exception for 14:0 in PL) support an inverse association between SAD and the intake of dairy fat. However, since this association was not fully supported by our dietary data, one might speculate if the fatty acids in PL and AT possibly could also reflect something other than dairy fat. It is notable that 15:0 in PL and AT correlated weaker to SAD compared with 17:0, since 15:0 correlated considerably stronger to the intake of dairy fat. It would be expected that SAD correlated stronger to 15:0 compared with 17:0, since 15:0 was the better marker for dairy fat. The cross-sectional design of this study precludes inferences on causal effects and an alternative hypothesis, which is supported by the data in the non-URs, is that the fatty acid composition in PL and AT is affected by both dairy fat intake and SAD independent of each other.

Inverse associations between the metabolic syndrome and intake of dairy fat or dairy products have been found in other studies,<sup>5,6,8</sup> although in one study this was only seen in overweight people.<sup>8</sup> The favorable effect of calcium or other components associated with dairy fat such as potassium, magnesium, vitamins, and bioactive peptides give grounds for a biological explanation for the inverse association.<sup>4,42</sup> In the present study, we found inverse correlations between SAD and the intake of calcium in both URs and non-URs (Table 3). These correlations were also stronger in the URs. However, since the difference between URs and non-URs was not statistically significant, this finding may support an effect of calcium on SAD. Another possibility is that the intake of dairy products is a marker of a healthy diet or a healthy lifestyle. For example, low-fat dairy products may contribute to increased intake of micronutrients as the energy content is low. Milk also contains conjugated linoleic acid and supplementation of higher doses of this acid has been shown to reduce abdominal obesity in obese men.<sup>43</sup>

The intake of dairy products could also be associated with a physical active life. We investigated if the intake of dairy fat was related to physical activity and found no such association in our study (Rosell *et al*, submitted manuscript). To fully elucidate the health effects of the intake of dairy fat and to bring to light possible influences of under-reporting to these associations, a long-term, controlled intervention study would be desirable.

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#### References

- 1 Seidell JC. Obesity, insulin resistance and diabetes—a worldwide epidemic. *Br J Nutr* 2000; **83** (Suppl 1): 5–8.
- 2 Grundy SM. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am J Cardiol* 1999; **83**: 25F–29F.
- 3 Mann JI. Diet and risk of coronary heart disease and type 2 diabetes. *Lancet* 2002; **360**: 783–789.
- 4 Pfeuffer M, Schrezenmeir J. Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. *Br J Nutr* 2000; **84**: S155–S159.
- 5 Smedman AEM, Gustafsson I-B, Berglund LGT, Vessby BOH. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. *Am J Clin Nutr* 1999; **69**: 22–29.
- 6 Mennen LI, Lafay L, Feskens EJM, Novak M, Lépinay P, Balkau B. Possible protective effect of bread and dairy products on the risk of the metabolic syndrome. *Nutr Res* 2000; **20**: 335–347.
- 7 Wirfält E, Heldblad B, Gullberg B, Mattisson I, Andren C, Rosander U, Janson L, Berglund G. Food patterns and components of the metabolic syndrome in men and women: A cross-sectional study within the Malmö Diet and Cancer Cohort. *Am J Epidemiol* 2001; **154**: 1150–1159.
- 8 Pereira MA, Jacobs DR, Horn LV, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults. The CARDIA Study. *J Am Med Assoc* 2002; **287**: 2081–2089.
- 9 Phillips SM, Bandini LG, Cyr H, Colclough-Douglas S, Naumova E, Must A. Dairy food consumption and body weight and fatness studies longitudinally over the adolescent period. *Int J Obes Relat Metab* 2003; **27**: 1106–1113.
- 10 Trabulsi J, Schoeller D. Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake. *Am J Physiol Endocrinol Metab* 2001; **281**: E891–E899.
- 11 Price GM, Paul AA, Cole TJ, Wadsworth MEJ. Characteristics of the low-energy reporters in a longitudinal national dietary survey. *Br J Nutr* 1997; **77**: 833–851.
- 12 Pryer AJ, Vrijheid M, Nichols R, Kiggins M, Elliott P. Who are the 'low energy reporters' in the dietary and nutritional survey of British adults? *Int J Epidemiol* 1997; **26**: 146–154.
- 13 Lafay L, Basdevant A, Charles MA, Vray M, Balkau B, Borys JM, Eschwege E, Romon M. Determinants and nature of dietary underreporting in a free-living population: the Fleurbaix Laventie Ville Santé (FLVS) study. *Int J Obes Relat Metab* 1997; **21**: 567–673.

