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Trends of serum phospholipid fatty acids over time in rural Uganda: evidence of nutritional transition?

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Abstract

Non-communicable diseases are projected to become the most common causes of death in Africa by 2030. The impact on health of epidemiological and nutritional transitions in sub-Saharan Africa remains unclear. To assess the trends of dietary fatty acids over time in Uganda, we examined fatty acids in serum collected from individuals in rural south-west Uganda, at three time points over two decades. Independent cross-sectional samples of 915 adults and children were selected from the general population cohort in 1990 (n 281), 2000 (n 283) and 2008 (n 351). Serum phospholipid fatty acids were measured by GC. Multivariate regression analyses were performed to compare the geometric means of fatty acids by time period. Serum fatty acid profiling showed high proportions of SFA, *cis*-MUFA and industrial *trans*-fatty acids (iTFA), likely to be biomarkers of high consumption of palm oil and hydrogenated fats. In contrast, proportions of *n*-6 and *n*-3 PUFA from vegetable oils and fish were low. From 1990 to 2008, serum phospholipids showed increases in absolute amounts of SFA (17.3% increase in adults and 26.4% in children), MUFA (16.7% increase in adults and 16.8% in children) and *n*-6:*n*-3 PUFA (40.1% increase in adults and 39.8% in children). The amount of elaidic acid, iTFA from hydrogenated fats, increased in children (60.1% increase). In this rural Ugandan population, we show evidence of unfavourable trends over time of dietary fatty acids.

Key words: Nutrition transition: Fatty acids: Biomarkers: Epidemiology: East Africa

Non-communicable diseases (NCD) are projected to become the most common causes of death in Africa by 2030⁽¹⁾. Despite this fact, risk factors – including nutritional factors – have not been fully studied in African populations undergoing epidemiological and nutritional transitions, with concomitant changes in lifestyle and diet. In high-income countries, diets began to shift in the 1970s, from a traditional pattern of high carbohydrate and fibre and low fat and sugar, toward a more globalised ‘Western’ pattern characterised by increased consumption of processed foods, greater use of edible oils and sugar-sweetened beverages⁽²⁾. Similar changes started to emerge in the 1990s in low- to middle-income countries^(2–4). The nutritional transition has led to an increased consumption of products that can be harmful to health at high levels, such as sugar, Na and fat, particularly SFA and industrial *trans*-fatty acids (iTFA). In turn, this has had an impact on patterns of cancers,

from one dominated by infection-related cancers to one characterised by cancers related to changing reproductive patterns, poor diet, excess body fatness and reduced physical activity⁽⁵⁾.

Countries in sub-Saharan Africa are clearly undergoing a nutrition transition⁽³⁾. More than half of them are still in the early stage, as illustrated by the finding that fat, protein and carbohydrate intake are still within those recommended, while a few have already reached a situation where changes in dietary patterns are affecting health outcomes in the population^(3,6). Data from the FAO indicated a steady increase in the energy supply in most sub-Saharan countries over a period of three decades, protein and fat intake displaying a similar trend⁽⁷⁾. An increase in fat consumption has been shown to be highly associated with global dietary changes⁽³⁾. Thus, these data suggest that measurement of fatty acid intakes might serve as an indicator of nutrition transition in general.

Abbreviations: GPC, general population cohort; iTFA, industrial *trans*-fatty acid; NCD, non-communicable disease; TFA, *trans*-fatty acid.

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Measurement of dietary fatty acids in nutritional epidemiology studies has been hampered by substantial measurement error when using dietary questionnaires. Plasma or serum phospholipid fatty acid level measurements offer specific biomarkers of medium-term (weeks to months) dietary intake of bioavailable amounts of these nutrients, particularly for fatty acids that are not endogenously synthesised, such as *i*TFA and *n*-6 and *n*-3 PUFA^(8,9). Thus, the use of specific biomarkers of fatty acids is a more rigorous approach to provide a better understanding of changes in dietary fatty acids in low- to middle-income countries.

The general population cohort (GPC) set-up in rural south-western Uganda provided a unique framework to examine the biomarkers of dietary fatty acids. We measured serum phospholipid fatty acid levels at three time periods, in 1990–1991, 1999–2000 to 2007–2008, and monitored changes over this period in a representative sample within the GPC.

Methods

Population

The GPC was set up as a population-based open cohort study established in 1989 to examine trends in HIV prevalence and incidence in rural south-western Uganda. Since 2010, the scientific research questions have incorporated the epidemiology and genetics of both communicable and NCD. The study area is located in Kyamulibwa sub-county of Kalungu district. The initial study population of about 10 000 residents comprised a cluster of fifteen neighbouring villages. From 1999, ten more adjacent villages with comparable characteristics were added to the cohort, thereby doubling the population⁽¹⁰⁾.

