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STUDIES IN HUMANS

Dietary sources of polyphenols in the Mediterranean healthy Eating, Aging and Lifestyle (MEAL) study cohort

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ABSTRACT

The aim of this study was to estimate the dietary intake and major food sources of polyphenols in the Mediterranean healthy Eating, Aging and Lifestyles (MEAL) study cohort. A total of 1937 individuals (18 + y) of urban population of Catania, Italy, completed a validated 110-item food frequency questionnaire; Phenol-Explorer database was used to estimate polyphenol intake. Mean intake of polyphenols was 663.7 mg/d; the most abundant classes were phenolic acids (362.7 mg/d) and flavonoids (258.7 mg/d). The main dietary sources of total polyphenols were nuts, followed by tea and coffee as source of flavanols and hydroxycinnamic acids, respectively, fruits (i.e. cherries were sources of anthocyanins and citrus fruits of flavanones) and vegetables (i.e. artichokes and olives were sources of flavones and spinach and beans of flavonols); chocolate, red wine and pasta contributed to flavanols and tyrosols, respectively. These findings will be useful to assess the potential benefits of foods with high polyphenol content.

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KEYWORDS


Polyphenols; intake; general population; food sources; Italian adults

Introduction

Polyphenols are compounds naturally occurring in a variety of plant-derived foods and commonly consumed beverages, such as fruit, vegetable, coffee, tea and cocoa (Crozier et al. 2010). Polyphenols are the widest group of phenolic compounds existing in nature, which are grouped according to their chemical structures in flavonoids and nonflavonoids (such as phenolic acids, stilbenes, lignans, alkylphenols and others) (Del Rio et al. 2013; Rodriguez-Mateos et al. 2014). Flavonoids, characterized by a common skeleton of diphenylpropanes (C6-C3-C6), are divided into classes, including flavonols, flavones, flavanones, flavan-3-ols (including catechins and their oligomers proanthocyanidins), isoflavones and anthocyanins. Each class may contain up to hundreds of individual compounds that have been demonstrated, to a various extent, to activate molecular pathways regulating antioxidant and anti-inflammatory gene expression (Del Rio et al. 2010). Moreover, among flavonoids and nonflavonoids compounds, isoflavones (named also isoflavonoids) and lignans may exert estrogen-like activity, thus interacting with hormonal metabolism.

Research on polyphenols widely grown over the last years, providing the rationale for the potential beneficial effects of these compounds and polyphenol-rich foods (Hooper et al. 2008; Liu et al. 2014; Grosso et al. 2016a). Several studies have been conducted in order to estimate polyphenol consumption across countries, showing important differences depending on the origin of cohort investigated: for instance, flavonoids and phytoestrogens (such as isoflavones) are highly consumed in US and Asian countries, as their major food sources are tea and soy products; in Europe, northern and eastern countries have been reported to have high intake of phenolic acids, which are highly contained in coffee, while southern Mediterranean countries consume more flavonoids contained in fruits and vegetables (Hertog et al. 1993; Knekt et al. 1996; Dragsted et al. 1997; Knekt et al. 2002; Chun et al. 2007; Perez-Jimenez et al. 2011; Tresserra-Rimbau et al. 2013; Bai et al. 2014; Grosso et al. 2014b; Vogiatzoglou et al. 2015; Zamora-Ros et al. 2016). However, data are not univocal: considering the strong differences in dietary habits across countries, further information on consumption and

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major food sources is needed to better characterize their potential impact that such compounds may have on the general population. Thus, the aim of this study was to assess dietary intake of total, major classes and some individual polyphenols as well as dietary sources and contribution of specific foods in the Mediterranean healthy Eating, Aging and Lifestyles (MEAL) study cohort.

Materials and methods

Study population

The MEAL study is an observational investigation primarily focused on nutritional habits and their relation with a cluster of lifestyle behaviors characterizing the classical Mediterranean lifestyle. Details of the study protocol have been described elsewhere (Grosso et al. 2016b). Briefly, the cohort consisted of a random sample of 2044 men and women (age 18 + y) recruited during 2014–2015 through general practitioners ambulatories serving the urban area of Catania, one of the largest cities in the east coast of Sicily, southern Italy. All the study procedures were carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association and participants provided written informed consent after accepting to participate. The study protocol was approved by the ethic committee of the referent health authority.

