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ORIGINAL COMMUNICATION

Dietary habits and nutritional biomarkers in Italian type 1 diabetes families: evidence of unhealthy diet and combined-vitamin-deficient intakes

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Objective: Nutritional status and lifestyle can have profound effects on health. To analyse behaviour patterns in population subgroups of public health importance, we compared lifestyle, dietary intake of energy and selected nutrients, and nutritional biomarkers of type 1 diabetes (T1DM) patients and nondiabetic first-degree relatives against control subjects with no family history of T1DM.
Design: A cross-sectional study.
Setting: Department of Internal Medicine, University of Pisa, Italy.
Subjects: A total of 209 individuals including 38 type 1 patients, 76 relatives, and 95 healthy subjects.
Interventions: We used the European Prospective Investigation of Cancer and Nutrition questionnaires to assess dietary intake and lifestyle. Anthropometric indices and nutritional biomarkers (such as plasma levels of albumin, iron, lipids, homocysteine, vitamin B_9 and vitamin B_{12} as well as urinary outputs of nitrogen, sodium and potassium) were evaluated.
Results: Emerging health issues:
(1) In total, 45% of controls were overweight. Increasing age was associated with increasing body mass and decreasing activity in sport in front of an unchanged energy intake.
(2) The distribution of energy sources was incorrect. The proportion of caloric intake derived from total fat and cholesterol did not match general guidelines. Total dietary fibre consumption was assessed to be adequate (25 g/day) in only 27% of all the participants.
(3) Estimated daily intakes of water-soluble vitamin B_9 and fat-soluble vitamin D and vitamin E were deficient in comparison with dietary reference intakes.
(4) The prevalence of adoption and maintenance of healthful eating and physical activity habits was higher in women and T1DM patients (probably as a consequence of the medical educational intervention). On the contrary, supportiveness of the family in term of changing the undesirable behaviours at home seemed to fail.
Conclusions: This study provides first evidence indicating unhealthy dietary behaviours, which could even predispose to the development of diabetes and cardiovascular complications, in subjects living in Pisa. The combination of vitamin B_9 and vitamin E deprivation could be deleterious for endothelial function, since these antioxidants have been implicated in the modulation of nitric oxide and eicosanoid signalling.
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Keywords: type 1 diabetes families; food-frequency questionnaire; lifestyle; nutritional biomarkers

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Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease inherited in a non-Mendelian way (Pociot & McDermott, 2002). It is thought that environmental factors (both protective and inductive) do determine the penetrance of T1DM susceptibility genes (Akerblom *et al*, 2002). However, whether viral infections, dietary habits, lifestyle, and socioeconomic status may be important in

the development of T1DM is debatable (Lipton *et al*, 1999; Littorin *et al*, 2001). Moreover, behavioural science research in diabetes promotes lifestyle changes related to nutrition and physical activity (Wing *et al*, 2001), because these factors play a major role in the prevention and treatment of diabetes and related complications. However, it is often difficult to discern the efficacy of diabetes education. Thus, there is insufficient awareness on the part of physicians of the effectiveness of their own interventions.

To analyse behaviour patterns in population subgroups of public health importance, we compared lifestyle, dietary intake of energy and selected nutrients, and nutritional biomarkers of type 1 patients and nondiabetic first-degree relatives against control subjects with no family history of T1DM. This study is among the first to use the European Prospective Investigation of Cancer and Nutrition food frequency and lifestyle questionnaires to evaluate Italian type 1 diabetes families.

Materials and methods

Subjects

This cross-sectional study was carried out in the following groups of subjects:

(1) A total of 38 T1DM patients with duration of disease ranging from 4 months to 41 y (mean 18 ± 11 y). From the roster of all patients with T1DM who visited the Clinic between 31 January 2001 and 19 July 2001, we selected those patients who resided in Pisa, who had at least one living biological relative alive, who fulfilled the inclusion criteria, and who gave informed consent. The group consisted of 19 patients without diabetic complications, 10 patients with retinopathy (background or proliferative, determined by fluorescein angiography following fundus examination), and nine patients with nephropathy. All patients with nephropathy had also diabetic retinopathy. All patients had been treated from time of diagnosis with at least two daily insulin injections and were now receiving at least four daily insulin injections. None received medical treatment except insulin (0.6 ± 0.2 U/kg) and possibly antihypertensive drugs.

(2) In total, 76 first-degree relatives of T1DM patients, 44 parents and 32 siblings (2 ± 1 relative per proband). None of the relatives had clinical evidence of illness or was taking any drugs. If fasting plasma glucose was ≥ 7 mmol/l, a 75-g oral glucose tolerance test (OGTT) was performed.