The study population was recruited through annual house-to-house rounds of census through which participants for the medical survey are selected. All residents aged 13 years and above were included in all the medical survey rounds 1–22 (1989–2011). Children <13 years were also recruited every third round. Data on health and lifestyle are collected using a standard individual questionnaire, and blood samples were obtained and biophysical measurements taken. Blood samples were transported to MRC/UVRI Laboratories in Entebbe and stored at -80°C . Overall, more than 95% of households approached for census participated.

Ethics statement

Study participants provided written informed consent to participate in the study. Ethical approval for this study was granted by London School of Hygiene and Tropical Medicine and the Uganda Virus Research Institute Research and Ethics Committee, and the study was approved by the Uganda National Council for Science and Technology. It was also approved by the Ethics Committee of the International Agency for Research on Cancer (IARC).

Selection criteria

Census rounds 3, 11 and 19 were chosen because of the inclusion of children. A total of three independent random

samples of 281 individuals (149 adults and 132 children), 283 individuals (166 adults and 117 children) and 351 individuals (238 adults and 113 children) were selected on the basis of availability of serum sample and stratified to provide a 1:1 sex ratio. Serum samples were shipped to the IARC in Lyon, France.

Laboratory analysis

All measurements of fatty acids were performed at the IARC. Serum samples were profiled for phospholipid fatty acid composition by batches of twenty samples, including samples from eighteen subjects at three time periods (six men, six women and six children) in random order, and two independent serum samples as quality controls. The laboratory staff was blinded to the status of the sample (subject, sex, time period or quality control). As previously described, total lipids were extracted from serum samples, phospholipids were purified by adsorption chromatography, and fatty acid methyl esters separated through GC⁽¹¹⁾. The relative concentration of each fatty acid, expressed as percentage of total fatty acids, was quantified by integrating the area under the peak and dividing the result by the total area. Fatty acids were also expressed as absolute concentrations in serum ($\mu\text{mol/l}$).

Overall (intra-batch and inter-batch) CV for fatty acids, which were calculated using two serum samples as quality controls added to each batch, ranged from 0.29% for large peaks such as palmitic acid, to 7.74% for the smallest peaks such as 18:3*trans*. Specifically for *trans*-fatty acids (TFA), the overall CV were 0.112 for 16:1*n*-9*trans*, 0.137 for 18:1*n*-9*trans*, 0.376 for 18:2*trans,cis*, 0.198 for 18:2*trans,trans*, 7.74 for 18:3*n*-3*trans,cis,cis*, 0.282 for 18:1*n*-7*trans*, 1.017 for CLA 10*trans,12cis* and 0.097 for CLA 9*trans,11cis*.

Statistical analysis and data treatment

Multivariate regression analyses were performed to compare the geometric mean levels (mean 95% confidence intervals) of fatty acids by time period. We calculated the percentage and absolute amounts of the following groups: SFA, *cis*-MUFA, ruminant TFA, *i*TFA, *cis*-*n*-6 PUFA, long-chain *n*-6 PUFA, *n*-3 PUFA, long-chain *n*-3 PUFA and the ratio of *n*-6:*n*-3 PUFA. Analyses were conducted separately in children, men and women and in men and women combined. Tests for trend were computed using the time period-specific geometric means of each fatty acid. The models were adjusted for age at baseline for men and for women separately. For children and for adults combined, the models were adjusted for age at baseline and for sex. All analyses were performed with STATA version 13 software.

Results

Subject characteristics

Characteristics of the study population are described above and in a previous report⁽¹⁰⁾. Briefly, the cohort includes all residents (52% aged ≥ 13 years, men and women in equal proportions) within one-half of a rural sub-county, residing in scattered houses. Characteristics of the study population showed a

relatively young population, with about 90% of the population <50 years old, predominantly farmers of three major ethnic groups. Only 13% of the population attained education beyond primary level.

Serum phospholipid fatty acid profile

The separation of serum phospholipid fatty acids, particularly TFA isomers, is shown in Fig. 1. The serum phospholipid fatty acid profile in the adult study population at baseline (1990–1991) is provided in Table 1. Due to a lack of appropriate reference values, we used serum phospholipid fatty acid profiles reported at the same time in other cross-sectional studies of healthy adults to determine whether our population had been exposed to low or high fatty acid levels. Serum phospholipid fatty acid proportions reported in the European Prospective Investigation into Cancer and Nutrition (EPIC) study^(8,9), the Japan Public Health centre-based prospective study on cancer and CVD study⁽¹²⁾ and the New Zealand National Nutrition Survey⁽¹³⁾ are provided for comparison. In all studies, fatty acids are expressed in percentage of total fatty acids. Because the proportions of fatty acids are similar in adult men and women in the Ugandan population study (data not shown), combined data are provided for men and women.