Data collection

Demographics (including age, gender, educational and occupational level) and lifestyle characteristics (including physical activity, smoking and drinking habits) were collected. Educational level was categorized as (i) low (primary/secondary), (ii) medium (high school) and (iii) high (university). Occupational level was categorized as (i) unemployed, (ii) low (unskilled workers), (iii) medium (partially skilled workers) and (iv) high (skilled workers). Physical activity level was evaluated through the International Physical Activity Questionnaires (IPAQ) (Craig et al. 2003), which comprised a set of questionnaires (five domains) investigating the time spent being physically active in the last 7 days: based on the IPAQ guidelines, final scores allow to categorize the physical activity level as (i) low, (ii) moderate and (iii) high. Smoking status was categorized as (i) nonsmoker, (ii) ex-smoker and (iii) current smoker. Alcohol consumption was categorized as (i) none, (ii) moderate drinker (0.1–12 g/d) and (iii) regular drinker (>12 g/d).

Dietary assessment and estimation of polyphenol intake

Dietary data was collected using two (a long and a short version) food frequency questionnaires (FFQs) specifically developed and validated for the Sicilian population (Buscemi et al. 2015; Marventano et al. 2016). The FFQs consisted of 110 food and drink items representative of the diet during the last 6 months. Participants were asked how often, on average, they had consumed foods and drinks included in the FFQ, with nine responses ranging from “never” to “4–5 times per day”. Intake of food items characterized by seasonality referred to consumption during the period in which the food was available and then adjusted by its proportional intake in one year. After exclusion of 107 entries with unreliable intakes (<1000 or >6000 kcal/d, controlled case by case and validated due to missing food items or unreliable answers), a total of 1937 individuals were included in the analyses for the present study.

Estimation of polyphenol intake

Data on the polyphenol content in foods from the long version of the FFQ was obtained from the Phenol-Explorer database (www.phenol-explorer.eu) (Phenol-Explorer: an online comprehensive database on polyphenol contents in foods 2010). A new module of the Phenol-Explorer database containing information on the effects of cooking and food processing on polyphenol contents was used whenever possible in order to apply polyphenol-specific retention factors (Rothwell et al. 2013). After exclusion of foods that contained no polyphenols, a total of 75 items were included for the analyses. The average food consumption was calculated (in g or ml) by following the standard portion sizes used in the study and then converted into 24 h intake. Finally, a search was carried out in the Phenol-Explorer database to retrieve mean content values for all polyphenols contained in the foods obtained and polyphenol intake from each food was calculated by multiplying the content of each polyphenol by the daily consumption of each food. The polyphenol content of foods included in the FFQ that could correspond to several entries in the Phenol-Explorer database (i.e. “jams”) was weighted based on data on 24 h recalls available from the FFQ validation process. Intake of the main classes of polyphenols (flavonoids, phenolic acids, lignans, stilbenes, others) was estimated and total polyphenol intake was calculated as their sum; additional sub-class and individual polyphenols were also estimated.

Statistical analysis

Total polyphenols in the MEAL study cohort were determined for the whole study sample as well as according to the sociodemographic and lifestyle characteristics. Mean intakes of all individual polyphenols, polyphenol classes and major contributing food groups were also determined. Continuous variables are presented as means and standard deviations. Polyphenol intake distribution was tested for normality distribution with the Kolmogorov–Smirnov test and it followed a slightly asymmetric normal distribution due to extreme values of the upper side. Mann–Whitney *U* test and Kruskal–Wallis test were used to compare differences in intakes between groups, as appropriate. All reported *p* values were based on two-sided tests and compared to a significance level of 5%. SPSS 17 (SPSS Inc., Chicago, IL) software was used for all the statistical calculations.

Results

Total polyphenols intake

The analyses were conducted on a final sample of 1937 individuals. The mean intake of polyphenols was 663.7 mg/d. Polyphenol consumption was higher in men and was unequally distributed between age groups, despite with no specific trend (Table 1). No differences were found based on educational and occupational level, as well as smoking status and physical activity. However, higher polyphenol intake was found among alcohol drinkers.