(3) In all, 95 healthy subjects were recruited from the local community to achieve a similar distribution of age and sex to the families. They had no family history of T1DM, were taking no drugs, and had no clinical signs or symptoms of illness.

All subjects gave informed consent and the ethical committee of the hospital approved the study proposal.

Data collection

To assess dietary intake the country-specific questionnaire designed to capture local dietary habits in the prospective collaborative study European Prospective Investigation of Cancer and Nutrition (EPIC) was administered (Riboli & Kaaks, 1997). EPIC study was conducted in 10 European countries and used a self-administered, scanner-readable food-frequency questionnaire (FFQ) whose development, reproducibility, and relative validity were described in detail previously (Franceschi *et al*, 1993, 1995; Decarli *et al*, 1996; Pisani *et al*, 1997; Pasanisi *et al*, 2002). Energy and nutrient intakes from dietary assessment were calculated by using data from the Italian food code (Salvini *et al*, 1998). Daily amount (vq, g/day) and daily frequency of consumption (vf) of specific food items consumed were also provided.

Demographic, socioeconomic and lifestyle information was obtained by Lifestyle EPIC questionnaire (including questions on history of previous illness and surgical operations, lifetime history of consumption of tobacco and alcoholic beverages, and physical activity) (Salvini *et al*, 2002). Current smokers were defined as subjects smoking one or more cigarette, cigar or pipe per day. Ex smokers were subjects who had smoked in the past. Subjects reported the daily number of hours they engaged in housework as well as the weekly number of hours they engaged in physical activities during leisure (walking, cycling, gardening, repairing, exercise; through the year or seasonally) and during work (sedentary; standing; manual work; heavy manual work). Exercise (or sporting activities) included gymnastics, running, playing tennis, swimming, etc.

The body mass index (BMI) was calculated as body weight/height^2 (in kg/m^2). For all subjects basal metabolic rate (BMR) was calculated based on the formulas of Harris–Benedict (Harris & Benedict, 1919). The energy ratio equals daily energy intake divided by BMR. Sitting systolic and diastolic blood pressure (Korotkoff V) were measured twice and averaged after 10 min rest. Mean blood pressure (MBP) was calculated as diastolic BP + 1/3 (systolic BP − diastolic BP).

Assuming that urea nitrogen excretion is a constant proportion (85%) of total urinary nitrogen, protein intake was derived from the following formula (Isaksson, 1980):

$$\text{Calculated protein intake (g)} = 6.25 \times (\text{urine nitrogen output (g/day)} + 2g)$$

The protein ratio equals daily protein intake divided by calculated protein intake. Na and K ratios equal the daily intakes of either sodium or potassium divided by respective daily urinary excretions. Recommended Dietary Allowances (RDAs) and Adequate Intakes (AIs) were from Dietary reference intakes for energy, carbohydrate, fibre, fatty, fatty acids, cholesterol, protein and amino acids (Institute of Medicine, 1997, 1998, 2000; Food and Nutrition Board, Institute of Medicine, 2002, 2003).

Collection of samples and laboratory methods

Fasting venous blood samples and 24-h urine collections were obtained on a single occasion during the same period. Measurements were performed in freshly obtained material immediately after withdrawal, except insulin that has been measured on samples frozen at −20°C.

Creatinine, glucose, albumin, cholesterol, and triglycerides were measured by MODULAR ANALYTICS SWA and reagents from Roche Diagnostics (Milano, Italy); circulating iron by ADVIA 1650 (Bayer Divisione Diagnostici, Milano, Italy). HbA1c was evaluated by Bio-Rad DIAMAT™ fully automated glycosylated haemoglobin Analyzer System. Fasting plasma tHcy concentrations were determined by enzyme-linked immunoassay and automated fluorescence polarization analyser (FPIA, IMX System, Abbott Diagnostics, Roma, Italy). Plasma concentrations of folate and vitamin B_{12} were measured by AxSYM System (Abbott Diagnostics, Roma, Italy).

Immunoreactive insulin (IRI) was measured by commercial radioimmunoassay (Medgenix Diagnostics, Fleurus, Belgium). Insulin resistance was estimated by homeostasis model assessment ($HOMA_{IR}$) (Matthews *et al*, 1985).