In 1990–1991, the fatty acid profile in Ugandan adults is characterised by a high proportion of SFA (44.53% of total fatty

acids), mainly palmitic acid (27.56%). More striking is the very high proportion of *cis*-MUFA (26.30%), mainly oleic acid (20.50%). In contrast, the proportions of *n*-6 PUFA (21.66%), mainly linoleic acid (12.59%) and *n*-3 PUFA (3.22%) are low. The ratio of *n*-6:*n*-3 PUFA is 6.73. The proportion of TFA from industrial processing (1.72%) is relatively high, mainly as the consequence of a high proportion of palmitelaidic acid, sixteen-carbon TFA (Fig. 1). In contrast, the proportion of elaidic acid, the main TFA from industrial processing, is relatively low, as is the proportion of TFA from natural sources mainly represented by vaccenic acid.

Trends in serum fatty acids over time

Trends in serum phospholipid fatty acids, expressed in absolute amounts and in proportions, from 1990 to 2008 are shown in Table 2 for adults and children.

Notably, there were statistically significant increases in absolute amounts of SFA (17.3% increase in adults and 26.4% in children) (mainly palmitic acid), of *cis*-MUFA (16.7% increase in adults and 16.8% in children) (mainly oleic acid) and of total *n*-6 PUFA (20.3% increase in adults and 33.8% in children), as a consequence of the increasing levels of linoleic acid and long-chain *n*-6 PUFA. These trends were present in children and adults but were stronger in children; an unexplained peak level of SFA and MUFA was seen in adults in the 1999–2000 collection. There was an increasing ratio of *n*-6:*n*-3 PUFA, rising to