Polyphenol intake and major food group contributors

The main polyphenol groups were phenolic acids (362.7 mg/d, 54.6% of total intake), flavonoids (258.7 mg/d, 38.9% of total intake), whereas stilbenes, lignans and others accounted for the remaining part (Table 2). Among flavonoids, the most consumed were flavanols (93.9 mg/d) while among phenolic acids were hydroxybenzoic acids (211.2 mg/d). Comparable mean intakes were found for flavonols, flavanones and anthocyanins, while other classes of flavonoid intake were lower.

The main dietary sources of total polyphenols were nuts, followed by nonalcoholic beverages, fruits and vegetables (Table 2). However, the various food groups contributed to different polyphenol classes and subclasses. Indeed, cereals mainly contributed to phenolic acids and, specifically, hydroxybenzoic acids. Nonalcoholic beverages, fruits and vegetables

Table 1. Total polyphenol intake according to demographic characteristics and distribution of MEAL study cohort (*n* = 1947).

	Median (IQR)	Mean (SD)	<i>p</i>
Age group			<.001
<30	467.1 (460.6)	594.2 (416.1)	
30–39	489.1 (464.9)	672.6 (806.5)	
40–49	575.5 (485.1)	792.0 (836.2)	
50–59	545.4 (431.2)	677.4 (547.3)	
60–69	550.2 (407.3)	631.1 (359.4)	
≥70	489.6 (366.0)	586.5 (386.1)	
Gender			.039
Male	508.5 (417.3)	697.6 (796.3)	
Female	527.4 (444.4)	639.8 (425.7)	
Educational level			.803
Low	528.4 (445.3)	655.1 (489.9)	
Medium	541.4 (361.1)	662.0 (606.5)	
High	465.1 (507.4)	678.2 (740.5)	
Occupational level			.263
Unemployed	540.9 (479.3)	656.1 (405.7)	
Low	526.0 (394.0)	615.1 (393.2)	
Medium	526.4 (381.5)	664.2 (571.4)	
High	491.8 (418.9)	614.0 (454.1)	
Smoking status			.173
Nonsmoker	514.0 (432.2)	671.3 (672.9)	
Ex-smoker	516.0 (364.4)	681.7 (556.8)	
Current smoker	566.9 (476.1)	601.2 (330.2)	
Physical activity			.379
Low	502.8 (473.0)	696.2 (789.0)	
Medium	514.4 (449.2)	671.0 (640.0)	
High	526.7 (394.8)	637.6 (469.0)	
Alcohol consumption			<.001
No	443.3 (299.4)	508.5 (308.8)	
Moderate (<12 g/d)	486.5 (330.9)	633.1 (673.0)	
Regular (≥12 g/d)	805.4 (382.2)	930.6 (527.7)	

contributed to both flavonoids and phenolic acids, but with some differences: nonalcoholic beverages were important sources of flavanones and flavanols, fruits mainly contributed to intake of anthocyanins and flavanones, and vegetables of flavonols; all these food groups were major sources of hydroxycinnamic acids.

Intake of a selected variety of individual polyphenols was explored (Table S1). The most consumed compounds among those selected were catechins (class flavanols); the major food contributors were nonalcoholic beverages (i.e. tea). Among other commonly consumed polyphenols, hesperetin and naringenin (class flavanones) were mainly contained in fruits. Finally, certain isoflavones (i.e. daidzein, genistein, glycitein) were consumed in minor quantities while other phytoestrogens (such as coumestrol, formononetin, matairesinol and biochanin A) were only consumed in traces or not at all.

Major food contributors of polyphenol intake

The main food sources of polyphenol in our cohort were nuts, which accounted for 28% of total polyphenol intake, followed by coffee (7%), cherries (7%), red wine (6%) and tea (5%). However, food contributors of individual classes and subclasses were different,

Table 2. Total, classes and subclasses of polyphenol intake according to food group sources.