Statistical analysis

The results are presented as mean ± standard deviation or median and range (food items vq and vf). Data not normally distributed were logarithmically transformed to achieve normal distributions before *t*-tests and ANOVA. The cutoff level for statistical significance was set at $P<0.05$. The unpaired Student's *t*-test (two-tailed) and Mann–Whitney *U*-test were used to determine significant differences between independent groups. Statistical analyses comparing multiple variables were performed by using ANOVA (followed by Bonferroni correction) and Kruskal–Wallis rank test. Comparison of categories was by χ^2 test. We estimated the associations by linear regression analysis (Spearman's rank correlation analysis) as well as by stepwise regression analysis and multiple linear regression (Statview Package, Abacus Concepts, Berkeley, CA, USA). When regressing the energy ratio on BMI, we adjusted for levels of physical activity. Besides analysing data from the whole study population, we also performed analyses after including separate subsets of individuals (either control subjects or T1DM patients) in the multiple regression analyses relating circulating concentrations of folate or tHcy to explanatory variables. For the purpose of making between-sex comparisons, estimated total energy and nutrient intakes were divided into body surface area (BSA, $m^2 = kg^{0.425} cm^{0.725} 0.007184$). In search of systematic measurement error (Heerstrass *et al*, 1998), mostly in the form of under-reporting, we performed linear regression analysis to analyse the relationship between estimated and calculated (on the basis of laboratory measurements) nutrient intakes as well as between BMI and energy ratio.

Results

Healthy control subjects

Table 1 displays general characteristics of the healthy control population. A total of 43 controls (45%) could be defined overweight (BMI ≥ 25 kg/m^2) and overweight was prevalent in the male gender.

Among the healthy control subjects, current smokers were 56%. Despite the mainly seated/standing occupation (73%), the energy ratio was 1.6 ± 0.3. The percentage of energy from protein, carbohydrates, and fat was 16 ± 2, 51 ± 6, 33 ± 5%, respectively, with P:S ratio 0.32 ± 0.07. The median value of alcohol consumed was 5.3 g/day (range 0–59), that is, less than 1.4% of the total calories.

In comparison with RDAs/AIs, ADA and AHA dietary guidelines, daily intake of total fat (97 ± 31 g) and cholesterol (413 ± 136 mg) was abundant, whereas intakes of fibre (22 ± 6 g), folic acid (282 ± 73 μg), vitamin D (3.2 ± 1.4 μg) and vitamin E (8.3 ± 2.4 mg) were inadequate.

A marginal plasma folate deficiency (10.4 ± 4.7 nmol/l) was observed, consistent with the low estimated folate intake. About 82% of our control subjects had serum folate ≤ 15 nmol/l. The mean plasma tHcy concentration (11.8 ± 7.0 μmol/l) was in the upper normal range. Among control subjects, serum folate showed a negative association with total energy ($r=-0.3$, $P<0.001$), protein energy ($r=-0.3$, $P<0.001$), and alcohol ($r=-0.2$, $P<0.01$) intakes. Plasma tHcy concentration was associated (multiple $r=0.5$, $P<0.001$) positively with plasma creatinine (coefficient = 0.005, s.e. = 0.001, *t*-value = 4, $P<0.001$) while negatively with plasma concentrations of vitamins B_9 (−0.02, 0.01, −2, $P<0.05$; Figure 1) and B_{12} (−0.0004, 0.0001, −3, $P<0.01$).

Women had lower BMI, MBP, fasting plasma glucose (FPG) and insulin (FPI), HOMA IR (1.9 ± 1.0 *vs* 2.4 ± 1.3, $P<0.05$), triglicerydes, and homocysteine, while higher HDL cholesterol, and plasma folate than men. The frequency of smoking was similar in both sexes. Women reported lower levels of regular exercise (2.0 ± 1.5 *vs* 3.0 ± 2.1 h/week, $P<0.01$), but were the only persons who spent time in household activities (2.5 ± 1.2 *vs* 0 h/day, $P<0.001$). Women tended to consume more soluble carbohydrates, fibre, vitamins B_9, C, A, and E (adjusted for BSA) than men. A total of 12 women (23%) were nondrinkers, and the remaining drank 3.5 g alcohol daily. A total of four men (9%) were nondrinkers, and the remaining drank 7.5 g alcohol daily ($P<0.01$ *vs* women).

TIDM families *vs* matched controls

T1DM patients had higher FPG, HbA1c, and plasma folate (Table 2). Their dietary habits appeared healthier than those observed in controls. Diabetes was associated with reduced intake of total calories, soluble carbohydrate, total and saturated fat. Even though daily cholesterol intake did not reach the suggested value (< 300 mg/day), their P:S ratio was higher than that of controls. The mean plasma folate