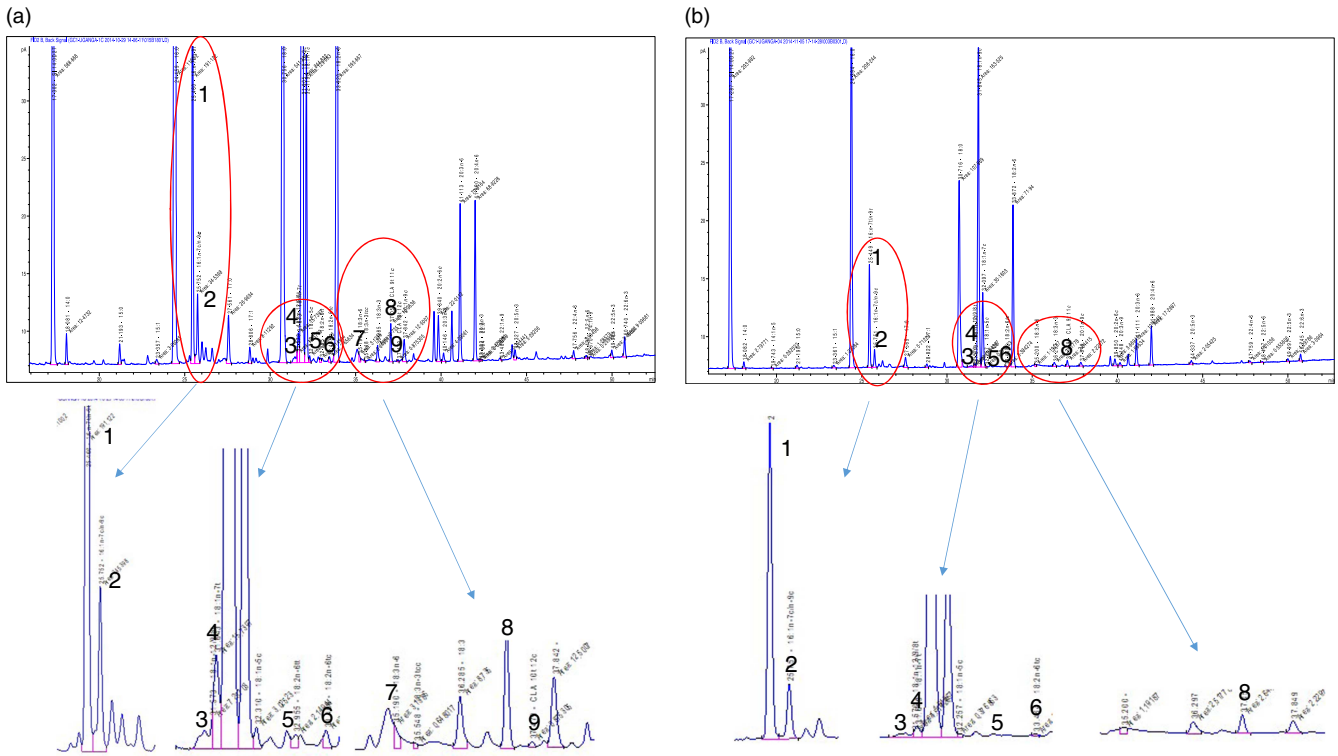


Fig. 1. Separation of serum phospholipid fatty acids through GC. (a) The separation of fatty acids in a sample from the population study with a high proportion of *trans*-fatty acid isomers, while (b) shows a sample with a low proportion of *trans*-fatty acid isomers. 1, Palmitelaidic acid (16:1*n*-9*trans*); 2, palmitoleic acid (16:1*n*-9*cis*); 3, elaidic acid (18:1*n*-9*trans*); 4, vaccenic acid (18:1*n*-7*trans*); 5 and 6, linolelaidic acid isomers (18:2*n*-6*trans,trans*, 18:2*n*-6*trans,cis*); 7, linolenelaidic acid (18:3*n*-3*trans,cis,cis*); 8, conjugated linoleic acid (CLA) 9*trans*,11*cis*; 9, CLA 10*trans*,12*cis*. Industrial *trans*-fatty acids (iTFA) included peaks 1, 3, 5, 6 and 7, while ruminant *trans*-fatty acids included peaks 4, 8 and 9.

Table 1. Serum phospholipid fatty acid ranges in the general population cohort (GPC) in Uganda, the European Prospective Investigation into Cancer and Nutrition study, the Japan Public Health centre-based prospective study on cancer and CVD study and a New Zealand National Nutrition Survey (Mean values, 95% confidence intervals and ranges)

Fatty acids (% total fatty acids)	Adults, GPC Uganda (1990; n 149)		Adults, Europe ⁽⁸⁾ (1992–1998; n 3262)		Adults, Japan ⁽¹²⁾ (1994–1995; n 87)		Adults, New Zealand ⁽¹³⁾ (1993–1998; n 2416)	
	Mean	95% CI	Mean	95% CI	Mean	Range	Mean	Range
Total SFA	44.53	44.12, 44.93	40.46	40.38, 40.53	47.60	42.20–58.90	50.25	43.19–61.22
Palmitic acid (16:0)	27.56	27.11, 28.01	25.41	25.33, 25.49	28.70	23.20–36.90	31.73	28.41–37.56
Stearic acid (18:0)	15.55	15.19, 15.91	13.99	13.94, 14.05	15.10	11.70–20.80	14.34	11.95–17.79
Total <i>cis</i> -MUFA	26.30	25.56, 27.07	12.80	12.72, 12.88	13.60	10.30–18.40	12.56	9.04–16.85
Palmitoleic acid (16:1 <i>n</i> -9 <i>cis</i>)	1.32	1.24, 1.40	0.60	0.59, 0.60		NA	0.83	0.38–1.43
Oleic acid (18:1 <i>n</i> -9 <i>cis</i>)	20.50	19.85, 21.16	10.09	10.02, 10.17	8.80	6.30–12.60	10.00	7.57–13.00
Total <i>cis</i> - <i>n</i> -6 PUFA	21.66	20.93, 22.41	37.38	37.25, 37.51	26.50	15.30–33.20	26.26	18.73–38.00
Linoleic acid (18:2 <i>n</i> -6 <i>cis,cis</i>)	12.59	12.03, 13.19	21.83	21.63, 22.02	17.30	9.30–24.30	19.01	12.72–24.54
Long-chain <i>n</i> -6 PUFA	8.76	8.41, 9.12	15.17	15.07, 15.26		NA	7.25	6.01–13.46
Total <i>cis</i> - <i>n</i> -3 PUFA	3.22	2.99, 3.47	6.90	6.83, 6.97	12.40	7.00–20.20	4.74	2.31–7.59
α -Linolenic acid (18:3 <i>n</i> -3 <i>cis,cis,cis</i>)	0.15	0.14, 0.17	0.18	0.18, 0.19	0.20	0.10–1.20	0.24	0.12–0.42
Long-chain <i>n</i> -3 PUFA	2.84	2.63, 3.06	6.69	6.62, 6.76	12.20	5.80–20.80	4.50	2.19–7.17
<i>n</i> -6: <i>n</i> -3 PUFA	6.73	6.21, 7.29	5.42	5.36, 5.48	2.30	0.80–4.40	5.54	5.01–8.11
Total industrial <i>trans</i> -fatty acids	1.72	1.58, 1.86	0.48	0.47, 0.49		NA		NA
Palmitelaidic acid (16:1 <i>n</i> -9 <i>trans</i>)	1.28	1.16, 1.41	0.28	0.27, 0.29		NA		NA
Elaidic acid (18:1 <i>n</i> -9 <i>trans</i>)	0.18	0.16, 0.19	0.31	0.30, 0.31		NA		NA
Total natural <i>trans</i> -fatty acids	0.22	0.19, 0.25	0.80	0.79, 0.82		NA		NA
Vaccenic acid (18:1 <i>n</i> -7 <i>trans</i>)	0.08	0.06, 0.09	0.26	0.25, 0.27		NA		NA

NA, not available.