	Food groups							
	Total foods	Nonalcoholic beverages	Alcoholic beverages	Fruits	Vegetables	Nuts	Grain-derived	Oils
	<i>mg/d per person, mean (SD)</i>							
Total polyphenols	663.7 (608.1)	150.5 (165.1)	44.5 (86.3)	133.5 (126.3)	100.2 (112.8)	190.8 (499.8)	21.4 (32.3)	11.2 (10.6)
Flavonoids	258.7 (199.1)	78.2 (128.2)	31.7 (64.7)	86.2 (84.1)	51.5 (51.4)	0.3 (0.5)	–	–
Flavanols	57.0 (45.6)	6.7 (12.2)	2.7 (5.4)	5.8 (7.1)	40.9 (40.0)	0.1 (0.1)	–	–
Flavanols	93.9 (118.2)	56.4 (108.3)	18.2 (36.9)	10.1 (9.6)	1.9 (2.4)	0.2 (0.4)	–	–
Flavanones	37.9 (42.0)	13.1 (26.8)	0.4 (0.7)	24.4 (28.9)	0.1 (0.1)	–	–	–
Flavones	8.4 (10.2)	2.0 (4.1)	–	1.1 (1.2)	5.4 (8.9)	–	–	–
Anthocyanins	55.4 (55.3)	–	8.4 (17.4)	44.9 (52.5)	2.2 (6.2)	–	–	–
Isoflavones	4.0 (14.4)	–	–	–	1.1 (8.8)	–	–	–
Dihydroflavonols	2.1 (4.3)	–	2.1 (4.3)	–	–	–	–	–
Phenolic acids	362.7 (516.0)	70.8 (59.0)	9.0 (15.1)	45.0 (43.6)	35.8 (49.4)	190.5 (499.7)	3.8 (4.9)	7.1 (10.5)
Hydroxybenzoic acids	211.2 (503.9)	11.2 (21.2)	4.4 (7.0)	1.9 (2.0)	3.0 (7.5)	190.5 (499.7)	0.2 (0.3)	–
Hydroxycinnamic acids	150.8 (90.4)	59.6 (52.6)	4.5 (8.2)	43.1 (42.1)	32.3 (43.0)	–	3.6 (4.7)	7.1 (10.5)
Stilbenes	1.9 (3.5)	–	1.8 (3.5)	0.1 (0.1)	–	–	–	–
Lignans	2.8 (2.6)	–	–	2.2 (2.5)	0.3 (0.4)	–	0.1 (0.1)	0.2 (0.1)
Others	37.6 (48.7)	1.5 (1.7)	2.1 (3.6)	0.0 (0.1)	12.6 (33.5)	–	17.6 (29.4)	3.8 (1.7)
Hydroxyphenilacetic acid	0.5 (1.1)	–	0.1 (0.2)	–	0.4 (1.1)	–	–	–
Hydroxybenzaldehydes	0.3 (0.6)	–	0.3 (0.6)	–	–	–	–	–
Tyrosols	15.0 (26.1)	–	1.6 (2.9)	–	9.6 (25.7)	–	–	3.8 (1.7)

Table 3. Total, classes and subclasses of polyphenol intake according to major individual food sources.

	Major food sources (%)				
	Nuts (28)	Coffee (7)	Cherries (7)	Red wine (6)	Tea (5)
Total polyphenols	Cherries (11)	Red wine (11)	Tea (10)	Spinach (10)	Fruit tea (7)
Flavonoids	Spinach (49)	Beans (10)	Apples (7)	Tea (6)	Red wine (4)
Flavanols	Tea (27)	Red wine (18)	Fruit tea (17)	Green tea (17)	Dark chocolate (7)
Flavanones	Confectionated orange juice (30)	Citrus fruits (26)	Orange juice (24)	Red orange (19)	Red wine (1)
Flavones	Artichokes (44)	Orange juice (21)	Confectionated orange juice (11)	Black olives (8)	Mellon (6)
Anthocyanins	Cherries (52)	Strawberries (25)	Red wine (15)	Black olives (4)	Red oranges (4)
Isoflavones	Soy (95)	Beans (5)	Nuts (1)	Beer (0)	–
Dihydroflavonols	Red wine (96)	White wine (4)	–	–	–
Phenolic acids	Nuts (53)	Coffee (13)	Artichokes (4)	Cherry (4)	Decaffeinated coffee (3)
Hydroxybenzoic acids	Nuts (90)	Tea (3)	Fruit tea (2)	Red wine (1)	Green tea (1)
Hydroxycinnamic acids	Coffee (32)	Artichokes (10)	Cherries (10)	Decaffeinated coffee (6)	Pear (6)
Stilbenes	Red wine (85)	White wine (7)	Strawberries (3)	Grapes (2)	Berry fruits (1)
Lignans	Citrus fruits (44)	Red orange (32)	Garlic (11)	Olive oil (7)	Bread (3)
Others	Integral bread (35)	Black olives (19)	Green olives (16)	Olive oil (10)	Pasta (7)
Hydroxyphenilacetic acid	Green olives (65)	Black olives (13)	Red wine (12)	Beer (8)	White wine (2)
Hydroxybenzaldehydes	Red wine (91)	White wine (5)	Beer (3)	Black olives (1)	Olive oil (0)
Tyrosols	Black olives (46)	Olive oil (24)	Green olives (20)	Red wine (8)	Beer (1)

