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# Non-enzymatic antioxidant capacity and risk of gastric cancer

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ABSTRACT

*Background:* Consumption of fruit and vegetables has been inversely related to gastric cancer. Two studies found that dietary antioxidant capacity has some role in explaining this association. We investigated the overall antioxidant effect from diet on gastric cancer using three measures of non-enzymatic antioxidant capacity (NEAC).
*Methods:* We used data from an Italian case-control study including 230 patients with incident, histologically confirmed gastric cancer, and 547 frequency matched controls admitted to the same hospitals for acute non-neoplastic diseases. A reproducible and valid food frequency questionnaire was used to assess subjects' usual diet. NEAC was measured using Italian food composition tables in terms of Trolox equivalent antioxidant capacity (TEAC), Ferric reducing-antioxidant power (FRAP) and Total radical-trapping antioxidant parameter (TRAP). We estimated the odds ratios (OR) of gastric cancer and the corresponding 95% confidence intervals (CI) using conditional logistic regression models including terms for recognized gastric cancer risk factors and total energy intake.
*Results:* NEAC was inversely related with gastric cancer risk with ORs for the highest versus the lowest quintile of 0.54 (95%CI, 0.33–0.88) for TEAC, 0.67 (95%CI, 0.42–1.07) for FRAP and 0.57 (95%CI, 0.36–0.90) for TRAP.
*Conclusions:* A diet rich in antioxidant capacity reduced gastric cancer risk, suggesting a high consumption of fruit and vegetables and a moderate consumption of wine and whole cereals.


## 1. Introduction

Gastric cancer is the fifth most common cancer and the third leading cause of cancer death over both sexes worldwide, with almost 1 million cases and over 700,000 deaths estimated in 2012 [1]. *Helicobacter Pylori* is a key determinant of gastric cancer. However, other characteristics, including genetic, environmental, and lifestyle (notably, tobacco smoking and diet) factors [2] also have a role [3]. In particular, gastric cancer has been inversely related to fruit and vegetable intake [4–6]. This has been attributed to several antioxidants contained in fruit and vegetables that may act against oxidative stress and prevent cell damage [7,8]. Several investigations considered the role of single antioxidants on gastric cancer risk reporting inverse associations with vitamin C, vitamin E, beta-carotene and flavonoids [6,9–13]. It is still unclear, however, whether a specific compound or a group of compounds – through possible synergic effects – is responsible for a protective role against gastric cancer.

*Abbreviations:* BMI, body mass index; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; FRAP, ferric reducing-antioxidant capacity; ICD, International Classification of Diseases; NEAC, non-enzymatic antioxidant capacity; OR, Odds ratio; SD, standard deviation; TAC, total antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter.

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In order to capture the potential overall antioxidant effect of the diet, a few studies have analyzed the non-enzymatic antioxidant capacity (NEAC), also known as total antioxidant capacity (TAC). NEAC is defined as the moles of oxidants neutralized by 1 L of plasma, food extracts or single molecules, and represents a marker of antioxidant potential, including single and synergistic redox interactions [14]. The most common measures of NEAC are Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant-power (FRAP) for single-electron transfer mechanism, and total radical-trapping antioxidant parameter (TRAP) for hydrogen atom transfer mechanism. Two studies investigated the relation between dietary NEAC and gastric cancer risk [15,16]. A Swedish case-control study, including 505 cases, showed that a high TRAP from fruit and vegetables was associated with a 35% reduction in gastric cancer risk [15]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study, including 444 cases of gastric cancer, found a significant reduction of risk for an increase of NEAC, with an hazard ratio for the highest versus the lowest quintile of 0.61 for FRAP and 0.66 for TRAP [16].

To provide further information on this issue, we investigated the relation between three NEAC indices (TEAC, FRAP and TRAP) and gastric cancer risk using data from an Italian case-control study [5].

## 2. Methods

### 2.1. Participants and study design

We analyzed data from a case-control study of gastric cancer conducted between 1997 and 2007 in the greater Milan area in Italy [5]. Cases were 230 patients (143 men, 87 women; median age 63 years, range 22–80 years), admitted to major teaching and general hospitals with incident, histologically confirmed stomach cancer (ICD IX 151.0–151.9), diagnosed no longer than 1 year before the interview, and with no earlier diagnosis of cancer. Controls were 547 patients (286 men, 261 women; median age 63 years, range 22–80 years), frequency matched to cases by age and sex, and admitted to the same hospitals as cases for a wide spectrum of acute, non-neoplastic conditions, unrelated to risk factors for stomach cancer and long term diet modification. Of these, 20% were admitted for traumas, 23% for other orthopaedic conditions, 22% for acute surgical and 35% for other miscellaneous disorders. Less than 5% of cases and controls contacted refused to participate.