9.74:1 and 8.43:1 in adults (40.1% increase) and children (39.8% increase), respectively, in 2007–2008. There was a significant increase (of around 60%) in the mean level of elaidic acid, the main eighteen-carbon monoenoic TFA isomer from hydrogenated fats in children, with far less of a rise in adults. In contrast, the levels of palmitelaidic acid showed a decreasing trend in adults (9% decrease) and children (8% decrease). TFA from natural animal sources remained very low over time.

Expression of fatty acids, both in proportions and in absolute amounts, showed similar directions of changes over time; however, changes are generally more pronounced when expressed in absolute amounts (Table 2).

Discussion

This study provides the first direct evidence of the transition in serum fatty acid profiles in a rural African population over time. The profile is characterised by high proportions of SFA, *cis*-MUFA and *i*TFA, low proportions of PUFA along with a high ratio of *n*-6:*n*-3 PUFA. This profile persisted from 1990 to 2008, but with increases in the absolute concentrations of SFA, MUFA, *n*-6 PUFA and the ratio of *n*-6:*n*-3 PUFA. A specific TFA isomer from industrial processing increased in children. Current profiles show high levels of fatty acids that are known to be deleterious to health.

Compared with data reported for adults in Europe⁽⁸⁾, Japan⁽¹²⁾ and New Zealand⁽¹³⁾, the fatty acid profile in Ugandan adults in 1990–1991 is markedly different. The most prominent difference in Uganda is the far higher proportion of MUFA, mainly oleic acid. The Ugandan population is also characterised by higher proportions of SFA, mainly palmitic acid, than that reported in Europe, close to Japan and lower than New Zealand. The proportion of *n*-6 PUFA in Uganda is the lowest of

the four populations compared. Similarly, the proportion of *n*-3 PUFA is much lower in Uganda than in the three other populations, particularly Japan. These differences result in Uganda having the highest ratio of *n*-6:*n*-3 PUFA. Among the different TFA isomers, the proportion of palmitelaidic acid, sixteen-carbon monoenoic *n*-9 TFA from industrial processing, is much higher in Uganda than in Europe, while the proportion of elaidic acid, the main eighteen-carbon monoenoic *n*-9 TFA from industrial processing, is lower in Uganda than in EPIC. The proportion of TFA isomers from dairy product intake is much lower in Uganda than in Europe.

High proportions of SFA palmitic acid and MUFA oleic acid in the GPC in Uganda are likely to be the consequence of high consumption of palm oil rich in palmitic acid and oleic acid, commonly used for cooking in Africa⁽¹⁴⁾. High proportions of TFA from industrial processing are likely to be the consequence of high dietary intake of palmitelaidic acid, TFA isomer from partially hydrogenated vegetable oils (PHVO) – these are also used for cooking as well as being added to a myriad of processed foods to cheaply improve the shelf life and palatability. Major dietary sources of this specific TFA isomer are deep-fried foods, bakery products, packaged snack foods, margarines^(7,15,16) and also heating/frying and reuse of edible fats/oils⁽¹⁷⁾. Low proportions of natural TFA in the population study are likely to be the consequence of low consumption of dairy foods. Low proportions of *n*-6 PUFA likely result from low intake of vegetable oils rich in *n*-6 PUFA, as reported in a previous cross-sectional study within the GPC⁽¹⁸⁾. Finally, low proportions of long-chain *n*-3 PUFA may reflect low intake of fish⁽⁸⁾.

Trends in the levels of fatty acids from 1990 to 2008 in the population study showed significant increases in SFA (particularly in children), mainly palmitic acid, *cis*-MUFA, mainly oleic acid, *n*-6 PUFA, along with an increased ratio of *n*-6:*n*-3 PUFA,

Table 2. Evolution trends of serum phospholipid fatty acids over time in the general population cohort, Uganda (Mean values and 95% confidence intervals)