Table 1 Characteristics of the healthy control participants

Characteristic	*Controls*	*Women*	*Men*	P
n	95	52	43	
Age (y)	45±13	44±14	45±13	0.68
Smoker/never/ex	53/22/20	31/13/8	22/9/12	0.34
Physical activity (h/day)	1.6±0.5	1.4±0.5	1.7±0.6	0.003
BMI (kg/m^2)	25±4	23±3	26±4	<0.0001
MBP (mmHg)	93±12	88±12	98±9	<0.0001
FPG (mmol/l)	5.1±0.5	4.9±0.5	5.2±0.5	0.003
FPI (μU/ml)	9.1±4.4	8.4±4.0	10.0±4.7	0.048
HbA1c (%)	5.4±0.4	5.4±0.4	5.3±0.3	0.81
P albumin (g%)	4.3±0.3	4.2±0.3	4.3±0.3	0.07
P iron (μg/%)	84±31	83±32	91±29	0.22
Tot. cholesterol (mmol/l)	5.0±1.0	5.0±1.0	5.2±0.9	0.47
HDL cholesterol (mmol/l)	1.5±0.4	1.7±0.3	1.3±0.3	<0.0001
Triglycerides (mmol/l)	1.0±0.6	0.9±0.3	1.3±0.7	0.0003
Homocysteine (μmol/l)	11.8±7.0	9.6±3.0	14.5±9.2	0.0005
P vitamin B_{12} (pmol/l)	276±114	288±118	262±108	0.28
P folate (nmol/l)	10.4±4.7	11.8±5.4	8.8±2.9	0.0017
Energy/BSA (kJ/m^2)	6073±1503	6121±1687	6013±1263	0.73
Protein /BSA (g/m^2)	57±14	57±15	57±13	0.79
Carbohydrates/BSA (g/m^2)	185±53	188±58	182±47	0.58
Soluble carbohydrates (g/m^2)	64±26	70±31	57±17	0.019
Fat/BSA (g/m^2)	54±16	56±18	53±14	0.38
Saturated fat/BSA (g/m^2)	19±7	20±7	19±6	0.61
Cholesterol/BSA (mg/m^2)	230±73	231±82	228±60	0.81
P:S ratio	0.33±0.07	0.33±0.07	0.32±0.07	0.53
Fiber/BSA (g/m^2)	12±4	13±4	11±3	0.045
Folic acid/BSA (μg/m^2)	158±42	165±45	149±36	0.009
Vitamin C/BSA (mg/m^2)	68±28	76±30	58±21	0.0014
Retinol equivalent/BSA (μg/m^2)	596±292	643±277	540±303	0.019
Vitamin E/BSA (mg/m^2)	4.7±1.3	5.0±1.5	4.3±1.0	0.009

Demographics, biomarkers, the composition of dietary intakes estimated from the food-frequency questionnaire (adjusted for BSA) by gender.

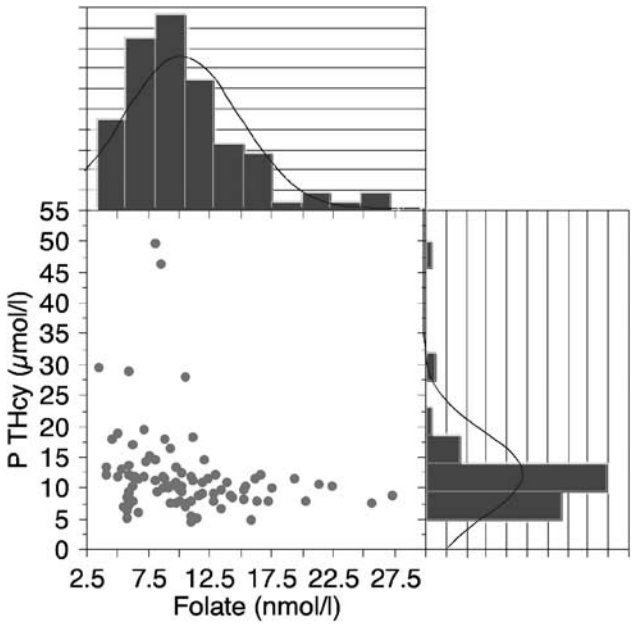

Figure 1 Relationship between plasma concentrations of homocysteine and folates in healthy control subjects.

concentration was elevated in the T1DM subset in which, however, folate intake was not correspondingly increased. Plasma tHcy concentration showed a stronger association ($r=0.8$) with the same variables as in control subjects.

There was a distinct pattern in the negative association between plasma folate concentration and diastolic blood pressure among healthy controls ($y=19.65-0.14x$, $P<0.05$) and among T1DM patients ($y=27.92-0.19x$, $P=0.10$). The regression equation significantly differs between groups in the parameter a (Figure 2). Moreover, the levels of plasma folate increased progressively with increasing category of microangiopathic complications (9.1±3.8 nmol/l in controls, 12.7±5.4 in T1DM without complications, 15.6±7.9 in T1DM with retinopathy, 15.4±7.7 in T1DM with nephropathy, $P<0.001$) (Figure 3). Depicted groups did not differ as to age, gender, BMI, and MBP (19 controls, 44±11 y, 25±4 kg/m^2, 88±9 mmHg; 10 T1DM without complications, 37±8, 26±4, 89±9; 10 T1DM with retinopathy, 45±9, 25±3, 85±9; 9 T1DM with nephropathy, 42±11, 25±3, 93±11).