- 14 Lafay L, Mennen L, Basdevant A, Charles MA, Borys JM, Eschwege E, Romon M. Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Santé (FLVS) study. *Int J Obes Relat Metab* 2000; **24**: 1500–1506.
- 15 Poppitt SD, Swann D, Black AE, Prentice AM. Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Int J Obes Relat Metab* 1998; **22**: 303–311.
- 16 Wolk A, Furuheim M, Vessby B. Fatty acid composition of adipose tissue and serum lipids are valid biological markers of dairy fat intake in men. *J Nutr* 2001; **131**: 828–833.
- 17 Wolk A, Vessby B, Ljung H, Barrefors P. Evaluation of a biological marker of dairy fat intake. *Am J Clin Nutr* 1998; **68**: 291–295.
- 18 Pedersen NL, Ringstad J, Almendingen K, Haugen TS, Stensvold I, Thelle DS. Adipose tissue fatty acids and risk of myocardial infarction—a case-control study. *Eur J Clin Nutr* 2000; **54**: 618–625.
- 19 Beyen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. *Am J Clin Nutr* 1985; **42**: 317–322.
- 20 Rosell M, Hellenius M-L, de Fair U, Berglund L, Gustafsson I-B, Johansson G. 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* 2003; **47**: 123–131.
- 21 Becker W. *Food Habits and Nutrient Intake in Sweden 1989 (In Swedish)*. The National Food Administration: Uppsala, Sweden; 1994.
- 22 Becker W, Lennernas M, Gustafsson I-B, Haraldsdottir J, Nydahl M, Vessby B, Ytterfors A. Precoded food records compared with weighed food records for measuring dietary habits in a population of Swedish adults. *Scand J Nutr* 1998; **42**: 145–149.
- 23 *Vikttabell*. The Swedish National Food Administration: Uppsala, Sweden; 1999.
- 24 *PC-Kost version 1/99*. The Swedish National Food Administration: Uppsala, Sweden; 1999.
- 25 Rosell MS, Hellenius M-LB, de Fair U, Johansson GK. The associations between diet and the metabolic syndrome vary with the validity of the dietary data. *Am J Clin Nutr* 2003; **78**: 84–90.
- 26 Bingham S, Cummings JH. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collection in man. *Clin Sci* 1983; **64**: 629–635.
- 27 Johansson G, Bingham S, Vahter M. A method to compensate for incomplete 24-h urine collections in nutritional epidemiology studies. *Publ Health Nutr* 1999; **2**: 587–591.
- 28 Bingham SA, Cummings JH. Urine nitrogen as an independent validity measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. *Am J Clin Nutr* 1985; **42**: 1276–1289.
- 29 Johansson G, Callmer E, Gustafsson J. Validity of repeated dietary measurements in a dietary intervention study. *Eur J Clin Nutr* 1992; **46**: 717–728.
- 30 Black A, Goldberg G, Jebb S, Livingstone M, Cole T, Prentice A. Critical evaluation of energy intake using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr* 1991; **45**: 583–599.
- 31 Black AE. 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 Relat Metab* 2000; **24**: 1119–1130.
- 32 Black AE. The sensitivity and specificity of the Goldberg cut-off for EI:BMR for identifying diet reports of poor validity. *Eur J Clin Nutr* 2000; **54**: 395–404.
- 33 Nordhamn K, Sodergren E, Olsson E, Karlstrom B, Vessby B, Berglund L. Reliability of anthropometric measurements in overweight and lean subjects: consequences for correlations between anthropometric and other variables. *Int J Obes Relat Metab* 2000; **24**: 652–657.
- 34 Ohrvall M, Berglund L, Vessby B. Sagittal abdominal diameter compared with other anthropometric measurements in relation to cardiovascular risk. *Int J Obes Relat Metab* 2000; **24**: 497–501.
- 35 Black AE, Prentice AM, Goldberg GR, Jebb SA, Bingham SA, Livingstone MB, Coward WA. Measurements of total energy expenditure provide insights into the validity of dietary measurements of energy intake. *J Am Diet Assoc* 1993; **93**: 572–579.
- 36 Black AE, Cole TJ. Biased over- or under-reporting is characteristic of individuals whether over time or by different assessment methods. *J Am Diet Assoc* 2001; **101**: 70–80.
- 37 Johansson G, Wikman Å, Åhrén A-M, Hallmans G, Johansson I. Underreporting of energy intake in repeated 24-h recalls related to gender, age, weight status, day of interview, educational level, reported food intake, smoking habits and area of living. *Publ Health Nutr* 2001; **4**: 919–927.
- 38 Heitmann BL, Lissner L. Dietary underreporting by obese individuals—is it specific of non-specific? *BMJ* 1995; **311**: 986–989.
- 39 Bingham SA, Cassidy A, Cole TJ, Welch A, Runswick SA, Black AE, Thurnham D, Bates C, Khaw KT, Key TJ et al. Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *Br J Nutr* 1995; **73**: 531–550.
- 40 Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH, KANWU study. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU study. *Diabetologia* 2001; **44**: 312–319.
- 41 Macdiarmid JJ, Vail A, Cade JE, Blundell JE. The sugar-fat relationship revised: differences in consumption between men and women of varying BMI. *Int J Obes Relat Metab* 1998; **22**: 1053–1061.
- 42 Massey L. Dairy food consumption, blood pressure and stroke. *J Nutr* 2001; **131**: 1875–1878.
- 43 Riserus U, Berglund L, Vessby B. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial. *Int J Obes Relat Metab* 2001; **25**: 1129–1135.