Fatty acids (μmol/l)	Time period	Adults				Children							
		Mean (μmol/l)	95% CI	<i>P</i> _{trend}	Mean (%)	95% CI	<i>P</i> _{trend}	Mean (μmol/l)	95% CI	<i>P</i> _{trend}	Mean (%)	95% CI	<i>P</i> _{trend}
Total SFA*	1990–1991	1040.82	944.84, 1146.54		44.53	43.41, 45.67		1087.15	987.6, 1196.75		45.11	44.69, 45.53	
	1999–2000	1370.70	1250.7, 1502.3		44.62	43.56, 45.70		1247.59	1126.6, 1381.6		46.52	46.06, 46.99	
	2007–2008	1221.04	1131.06, 1318.2	0.037	44.75	43.86, 45.66	0.752	1374.43	1238.9, 1524.8	0.001	46.02	45.55, 46.49	0.003
Palmitic acid (16:0)	1990–1991	673.69	583.74, 777.50		27.56	25.25, 30.07		674.57	613.94, 741.20		26.69	26.22, 27.17	
	1999–2000	844.79	737.53, 967.65		26.94	24.80, 29.26		802.82	726.39, 887.29		28.65	28.11, 29.19	
	2007–2008	789.31	704.69, 884.08	0.138	27.66	25.81, 29.64	0.896	833.65	752.96, 922.99	0.002	26.56	26.05, 27.08	0.936
Stearic acid (18:0)	1990–1991	332.77	286.90, 385.97		15.55	14.26, 16.95		377.39	340.11, 418.75		17.00	16.65, 17.37	
	1999–2000	390.25	339.09, 449.13		14.14	13.03, 15.35		407.08	364.50, 454.62		16.51	16.14, 16.89	
	2007–2008	391.95	348.55, 440.76	0.111	15.69	14.65, 16.80	0.677	503.76	450.20, 563.69	0.0001	18.25	17.84, 18.68	<0.0001
Total <i>cis</i> -MUFA†	1990–1991	562.28	511.25, 618.41		26.33	25.61, 27.07		562.70	508.55, 622.61		25.50	24.84, 26.17	
	1999–2000	737.16	673.62, 806.70		26.38	25.70, 27.08		635.61	570.84, 707.73		25.98	25.27, 26.71	
	2007–2008	656.06	608.48, 707.36	0.041	26.33	25.76, 26.91	0.992	657.37	589.28, 733.34	0.038	24.97	23.30, 24.65	0.301
Palmitoleic acid (16:1 <i>n</i> -9 <i>cis</i>)	1990–1991	30.84	27.47, 34.64		1.32	1.24, 1.40		30.30	27.05, 33.95		1.25	1.19, 1.32	
	1999–2000	43.48	38.95, 48.53		1.42	1.34, 1.51		36.96	32.75, 41.70		1.38	1.31, 1.46	
	2007–2008	35.49	32.37, 38.90	0.183	1.30	1.24, 1.37	0.523	34.00	30.07, 38.45	0.153	1.13	1.07, 1.20	0.022
Oleic acid (18:1 <i>n</i> -9 <i>cis</i>)	1990–1991	440.01	399.20, 484.99		20.50	19.88, 21.14		440.45	397.21, 488.39		19.84	19.25, 20.45	
	1999–2000	582.89	531.54, 639.20		20.77	20.18, 21.38		503.35	451.03, 561.73		20.48	19.83, 21.15	
	2007–2008	519.98	481.43, 561.61	0.029	20.77	20.27, 21.28	0.536	522.96	467.70, 584.74	0.025	19.96	18.35, 19.59	0.630
Total <i>cis</i> - <i>n</i> -6 PUFA‡	1990–1991	461.79	416.13, 512.47		21.66	20.93, 22.40		486.21	437.93, 539.82		22.00	21.30, 22.72	
	1999–2000	624.64	565.96, 689.41		22.27	21.56, 23.00		531.40	475.53, 593.84		21.71	20.97, 22.46	
	2007–2008	555.74	511.79, 603.46	0.022	22.35	21.76, 22.96	0.174	650.35	580.83, 728.18	0.0001	23.58	22.77, 24.41	0.006
Linoleic acid (18:2 <i>n</i> -6 <i>cis</i> , <i>cis</i> , <i>cis</i>)	1990–1991	266.48	239.17, 296.90		12.60	12.07, 13.14		275.20	247.25, 306.31		12.56	12.05, 13.09	
	1999–2000	383.21	345.90, 424.54		13.75	13.21, 14.31		314.06	280.29, 351.90		12.93	12.38, 13.51	
	2007–2008	318.52	292.41, 346.96	0.050	12.92	12.49, 13.36	0.596	348.41	310.33, 391.16	0.003	12.73	12.17, 13.31	0.642
Long-chain <i>n</i> -6 PUFA	1990–1991	188.53	169.26, 210.00		8.76	8.37, 9.16		204.74	183.08, 228.96		9.16	8.74, 9.60	
	1999–2000	234.26	211.51, 259.46		8.27	7.92, 8.63		208.84	185.46, 235.18		8.45	8.04, 8.88	
	2007–2008	228.45	209.77, 248.80	0.012	9.09	8.77, 9.43	0.098	290.55	257.48, 327.87	<0.0001	10.44	9.92, 10.98	0.001
Total <i>cis</i> - <i>n</i> -3 PUFA§	1990–1991	66.43	58.41, 75.55		3.27	3.04, 3.50		80.63	70.64, 92.03		3.78	3.49, 4.08	
	1999–2000	69.65	61.66, 78.68		2.60	2.43, 2.78		64.66	56.19, 74.42		2.76	2.54, 3.00	
	2007–2008	57.05	51.53, 63.17	0.041	2.44	2.31, 2.58	<0.0001	77.16	66.88, 89.02	0.588	2.92	2.68, 3.18	<0.0001
α -Linolenic acid (18:3 <i>n</i> -3 <i>cis</i> , <i>cis</i> , <i>cis</i>)	1990–1991	2.46	1.32, 4.59		0.15	0.10, 0.24		2.86	1.56, 5.24		0.17	0.11, 0.26	
	1999–2000	1.68	0.93, 3.04		0.09	0.06, 0.144		2.23	1.17, 4.25		0.12	0.08, 0.20	
	2007–2008	0.90	0.55, 1.48	0.011	0.06	0.04, 0.09	0.002	1.51	0.78, 2.90	0.149	0.08	0.05, 0.13	0.033
Long-chain <i>n</i> -3 PUFA	1990–1991	57.69	50.32, 66.14		2.89	2.68, 3.10		71.86	62.52, 82.60		3.40	3.13, 3.69	
	1999–2000	60.57	53.21, 68.95		2.29	2.14, 2.45		56.95	49.12, 66.03		2.47	2.26, 2.69	
	2007–2008	50.54	45.36, 56.31	0.092	2.20	2.08, 2.33	<0.0001	70.23	60.41, 81.64	0.745	2.68	2.46, 2.93	<0.0001
<i>n</i> -6: <i>n</i> -3 PUFA	1990–1991	6.95	6.45, 7.49		6.63	6.18, 7.11		6.03	5.58, 6.51		5.83	5.41, 6.27	
	1999–2000	8.97	8.35, 9.63		8.57	8.01, 9.16		8.22	7.57, 8.92		7.87	7.27, 8.51	
	2007–2008	9.74	9.18, 10.34	<0.0001	9.17	8.67, 9.70	<0.0001	8.43	7.75, 9.16	<0.0001	8.07	7.45, 8.74	<0.0001
Total ITFA	1990–1991	37.70	34.94, 40.68		1.72	1.58, 1.86		38.82	35.51, 42.44		1.70	1.58, 1.82	
	1999–2000	39.51	36.77, 42.46		1.39	1.29, 1.51		36.23	32.95, 39.82		1.44	1.34, 1.55	
	2007–2008	38.07	35.85, 40.43	0.938	1.51	1.42, 1.62	0.054	38.61	35.07, 42.51	0.896	1.38	1.28, 1.50	0.0001
Palmitelaidic acid (16:1 <i>n</i> -9 <i>trans</i>)	1990–1991	29.80	27.63, 32.13		1.28	1.15, 1.41		31.34	28.77, 34.13		1.30	1.19, 1.41	
	1999–2000	29.46	27.43, 31.65		0.96	0.88, 1.06		28.82	26.32, 31.56		1.08	0.98, 1.18	
	2007–2008	27.40	25.81, 29.08	0.069	1.01	0.93, 1.09	0.001	28.96	26.40, 31.76	0.205	0.97	0.88, 1.06	<0.0001
Elaidic acid (18:1 <i>n</i> -9 <i>trans</i>)	1990–1991	3.08	2.54, 3.73		0.18	0.16, 0.20		2.13	1.62, 2.81		0.13	0.11, 0.16	
	1999–2000	3.86	3.22, 4.63		0.17	0.15, 0.19		2.79	2.08, 3.73		0.14	0.12, 0.18	