Relatives had a higher plasma total cholesterol concentration (Table 3). The total amount of niacin they consumed per day was higher than corresponding controls did (higher consumption of chicken—vq 39, 0–128, in relatives *vs* 30, 0–77, in controls, $P<0.01$—and crisp bread—vq 4.0, 0–58, *vs*

Table 2 Characteristics of T1DM patients and matched controls

Characteristic	*Controls*	*T1DM*	P
F/M	22/16	21/17	0.82
Age (y)	38±11	37±12	0.74
Smoker/never/ex	21/7/10	18/15/5	0.09
Physical activity (h/day)	1.6±0.5	1.5±0.6	0.23
BMI (kg/m^2)	24±3	25±3	0.35
MBP (mmHg)	88±9	88±10	0.83
FPG (mmol/l)	4.8±0.5	12.2±5.3	<0.0001
HbA1c (%)	5.2±0.4	8.4±1.5	<0.0001
Tot cholesterol (mmol/l)	5.1±0.9	4.9±0.9	0.34
HDL cholesterol (mmol/l)	1.5±0.4	1.5±0.4	0.69
Triglycerides (mmol/l)	0.9±0.5	1.0±0.4	0.67
Homocysteine (μmol/l)	11.6±7.7	9.0±5.8	0.1
P Vitamin B_{12} (pmol/l)	306±124	331±151	0.44
P Folate (nmol/l)	9.1±3.8	14.0.±6.6	0.0001
Energy (kJ)	11 063±2427	9480±2939	0.012
Protein (g)	103±24	99±28	0.48
Carbohydrates (g)	341±83	282±120	0.016
Soluble carbohydrates (g)	118±48	82±36	0.0003
Fat (g)	98±28	82±21	0.0081
Saturated fat (g)	35±12	27±8	0.0003
Cholesterol (mg)	410±121	332±108	0.0041
P:S ratio	0.32±0.07	0.40±0.13	0.002
Fiber (g)	21±5	22±6	0.61
Folic acid (μg)	284±69	291±88	0.69
Vitamin C (mg)	123±51	124±51	0.93
Retinol equivalent (μg)	1.1±0.5	1.1±0.7	0.7
Vitamin E (mg)	8.2±2.3	8.0±2.3	0.76

1.2, 0–32; vf 0.4, 0–6.8, *vs* 0.2, 0–4.0, $P<0.05$). They reported lower physical activity levels, largely determined by their reduced routine use of cycle (0.3±0.2 *vs* 0.4±0.2 h/week, $P<0.01$). No difference in alcohol consumption emerged among families and controls (T1DM 4.2 g, 0–58, *vs* controls 4.3, 0–49; relatives 5.9, 0–95, *vs* controls 6.1, 0–59).

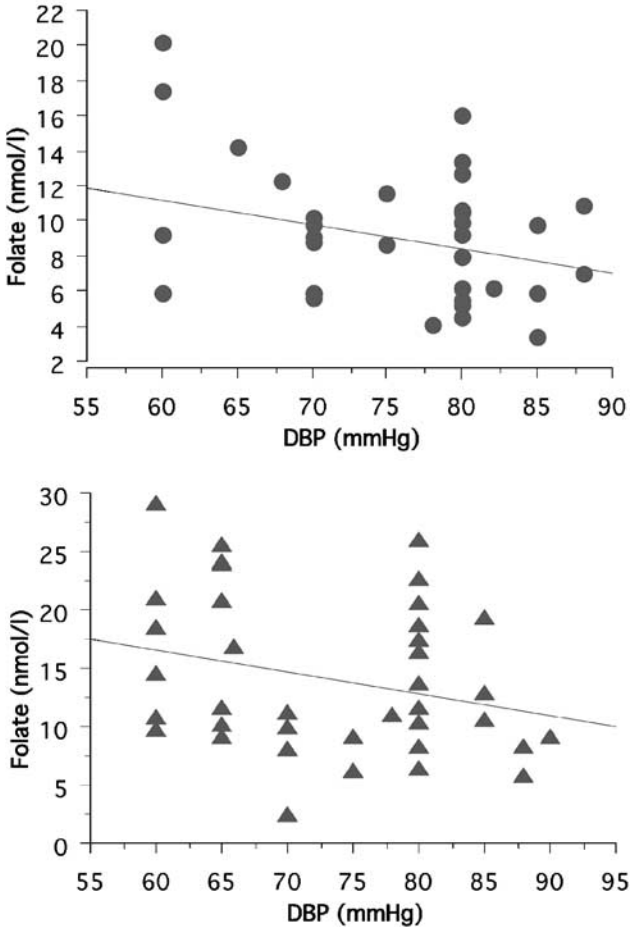

Figure 2 Relationship between plasma concentrations of folates and diastolic blood pressure in healthy control (upper panel) and T1DM subjects (lower panel).