mainly depending on the most representative polyphenols contained (Table 3). For instance, nuts and coffee were major sources of phenolic acids: specifically, nuts together with teas and red wine were major sources of hydroxybenzoic acids, coffee together with artichokes and cherries were major sources of hydroxycinnamic acids. Major sources of flavonoids were the following: (i) fruits, mostly cherries, but also strawberries as anthocyanins source, apples as sources of flavanols and orange/citrus juices as sources of flavanones; (ii) teas, including fruit, green and regular tea as sources of flavanols; (iii) red wine and chocolate, as sources of flavanols; vegetables, such as spinach and beans as sources of flavonols and artichokes and black olives as sources of flavones. Other foods characteristic of a Mediterranean diet, such as olives, wines and

pasta were major sources of other polyphenols, including tyrosols, hydroxyphenilacetic acid and hydroxybenzaldehydes.

Discussion

This study is the first to report a detailed description of the total and main classes of polyphenol intake in a nutritional cohort in Sicily. Despite it has been previously reported that diet of individuals living in this Mediterranean island still characterized by a good adherence to the Mediterranean diet among adults (Grosso et al. 2014a), we found relatively low intake of total polyphenols. A study from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort reporting individual center dietary

intakes of polyphenols using the same database to assess polyphenol intake showed slightly higher consumption, probably due to a methodological difference (i.e. calculation of total polyphenols by summing individual compounds rather than only main classes) (Zamora-Ros et al. 2016); however, they reported a north-to-south gradient, with southern countries showing lower consumption of polyphenols. Compared to international cohorts sharing similar methodology we found lower total polyphenol intake than in Polish (1740 ± 630) (Grosso et al. 2014b), French (1193 ± 510 mg/d) (Perez-Jimenez et al. 2011) and rather similar to Spanish (820 ± 323 mg/d) (Tresserra-Rimbau et al. 2013) population. When comparing individual polyphenol classes with European data, consumption of flavonoids reported in our study was lower than reported for the EPIC cohort (Zamora-Ros et al. 2016) and similar than the one reported for Italy from the European Food Safety Authority (EFSA) (291 mg/d) (Vogiatzoglou et al. 2015); when referring to individual studies, intake of flavonoids in our cohort was generally higher than reported in previous individual studies conducted in US (Chun et al. 2007), Denmark (Dragsted et al. 1997), Finland (Knekt et al. 1996; Knekt, et al. 2002), Netherlands (Hertog et al. 1993) and Japan (Arai et al. 2000), but similar when considering newer studies (Bai et al. 2014). Flavanols were the second most abundant flavonoid consumed in all cohorts: however, compared to consumption in another southern Italian cohort using the BioActive Substances in Food Information System (Eurofir-eBASIS) as referent database (Pounis et al. 2016), mean intake of all individual classes of flavonoids were higher in our cohort.