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Table 2. Continued

Fatty acids (µmol/l)	Time period	Adults					Children						
		Mean (µmol/l)	95% CI	P _{trend}	Mean (%)	95% CI	P _{trend}	Mean (µmol/l)	95% CI	P _{trend}	Mean (%)	95% CI	P _{trend}
Total rTFA†	2007–2008	3.64	3.13, 4.24	0.231	0.19	0.17, 0.21	0.454	3.41	2.54, 4.59	0.022	0.15	0.13, 0.19	0.04
	1990–1991	4.31	3.49, 5.32		0.21	0.18–0.25		3.52	2.85, 4.36		0.16	0.14, 0.19	
	1999–2000	6.74	5.92, 8.23		0.25	0.21, 0.28		4.02	3.20, 5.04		0.17	0.15, 0.20	
Vaccenic acid (18:1n-7trans)	2007–2008	5.59	4.73, 6.60	0.117	0.23	0.20, 0.26	0.429	4.24	3.37, 5.34	0.238	0.16	0.14, 0.19	0.910
	1990–1991	0.07	0.03, 0.14		0.01	0.006, 0.02		0.02	0.01, 0.04		0.004	0.002, 0.006	
	1999–2000	0.04	0.02, 0.09		0.007	0.004, 0.011		0.01	0.01, 0.03		0.003	0.002, 0.005	
	2007–2008	0.11	0.06, 0.19	0.245	0.01	0.009, 0.018	0.366	0.05	0.02, 0.12	0.085	0.007	0.004, 0.01	0.106