Whole study population

The principal components analysis identified some food items that were associated with decreased folate, vitamin E, and vitamin D consumption. The intake of vitamin D fell as fish consumption frequency decreased (vq 29, 0–172; vf 0.2, 0–0.8). Low intakes of vitamin E could be related to the preferential use of olive oil (vq 27, 0.9–73; vf 3, 0.1–8) rather than other vegetable oils (vq 0.2, 0–37; vf 0.1, 0–3); low folate intake to decreased consumption of vegetables (leafy vegetables vq 14, 0–86; vf 0.6, 1–1.8; cooked veg. 10, 0–114; 0.2, 0–0.5).

Estimated intakes of protein (103±31 g), sodium (2.8±1.1 g), and potassium (3.4±0.9 g) were correlated ($r=0.4$, 0.3, and 0.3, respectively, $P<0.001$) with calculated protein intake (165±55 g), sodium output (3.9±1.7 g) and potassium (2.5±0.9 g) output, respectively. In comparison with values derived from urinary measurements, FFQ-derived protein and sodium intakes were significantly ($P<0.001$) under-reported, whereas FFQ potassium intake was overreported ($P<0.001$).

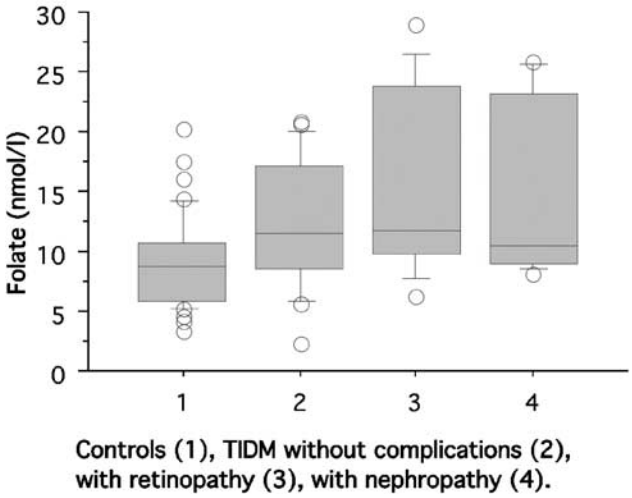

Figure 3 Levels of plasma folate in control subjects and T1DM patients gathered by microangiopathic complications.

Table 3 Demographics, biomarkers, the composition of dietary intakes estimated from the food-frequency questionnaire of all relatives and matched controls

Characteristic	*Controls*	*Relatives*	P
F/M	43/33	43/33	1
Age (y)	48±12	50±13	0.31
Smoker/never/ex	38/19/19	40/20/16	0.85
Physical activity (h/day)	1.6±0.6	1.4±0.6	0.035
BMI (kg/m^2)	25±4	26±4	0.11
MBP (mmHg)	93±12	95±12	0.44
FPG (mmol/l)	5.1±0.6	5.1±0.7	0.9
FPI (μU/ml)	9.1±4.4	9.9±6.6	0.4
HbA1c (%)	5.4±0.4	5.3±0.7	0.44
Tot cholesterol (mmol/l)	5.1±1.0	5.6±1.1	0.005
HDL cholesterol (mmol/l)	1.5±0.4	1.4±0.3	0.35
Triglycerides (mmol/l)	1.1±0.6	1.4±1.6	0.1
Homocysteine (μmol/l)	11.6±7.1	10.3±4.4	0.22
P vitamin B_{12} (pmol/l)	266±104	283±151	0.43
P folate (nmol/l)	11.1±4.8	12.0.±5.4	0.31
Energy (kJ)	10 743±2959	10 901±4037	0.78
T protein (g)	100±29	106±36	0.3
Carbohydrates (g)	328±98	326±139	0.91
Soluble carbohydrates (g/m^2)	112±43	112±60	0.99
T fat (g)	95±32	94±38	0.91
Saturated fat (g)	34±13	33±16	0.76
Cholesterol (mg)	395±139	398±168	0.9
P:S ratio	0.33±0.07	0.35±0.11	0.15
Fiber (g)	22±6	23±7	0.74
Niacin (mg)	20±5	22±7	0.031
Folic acid (μg)	280±75	292±102	0.39
Vitamin C (mg)	121±46	129±64	0.38
Retinol equivalent (μg)	1.1±0.5	1.0±0.5	0.28

When regressing the energy ratio on BMI ($r=0.2$, $P<0.001$), a degree of BMI-related under-reporting was apparent, but the significance was abolished after adjustment for exercise, that was decreasing with increasing BMI.