### 2.2. Data collection and assessment of non-enzymatic antioxidant capacity (NEAC)

Centrally trained interviewers administered a standard questionnaire to cases and controls during their hospital stay using a structured questionnaire covering socio-demographic and anthropometric measures, selected lifestyle habits including tobacco smoking, alcohol drinking, family history of cancer and personal medical history. A satisfactorily reproducible [17] and valid [18] food frequency questionnaire (FFQ) included the average weekly consumption of 83 foods and beverages. Intakes lower than once a week but at least once per month were coded as 0.5 per week.

Using Italian food tables, we developed a database for our FFQ in terms of three NEAC indexes: the Trolox Equivalent Antioxidant Capacity (TEAC), which measures the ability of antioxidant molecules to quench the long-lived $ABTS^{+}$ compared with that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox, the Ferric Reducing-Antioxidant Power (FRAP), which measures *in vitro* the reduction of the $Fe^{3+}$ (ferric ion) to $Fe^{2+}$ (ferrous ion) in the presence of antioxidants, and the Total Radical-trapping Antioxidant Parameter (TRAP), which measures the protection provided by antioxidants on the fluorescence decay of R-phycoerythrin (lag-phase) during a controlled peroxidation reaction.[19] The frequency of consumption of each food item of the FFQ was translated into average daily TEAC, FRAP and TRAP, taking the portion size into account. Measures were standardized as mmols of $Fe^{2+}$ equivalents per 100 g (solid foods) or 100 ml (beverages) for FRAP and mmols of Trolox per 100 g (solid foods) or 100 ml (beverages) for TEAC and TRAP.[20]

We excluded the contribution of coffee to NEAC since Maillard products (produced during the process of coffee roasting) are the main contributor to the high *in vitro* antioxidant capacity of coffee [21] and – due to their high molecular weight – may act in a peculiar way from other antioxidants. It is still unclear if they are efficiently absorbed, and hence display an antioxidant effect *in vivo*.[22]

Table 1 gives the contribution of main food groups to TEAC, FRAP and TRAP among controls in the two sexes and in both sexes combined. Among fruit, the stronger contributors to NEAC were citrus fruit (13% for TEAC and FRAP, and 9% for TRAP) and apple and pears (5% for TEAC and FRAP, and 7% for TRAP) among all controls. The three indices were strongly correlated, with linear correlation coefficients around 0.9.

Energy intake was computed using an Italian food composition database [23], supplemented with other published data.

### 2.3. Statistical methods

We categorized TEAC, FRAP and TRAP into sex-specific tertiles based on the control distribution. We estimated the corresponding odds ratios (OR) and 95% confidence intervals (CI) of gastric cancer using conditional multiple logistic regression models, conditioned on age (<50, 50–59, 60–69, ≥70 years old) and sex. Models included terms of year of interview (<2002, ≥2002), education (not having completed the middle school, <7 years; not having completed the high school, 7–11 years; having high or a higher

**Table 1**
Contribution of food groups to three dietary non-enzymatic antioxidant capacity indices (%) among controls in men, women and both sexes. Italy, 1997–2007.

| | | Alcoholic beverages | Fruits | Vegetables | Oils | Cereals | Legumes | Potatoes | Others |
|---|---|---|---|---|---|---|---|---|---|
| TEAC | Men | 42.0 | 24.8 | 12.9 | 9.5 | 7.0 | 0.7 | 0.7 | 2.4 |
| | Women | 17.9 | 37.8 | 20.4 | 11.1 | 7.8 | 1.0 | 0.9 | 3.0 |
| | Total | 32.5 | 30.0 | 15.9 | 10.1 | 7.3 | 0.8 | 0.8 | 2.7 |
| TRAP | Men | 53.1 | 22.3 | 15.1 | 0.0 | 7.7 | 0.3 | 0.7 | 0.9 |
| | Women | 25.1 | 37.8 | 24.8 | 0.0 | 9.5 | 0.6 | 1.0 | 1.2 |
| | Total | 42.7 | 28.0 | 18.7 | 0.0 | 8.3 | 0.4 | 0.8 | 1.0 |
| FRAP | Men | 44.1 | 25.0 | 17.8 | 0.0 | 9.8 | 0.7 | 1.3 | 1.3 |
| | Women | 18.9 | 38.9 | 26.9 | 0.0 | 11.1 | 1.0 | 1.6 | 1.7 |
| | Total | 34.2 | 30.4 | 21.4 | 0.0 | 10.3 | 0.8 | 1.4 | 1.5 |

TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter; FRAP, ferric reducing antioxidant-power.

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