rTFA, industrial trans-fatty acids; rTFA, ruminant trans-fatty acids.

* SFA, including 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0 and 24:0.

† cis-MUFA, including 14:1, 15:1, 16:1n-7/9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1 and 24:1.

‡ n-6 PUFA, including cis-18:2, 18:3, 20:2, 20:3, 20:4, 22:4 and 22:5.

§ n-3 PUFA, including cis-18:3, 18:4, 20:4, 20:5, 22:5, 24:5, 24:6 and 22:6.

|| Total iTFA isomers from industrial processes, including trans-16:1n-9, trans-18:1n-9, trans-18:2n-6 and trans-18:3n-3.

¶ Total rTFA isomers from animal sources, including trans-18:1n-7, conjugated linoleic acids.

in adults and children. Levels of total iTFA – with palmitelaidic acid and elaidic acid being the major contributors to total TFA from frying oils and PHVO – remained stable over time. However, when distinguishing the two TFA isomers, levels and proportions of elaidic acid, the main TFA occurring in PHVO, increased over time in children, while levels and proportions of palmitelaidic tended to decrease over time, albeit remaining high compared with European values. This set of data might suggest that the use of PHVO has further increased in the last two decades in this rural population in Uganda.

These data indicate unfavourable trends of dietary fatty acids over time. It is tempting to hypothesise that the unfavourable trend in this population study in Uganda might reflect a global change in this population's diet and be a surrogate for an early stage of nutrition transition in general. This is in line with the emergence and popularisation of soft drinks and fast foods in sub-Saharan Africa⁽³⁾. A study of the diet of urban Ethiopian adults showed the regular consumption of oil and fat, while the consumption of fruits and vegetables decreased over time⁽¹⁹⁾. A dietary survey conducted in Kenya showed significant urban/rural differences in the contribution of macronutrients to total energy intake, with higher energy from fat and lower energy from carbohydrates in urban areas⁽²⁰⁾. This study might also illustrate differences in nutrition transition stages between urban and rural areas.

The specific fatty acid profile in the Ugandan population study is of concern, as some epidemiological studies reported a positive association between intake of SFA and iTFA and mortality from CHD, ischaemic stroke and type 2 diabetes⁽²¹⁾. Intake of iTFA, even at low levels, is specifically associated with several adverse outcomes, including inflammation and cardiovascular mortality⁽²²⁾. Although limited, there is growing evidence that increasing blood proportion of iTFA is associated with an increased risk of weight gain⁽²³⁾ and breast cancer^(11,24).

If iTFA are a cause of NCD in the West and yet higher in levels in Africa, where NCD are less common, this could be attributable to other characteristics of the population, which mitigate the increased risks from higher exposure to iTFA. For examples, risk factors such as smoking, physical activity and harmful alcohol intake associated with the risk of NCD have been reported to be rare in Uganda⁽²⁵⁾. However, the impact of specific iTFA isomers on the risk of NCD has not been studied within the Ugandan population study where the proportion of some iTFA isomers is higher than that in Europe.

Key strengths of this study are the availability of blood samples collected from 1990 in adults and children. In addition, we were able to separate and quantify sixty fatty acids, including various TFA isomers from natural and industrial processes. Finally, we were able to examine the relative concentrations as well as absolute concentrations of fatty acids. Among limitations, long-term storage of blood samples might have affected fatty acids, particularly PUFA. However, samples stored between –196°C and –80°C are likely to be stable over time⁽²⁶⁾.

In conclusion, these data show evidence of an unfavourable trend over time of dietary fatty acids in a rural Ugandan population characterised by increasing levels of SFA, MUFA, iTFA (specifically elaidic acid) and n-6:n-3 PUFA. If the major

sources of these harmful fats are the types of oils and fats used for cooking, this offers opportunity for policy change to improve public health in low-income settings. Finally, we also demonstrate the power of this approach in characterising how serum fatty acid profiles have changed over time, providing a benchmark for future prospective studies and a comparator for countries at different stages of nutritional transition.

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