Increasing age was associated with unchanged energy intake ($r=0.01$, -0.00004, 0.0003, -0.1, $P=0.9$), reduced exercise ($r=-0.4$, -3.1, 0.5, -6.7, $P<0.001$), and increased BMI ($r=0.2$, 0.7, 0.25, 2.6, $P<0.01$).

In the whole study population, BMI correlated (multiple $r=0.4$, $P<0.001$) negatively with kJ/BMR (-0.52, 0.12, -4.4, $P<0.0001$) and time spent in housework (-0.32, 0.15, -2.0, $P<0.05$) and exercise (-0.28, 0.15, -1.9, $P=0.05$), whereas positively with age (0.06, 0.02, $P<0.0130$), and K ratio (0.9, 0.38, 2.3, $P<0.05$).

Discussion

Interesting findings emerged from this study that obtained for the first time in T1DM families a detailed assessment of nutrient intake levels and lifestyle using standardized EPIC questionnaires adapted for use in the Italian population. Indeed, current diet of residents from Pisa province may not provide appropriate amounts of macro- and micronutrients. EPIC data in the Italian EPIC cohorts (Pala *et al*, 2003), the Data Food Networking Reports (Trichopoulou & Naska, 2003), and evidence from a primary school health program carried out in Pisa (Giampietro *et al*, 2002) confirmed that Italian eating habits are undergoing marked changes with a tendency to less healthy eating.

The American Diabetes Association (ADA) recommends that dietary intake of fat is reduced to no more than 30% of total calories (saturated <10%, polyunsaturated ~10%). Dietary intake of cholesterol should be reduced to below 300 mg/day. A low fat/high carbohydrate (55–60%) diet, rich in fiber (25–30 g/day), is advised. It may be prudent to avoid protein intakes >15–20% of total daily energy (American Diabetes Association, 2003). American Heart Association (AHA) dietary guidelines advice limiting salt intake (<6 g/day) and engaging in a regular physical activity (30–60 min on most if not all days of the week) (Krauss *et al*, 2000). Energy intake in balance with energy expenditure is essential to prevent weight gain that accompanies aging. Polyunsaturated/saturated (P/S) ratio should be >1.0. Recent evidence supports that type 2 diabetic men in the P/S ratio >0.28 had a significantly lower risk for coronary heart disease death (Soinio *et al*, 2003).

An emerging health issue in our study is the fact that increasing age was associated with increasing body mass and decreasing activity in sport in front of an unchanged intake of total calories, contrary to recent findings in different populations (Mitchell *et al*, 2003). The distribution of energy sources was also incorrect. The proportion of caloric intake derived from total fat and cholesterol did not match general guidelines. Most controls (presumed to be consuming a free diet) ate up to 50% more protein (1.5±0.4 g/kg based on FFQ protein intake, 2.2±0.6 g/kg based on calculated protein intake) than recommended as RDAs/AIs. Total dietary fibre consumption was assessed to be adequate (25 g/day) in only 27% of all the participants. An even more relevant and unexpected health issue was the evidence of mild to moderate combined-vitamin-deficient intakes in otherwise well-nourished healthy adults. Disorders of vitamins are currently considered to be rarely epidemic especially in developed nations. Nevetheless, estimated daily intakes of water-soluble vitamin B_9 (or total folates) and fat-soluble vitamin D (sum of ergocalcipherol and cholecalcipherol) and vitamin E (sum of all tocopherols and tocotrienols) were deficient in comparison with RDAs and AIs (400 μg, 5–10 μg, and 15 mg, respectively). Taking into account that RDA values probably rely on outdated concepts of deficiency diseases and the special issue needs a reappraisal of current knowledge as to the optimal dietary intake for minimising degenerative diseases (Challem, 1999), the observed diet patterns may be seriously inadequate for health.

Despite many limitations, questionnaire assessments of dietary behaviours and physical activity levels were useful in numerous diseases, including cancer (Bingham *et al*, 2003) and coronary heart disease (Soinio *et al*, 2003). Biomarkers of dietary intake, such as urinary nitrogen and electrolytes, have been applied in nutritional validation studies. Based on our correlation coefficients, present data are within the range observed previously (Kroke *et al*, 1999), indicating

comparable relative validity for the FFQ used in the present study population. Furthermore, the direct measurement of plasma folate confirmed FFQ results. Energy and potassium ratios entered the regression model *vs* BMI. We did not observe differential underreporting depending on subject characteristics (obesity) when data were adjusted for differential physical activity.

