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Short Communication

Seven Countries Study cohort in Crete, Greece: gluteal adipose-tissue fatty-acid profiles of survivors, at 2010

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Abstract

Objective: To analyse the gluteal adipose-tissue fatty-acid profiles from Cretan cohort survivors of the Seven Countries Study (SCS) at 2010 and to compare them with those of survivors assessed in 2000, as well as with literature data on male Cretans at 1965.

Design: We analysed data concerning the gluteal adipose-tissue fatty acids (analysed by GC) from three studies.

Setting: The island of Crete (rural areas and the city of Heraklion).

Subjects: Twenty-two of the 2010 SCS survivors aged 90 years and over; seventy-eight men aged 80 years of the 2000 SCS survivors; and 280 men assessed in 1965.

Results: In comparison to 1965 and 2000, the SCS survivors in 2010 had a higher amount of 18 : 1*n*-9 ($P < 0{\cdot}05$) in their gluteal adipose tissue and a lower amount of PUFA ($P < 0{\cdot}05$). On the other hand, a constant decrease in adipose-tissue 14 : 1*n*-5 and 16 : 1*n*-7 was recorded between 1965 and 2010 ($P < 0{\cdot}001$), and between 2000 and 2010 ($P < 0{\cdot}05$), while 18 : 2*n*-6 appeared to decrease between the 1965 and 2010 assessments ($P < 0{\cdot}001$).

Conclusions: Comparison with a 1965 representative Cretan sample and 2000 SCS survivors indicated an increased concentration of oleic acid (known for its protective role against mortality) and a decreased concentration of PUFA (known for their susceptibility to oxidation) in our surviving sample at 2010. These changes may reflect internal physiological processes due to diet change within these years and/or ageing.

Keywords
Seven Countries Study
Adipose tissue
Fatty acids
Nonagenarians

Exceptional longevity is known to be influenced by lifestyle, environmental and genetic factors. Thus, a study evaluating lifestyle factors including physical activity, smoking, alcohol consumption and dietary habits in Ashkenazi Jews aged 95–109 years found no significant differences compared with the general American population. This finding suggests that people with exceptional longevity may interact with environmental factors differently from others[1] and may possess genetic traits that contribute to their exceptional longevity. On the other hand, non-genetic determinants were proved to play a significant role in the probability of an exceptional 90-year lifespan. Specifically, men with exceptional longevity who had healthy behaviours and less adverse factors (smoking, diabetes, obesity, hypertension or sedentary lifestyle) lived on average 10 years longer than men with less favourable behaviours/characteristics[2].

The mechanisms through which the favourable lifestyle behaviours may enhance life expectancy require further investigation. There is evidence that a healthy diet, like the Cretan Mediterranean diet, may play a substantial role in prolonging life[3]. The term 'Mediterranean diet' was first described during an epidemiological study called the Seven Countries Study (SCS) initiated in 1960 and is based on the nutrition patterns described in Crete[4]. The SCS determined that men of Crete in the 1960s had the lowest coronary and cancer mortality and the highest longevity among all fifteen cohorts of the SCS[5]; a fact attributed to their lifestyle those days and especially their dietary habits.

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However, the dietary habits of the SCS survivors evaluated in 2010 were partially different from those 50 years ago[6]. The survivors were found to exceed the recommended intake for total fat (42·9 (SD 10·1) % of energy, instead of 25–35 % of energy) and saturated fat (12·7 (SD 4·1) % of energy, instead of 8 % of energy); while their intake was below the recommendations for *n*-3 PUFA, dietary fibre, vitamins E, C, B_6, B_{12}, folic acid and potassium[6]. The fatty-acid composition of human subcutaneous adipose tissue is a useful biochemical marker of long-term fat consumption[7]. The present study aimed to use a reliable method to assess the gluteal adipose-tissue fatty-acid profile (an index of long-term fat intake) in the survivors of the Cretan cohort of the SCS at 2010 and compare it with that in the survivors assessed in 2000[8] and available data from Cretan men at 1965[7].

Materials and methods

We analysed data concerning the gluteal adipose-tissue fatty acids from three studies. The first study took place in 1965 on the island of Crete (rural areas and the city of Heraklion) by Christakis *et al.* and provided relevant data on 280 men aged about 60 years old[7]. The second study included data on fatty acids from seventy-eight Cretan SCS survivors aged 80 years old and over, collected during the 40-year follow-up of the SCS carried out in 2000[8]. The available data from the aforementioned studies were compared with those from our total cohort study in 2010 that consisted of twenty-seven men of the SCS Cretan cohort, aged 90 years old and over[6]. Of them, twenty-two were subjected to adipose-tissue fatty-acid analysis. Informed consent was obtained from all the participants. The ethical committee at the University of Crete had previously approved the protocol of the study.