Minor differences in polyphenol intake reported may depend on different methods to estimate their consumption; with exception of more recent databases (i.e. Phenol-explorer, USDA, ESFA, Eurofir-eBASIS), older studies often calculated by their own the content of certain polyphenol classes directly from selected foods, thus resulting in generally lower total estimates compared with newer studies. Besides these methodological differences, country-specific food consumption may be a major contributor for the reported polyphenol intakes. Indeed, the main food contributors to total polyphenol intakes in our cohort were cereals (grain-derived foods) while in the other cohorts using the same methodology for the estimation of polyphenols, were nonalcoholic beverages and fruit (Perez-Jimenez et al. 2011; Tresserra-Rimbau et al. 2013; Grosso et al. 2014b). Compared to non-Mediterranean cohorts, the main difference of food groups consumed relied mainly on general lower consumption of tea

and coffee: the predominantly consumed coffee in Italy is espresso coffee, which account for a more concentrated beverage (25–30 ml) compared with regular coffee (150–200 ml) (Zujko et al. 2012; Grosso et al. 2015a). Coffee and tea are major contributors of flavanols and hydroxycinnamic acids, which in our cohort were far less consumed than in non-Mediterranean countries (Zujko et al. 2012; Grosso et al. 2015a). Another important difference regard preferences in the type of fruits, as non-Mediterranean cohorts reported to consume more berry fruits and apples, which provides anthocyanins and flavanols; however, total intake of the former resulted higher in our cohort, suggesting that also other diet components contribute to anthocyanin intake, such as red wine and red oranges, which highly consumed among Sicilian inhabitants and are high sources of polyphenols (Grosso et al. 2013; Grosso, et al. 2014a; Maria Cova et al. 2015). Compared to other Mediterranean cohorts, besides mostly similar intake of vegetables, the main differences depended on nonalcoholic beverage consumption and type of fruits. However, the most peculiar difference with all the other cohorts was the contribution of nuts (in terms of quantity) and olives and olive oil (in terms of quality of polyphenols) in the Sicilian population: nuts were among the main contributors of hydroxybenzoic acids, which in other cohorts were generally provided by tea and red wine (Lee et al. 2016; Zeng et al. 2016), while in Mediterranean cohorts are also provided by olives (Perez-Jimenez et al. 2011; Tresserra-Rimbau et al. 2013; Grosso et al. 2014b). Such peculiarities may represent key points for the associated benefits of a Mediterranean-type diet, which is characterized by these components (NaAN EPoDP 2011; Grosso et al. 2015c; Kabiri et al. 2016). However, despite specific data on the role of such food products for the Sicilian center of the EPIC cohort was not available, there was no evidence of such peculiarity referred to Mediterranean countries in general. Thus, further attention on such issue should be paid in future investigations.

In our study, we found that independent of total energy intake, men were consuming more polyphenols than women: these results are in line with other studies showing similar results (Ovaskainen et al. 2008; Perez-Jimenez et al. 2011), especially concerning flavonoids (Chun et al. 2007; Zamora-Ros et al. 2010), due to higher consumption of specific foods, such as coffee and wine. Significant differences of polyphenol intake were also due to age but with different distribution compared with other cohorts: indeed, previous studies showed higher intake of polyphenols in younger

participants (Grosso et al. 2014b), while we found higher intake in more adult age groups. A reason can be the different attitude of younger and older population in different countries, as the former living in Mediterranean countries has been reported to slowly abandon traditional dietary patterns toward more Westernized dietary habits (Grosso et al. 2016c), while those living in non-Mediterranean countries are engaging in eating behaviors, such as increased physical activity and consumption of fruits and vegetables (Grosso et al. 2015b). As for alcohol drinking, we found that alcohol consumers (>12 g/d) had higher intake of polyphenols; this could be easily explained by the fact that alcoholic beverages (especially wine and beer) are themselves dietary sources of polyphenols.


Results of this study should be considered in light of some limitations. As previously stated, differences in absolute intakes of polyphenols with the other studies may depend on methodological differences. Even when the source of data on polyphenols was the same, the use of a FFQ may overestimate compared with 24 h recall the estimation of food consumption. However, the overall estimation, yet with some minor differences, was mainly comparable to other studies. Second, the FFQ used to collect dietary data may have lacked some of foods rich in polyphenols, such as spices, leading to possible underestimation. Third, due to the retrospective nature of the instrument, data may have been affected by recall bias.

In conclusions, this study provided a description of the total polyphenol intake and main food contributors of dietary polyphenols in a Southern Italian urban population. Besides the potential effects of fruit and vegetable on human health, it is important that future studies will further investigate the role of other highly consumed polyphenol-rich foods, such as grain-derived products, in order to underlie the potential benefits or threats for health of Italian individuals living in Sicily.

Disclosure statement

The authors report no conflicts of interest.

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