Nutritional status and nutrition can have profound effects on health and immune functions (Harbige, 1996). The deficiency of vitamin D, whose biologically active form is a potent modulator of the immune system, has been suggested among the dietary putative etiological factors in the pathogenesis of T1DM (Akerblom *et al*, 2002; Zella & DeLuca, 2003). However, vitamin D is rather a hormone. With adequate sunlight (as in Italy) no dietary supplements are usually needed. Since calcium homeostasis was not our target, we evaluated neither plasma concentrations nor urinary wasting of calcium and phosphate ions. Thus, we were unable to confirm a real state of hypovitaminosis D. Vitamin E supplementation, by a direct antioxidant effect or some other unknown mechanisms, could delay the progress of certain autoimmune diseases (Harbige, 1996) and prevent diabetes-induced endothelial dysfunction (Dhein *et al*, 2003). Unfortunately, we did not measure circulating levels of α-tocopherol to confirm antioxidant vitamin E reserve compromised. In contrast, a reduced intake of folic acid was confirmed by the measurement of its plasma concentration as well as by the correspondent increase in plasma homocysteine levels. The usual reference range for fasting total plasma homocysteine is 5–15 μmol/l, but some authors believe that the upper limit of the normal range should be 10–12 μmol/l. In addition to glomerular filtration rate, circulating level of vitamins B_9 and B_{12} are among main determinants of plasma tHcy. In subjects with plasma folate concentrations above 15 nmol/l (ie 7 ng/ml) the tHcy concentration is on a low, normal plateau. At lower levels of plasma folate, the plasma tHcy concentration increases steadily (Fonseca *et al*, 1999; Seshadri & Robinson, 2000). Dietary factors, such as an oral fat load, a single high-fat meal, an oral methionine load, or dietary animal protein, can cause temporary endothelial dysfunction in healthy volunteers. Indeed, among our control subjects, serum folate showed a negative association with total energy and protein energy intakes. The relationship between the median value of alcohol consumption intakes of micronutrients has been extensively investigated (Kesse *et al*, 2001). That the intake of folic acid decreased with alcohol consumption may partly contribute to lower folic acid intake of men.

In addition to lowering tHcy levels, folates possess antioxidant potential (Verhaar *et al*, 2002). The main form of folate in the circulation, 5-methyltetrahydrofolate, can reduce superoxide generation by xanthine oxidase/hypoxanthine and endothelial NO synthase (eNOS). Moreover, beneficial effects of folates on haem-containing oxygenase domain of eNOS may be explained by different mechanisms: (1) tetrahydrobiopterin (BH4) rescue, (2) antioxidant effects and (3) direct effects on eNOS. Our results from food frequency EPIC questionnaire exclude an increased folate intake in T1DM. Thus, why plasma folate is high remains unclear. Could higher serum folates be necessary to promote the regeneration of BH4 and, in turn, BH4-dependent restoration of eNOS? Redox status and circulating insulin could play a role (Das, 2003). Increased basal levels of plasma nitric oxide in type 2 diabetic subjects have been interpreted as a compensatory phenomenon for the decreased vascular responsiveness (Maejima *et al*, 2001). The ability of folate, BH4, insulin, and $O_2^{-\cdot}$ to interact with each other and influence NO generation, stability and action could pertain to our finding of a different association between plasma folate and diastolic blood pressure in T1DM patients and control subjects.

Niacin or vitamin PP (as the sum of nicotinic acid and nicotinamide) was the only micronutrient whose intake was estimated to differ in relatives *vs* controls, contributed for by different dietary patterns. Interestingly, niacin has been tested for the prevention of T1DM in subjects considered at risk of developing the disease (Beales *et al*, 1999).

The prevalence of adoption and maintenance of healthful eating and physical activity habits was higher in women (spontaneously?) and T1DM patients (probably as a consequence of the medical educational intervention). Thus, we could also assess achieved compliance with diabetes clinical practice recommendations by a reliable, objective measure of patient outcomes. Since making appropriate food choices is among patient-knowledge measures (Glasgow & Osteen, 1992), our patients did translate knowledge into improved eating patterns. On the contrary, judging by dietary habits of relatives indistinguishable from those of controls, supportiveness of the family in term of changing the undesirable behaviours at home seemed to fail. However, our study included individuals spanning a wide range of ages and many relative pairs currently living apart.

In conclusion, this study of Italian T1DM families provides first evidence indicating unhealthy dietary behaviours, which could even predispose to the development of diabetes and cardiovascular complications, in subjects living in Pisa. The combination of vitamin B_9 and vitamin E deprivation could be deleterious for endothelial function, since these dietary antioxidants have been implicated in the modulation of nitric oxide and eicosanoid signalling. Ignored mild to moderated deficiencies of essential nutrients, possibly involved in the development of cardiovascular disease, could account for the fact that, regardless of current intake level, large observational studies have shown a correlation between dietary or supplemental consumption of antioxidants and cardiovascular benefits (Morris & Carson, 2003).

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