Buttock subcutaneous tissue samples were collected by aspiration, as described by Beynen and Katan[9]. Samples were taken from the left upper outer quadrant of the gluteal area into a 10 ml Vacutainer tube. Prior to aspiration, sites were sprayed with local anaesthetic (ethyl chloride). Adipose-tissue samples were stored under N_2 at −80°C. Briefly, 20–30 mg of fat sample was saponified with 1·0 ml NaOH in methanol and fatty acid methyl esters (FAME) were prepared using 14 % (w/v) BF_3 in methanol followed by extraction with hexane after washing with saturated NaCl. The hexane (upper layer) containing the FAME was transferred to GC vials and stored at 20°C until analysis. The FAME were separated on a 100 mm × 0·25 mm internal diameter SP-2560 fused silica capillary column, coated with a 0·25 mm layer of cyanopropyl silicone provided by Supelco, using a Shimadzu GC-17A/FID gas chromatograph equipped with an AOC-20I auto injector. The Class-VP Chemstation software was used for quantification and identification of peaks. Baseline separation of over fifty FAME peaks was accomplished by means of mixed FAME standards (Sigma). The analytical conditions employed were as follows: volume injected = 1 µl, He as carrier gas (flow rate = 1·1 ml/min), injector temperature = 250°C, flame ionization detector temperature = 260°C, split ratio = 1:4 to 1:20 (depending on sample quantity) and oven temperature from 140°C to 245°C with a stepped temperature program, within total run time 54 min. The fatty acids were logged as percentage of the total fatty acids present in the chromatogram. We tested the normality of all variables by using the normal curve and the Kolmogorov–Smirnov test. Student's *t* test was employed to evaluate the differences in specific fatty acids between the 2010 study and those of the 1965 and 2000 studies. In the case of non-normality we used a non-parametric Mann–Whitney *U* test. The statistical software package IBM SPSS Statistics version 18·0 was used for all data analyses. The level of statistical significance was set at $P < 0·05$.

Results

Differences in the fatty-acid profiles in adipose tissue among the last survivors of the SCS in 2010 and those in 2000 (Table 1), as well between the 2010 survivors and the participants in the Christakis study (1965), were found. Concerning MUFA, a constant decrease in adipose-tissue 14 : 1*n*-5 between the 2000 and 2010 studies ($P < 0·001$), as well as between 1965 and 2010 ($P < 0·05$), was found. A significant decrease was also noted for adipose-tissue 16 : 1*n*-7 between 1965 and 2010 ($P < 0·001$), and between 2000 and 2010 ($P < 0·05$). On the other hand, adipose-tissue 18 : 1(*n*-9 & *n*-7) (estimated together in the present study) increased slightly between the studies of 1965 and 2010 ($P < 0·001$), as well as between 2000 and 2010 ($P < 0·05$). Concerning PUFA, 18 : 2*n*-6 decreased, mainly between the 1965 and 2010 studies ($P < 0·001$). Finally, the twenty-two survivors in 2010 had a lower concentration of 20 : 5*n*-3 ($P < 0·05$) when compared with the seventy-eight survivors in 2000. Overall, nonagenarians (SCS participants in 2010) had a lower amount of PUFA in their adipose tissue compared with 1965 ($P < 0·001$) and 2000 ($P < 0·05$).

Discussion

The survivors of the Cretan cohort of the SCS assessed in 2010 are characterized by an exceptional longevity. The reason for this is unknown but some assumptions can be made. The exact mechanism by which the fatty-acid profile in adipose tissue arises[10] is generally unclear. We compared their fatty-acid profile with that of survivors assessed in 2000, and that of a representative sample of Cretan men in 1965, in order to identify differences. SFA and MUFA can be synthesized *de novo* and so a close relationship with dietary fatty acids is not necessarily to be expected[10]. Concerning adipose-tissue SFA, the changes

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Table 1 Mean adipose-tissue fatty-acid measures in Cretan survivors of the Seven Countries Study in 2000 and 2010

Fatty acid (g/100 g total fatty acids)	Year 2000 (*n* 78)	Year 2010 (*n* 22)	*P* value*
14 : 0	1·4	1·23	0·022
14 : 1*n*-5	0·17	0·12	0·001
16 : 0	14·9	14·3	0·097
16 : 1*n*-7	3·3†	2·8	0·021
18 : 0	2·5	2·5	0·928
18 : 1(*n*-9 & *n*-7)	62·7	64·4	0·003
18 : 2*n*-6	7·8	7·6	0·455
18 : 3*n*-3	0·38	0·37	0·329
20 : 4*n*-6	0·33	0·31	0·208
20 : 5*n*-3	0·05	0·04	0·031
22 : 6*n*-3	0·15	0·13	0·079
SFA	19·5	18·6	0·071
MUFA	69·6	69·7	0·862
PUFA	9·6	9·1	0·033

**P* values for the comparison of mean values in 2000 *v.* 2010 using Student's *t* test and the Mann–Whitney *U* test in the case of 18 : 3*n*-3, 20 : 4*n*-6, 20 : 5*n*-3 and 22 : 6*n*-3.
†A different estimation, 0·84 %, was reported in a previous study[8] for the same sample.

observed in our study did not appear to be significant. However, their proportion in the overall composition of the adipose fat in the SCS participants was about 5–12 % less (18·5–19·5 % instead of 24–33 %) when compared with studies in non-SCS populations[7,11,12]. The effect of the long-term nutritional exposure to SFA of the SCS participants[13] in the above proportion – or simpler, the cumulative effect[14] of the Cretan/Mediterranean diet on adipose-tissue SFA proportion over an extended period of time – is difficult to quantify.

Overall, MUFA appeared not to change also, at least quantitatively; however, a constant decrease in adipose-tissue 14 : 1 and 16 : 1 between the 2000 and 2010 studies, as well as between 1965 and 2010, was found. According to Insull and Bartsch, 14 : 1*n*-5 occurs naturally in human adipose tissue at a proportion of about 0·5–0·7 % of total fatty acids, and 16 : 1*n*-7 at about 5·0–6·6 % of total fatty acids[15]. Although methodological and technical parameters may interfere when trying to compare biochemical data from different groups, these proportions are very close to the 1965 measurements in Cretan men[7], 0·5 % and 6·4 %, respectively; but decreased to less than half in the 2010 SCS survivors, to 0·12 % and 2·8 %, respectively. Adipose-tissue 14 : 1*n*-5 and 16 : 1*n*-7 seem to be indicators and reflective of endogenous lipogenesis (fat synthesis); they are products of this process[12]. A working hypothesis is that in the adipose tissue of the ageing SCS survivors the activities of lipogenic enzymes were lowered, as mentioned already in animal models[16], and this can be a base for discussion, although the specific hormonal/metabolic pathways responsible for this observation remain a challenge. Since stearoyl-CoA desaturase 1 is the enzyme responsible for the synthesis of adipose-tissue 16 : 1*n*-7 from 16 : 0[12], a decrease in the activity of this particular enzyme with ageing could explain the notable decrease in 16 : 1*n*-7 in the adipose tissue of the 2010 survivors.

On the other hand, the 18 : 1*n*-9 content in the adipose tissue of the SCS participants increased between the studies of 1965 and 2010, as well as 2000 and 2010. As shown elsewhere[15], the proportion of oleic acid increases significantly and in a linear manner with age; this may be the case in the present study also. Interestingly, the overall proportion of 18 : 1 (generally estimated as 18 : 1*n*-9 and 18 : 1*n*-7) in subcutaneous adipose tissue is reported in various studies to be 44–53 % of total fatty acids[12,15,17,18]; and especially when sampled from the buttock, averages from 48 %[17] to 58·7 %[19]. In all the measurements of adipose tissue in Cretan men[7,8], the average content of adipose-tissue 18 : 1 was higher than 61 %. The Christakis study[7] actually reports the same finding when comparing data from Cretan men with other American male groups. Previous studies suggest that diets sufficiently enriched in olive oil protect against all-cause mortality[20]. The high content of MUFA, especially 18 : 1, in the adipose tissue of Mediterranean populations may be due to the high availability of olive oil in their diet[11,21], although this effect is difficult to quantify due to the different biochemical methods used in various studies[21]. We have data however which indicate that the increase of BMI, combined with increase in meat and saturated fat, and decrease in fruit consumption, correlates with lower MUFA and higher SFA proportions in subcutaneous adipose tissue[22].

Adipose-tissue 18 : 2*n*-6 may decrease with increasing age, as already reported[15]. Although, as concerns fish consumption, there was an increase from 18 g/d in the 1960s to 38 g/d among the surviving elderly in 2010 ($P < 0{\cdot}001$)[6], they also had a lower adipose-tissue PUFA content compared with 1965 and 2000. PUFA are susceptible to peroxidation, triggering oxidative stress that is a risk factor for atherosclerosis, while especially some of the *n*-6 fatty acids are potential precursors of inflammation eicosanoids[13]. Baylin and Campos[23] found that increased adipose-tissue 20 : 4*n*-6 is associated in due course with myocardial infarction, independently of dietary and adipose-tissue 18 : 2*n*-6, as well as of other (*n*-6 and *n*-3) fatty acids. In the present study, the overall lowering of PUFA content in the adipose tissue of the twenty-two survivors may also be connected with less lipid oxidation and CVD.

The present study has some limitations. First, the comparison carried out between Cretan men assessed in 1965 and Cretan survivors of the SCS assessed in 2000 and 2010 was somehow limited by the biochemical data available from 1965, since the GC analysis methods were less advanced then. In addition, we did not assess whether the fatty-acid composition of the twenty-two survivors in 2010 changed when compared only with the same twenty-two individuals (and not the total sample of seventy-eight individuals) in 2000; or if it differed from those individuals in 2000 who did not survive in 2010. It would be very

interesting to present such comparisons; however, since a limited number (half) of the 2010 survivors were analysed in the 2000 study, the necessary data were not available.

Conclusion

In conclusion, the comparison among a 1965 representative Cretan sample, the 2000 SCS survivors and the 2010 SCS survivors indicated an increased concentration of oleic acid (known for its protective role against mortality) and a decreased concentration of PUFA (known for their susceptibility to oxidation) in the 2010 sample. These changes may reflect internal physiological processes due to diet change within these years and/or ageing. In any case, the correlation of the nonagenarians' fatty-acid profile with their exceptional longevity is not as yet fully understood and should be further examined.

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