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Original Paper

Higher Fruit Intake Is Related to *TNF-α* Hypomethylation and Better Glucose Tolerance in Healthy Subjects

Júlia Cristina Cardoso Carraro[a] Helen Hermana Miranda Hermsdorff[b] Maria Luisa Mansego[c] Maria Ángeles Zulet[c–e] Fermín I. Milagro[c, e] Josefina Bressan[b] J. Alfredo Martínez[c–e]

[a]Department of Clinical and Social Nutrition, Universidade Federal de Ouro Preto, and [b]Department of Nutrition and Health, Universidade Federal de Viçosa, Minas Gerais, Brazil; [c]Department of Nutrition, Food Science and Physiology, Centre for Nutrition Research, University of Navarra, [d]IdiSNA, Navarra Institute for Health Research, Pamplona, and [e]CIBERobn, Centro de Investigación Biomédica en Red-Fisiopatología de la Obesidad y la Nutrición, Carlos III Institute of Health, Madrid, Spain

Key Words
Biomarker · Inflammation · Epigenetics · DNA methylation · Diet · Fiber · Vitamin C

Abstract
Background/Aim: This study hypothesized an association between healthy dietary patterns, hypermethylation of the tumor necrosis factor-α *(TNF-α)* promoter and decreased risk of metabolic changes. ***Methods:*** Forty normal-weight young women were involved in this cross-sectional study. DNA was isolated from white blood cells, and CpG site methylation in *TNF-α* was analyzed by Sequenom EpiTyper. The quality of the diet was assessed by Healthy Eating Index (HEI-2005). ***Results:*** Contradicting our hypothesis, HEI-2005 score was negatively associated with CpG5 (r = –0.460, p = 0.003) and *TNF-α* total methylation (r = –0.355, p = 0.026). A higher intake of fruits was related to lower insulin, HOMA-IR, and *TNF-α* methylation. No other dietary pattern was related to *TNF-α* methylation. *TNF-α* total methylation correlated positively with systolic blood pressure (r = 0.323; p = 0.042) and CpG5 methylation with body mass index (r = 0.333, p = 0.036). Furthermore, fiber intake was negatively associated with the CpG5 (r = –0.324, p = 0.041) and *TNF-α* total methylation (r = –0.434, p = 0.005), whereas vitamin C intake was negatively associated with *TNF-α* total methylation (r = –0.411, p = 0.009). Intakes of apples and citrus fruits were negatively associated with *TNF-α* total methylation. ***Conclusion:*** A healthy dietary pattern and higher fruit intake (particularly apples and citrus fruits) were related to better glucose tolerance in healthy subjects, which could be mediated by lower *TNF-α* methylation.

Prof. J. Alfredo Martínez
C/ Irunlarrea 1
University of Navarra
ES–31008 Pamplona (Spain)
E-Mail jalfmtz@unav.es

Introduction

Healthy dietary patterns and diets rich in fruits and vegetables (FV) are widely recommended as a health promotion strategy due to high concentrations of vitamins, minerals, antioxidants, phytochemicals and dietary fiber [1]. The occurrence of these foods in the habitual diet contributes to explain the 'fiber hypothesis', which states that increased fiber intake protects against Western diseases [2]. In this context, several studies have reported an association between FV intake and lower risk of chronic illnesses, such as cardiovascular diseases [3], oxidative stress [4] including lower DNA oxidation [5], and inflammation, being the expression of inflammatory genes inversely proportional to the consumption of fruits [6]. A dietary pattern rich in fruits and dairy products has also been related to decreased odds of impaired blood glucose, hypertriglyceridemia and metabolic syndrome (MetS) risks [7]. In addition, FV have low energy density, making them interesting for body weight management [8].

MetS has been defined as a cluster of medical disturbances related not only to increased cardiovascular risk and type 2 diabetes, but also to higher mortality [9]. MetS risk factors include diet [10], lifestyle [11], oxidative stress [12], genetics [13] and epigenetic mechanisms such as DNA methylation [14, 15].

Chronic inflammation has been proposed as a potential link between excessive weight and adiposity and metabolic complications of obesity [16]. Indeed, tumor necrosis factor-α (TNF-α), one of the major mediators of inflammatory response [17], is usually overexpressed in obesity and with the number of MetS components [18], and is upregulated in white adipose tissue in obese individuals with insulin resistance [19].

On the other hand, several nutrients and bioactive compounds have been reported to affect epigenetic mechanisms involved in gene expression regulation, such as DNA methylation, thus contributing to the prevention of the development of metabolic disorders [20]. In fact, DNA methylation can be related to MetS phenotypes, and these relationships among epigenetics, diet and disease may be a cyclic interplay [21].

Overall, we hypothesized that healthy dietary patterns can change the relationship between epigenetic signature and metabolic traits, increasing the DNA methylation of the *TNF-α* promoter, and hence, decreasing its expression. Thus, this study aimed to evaluate the effect of healthy dietary patterns and food intake on DNA methylation of the *TNF-α* gene in white blood cells (WBC) in normal-weight healthy subjects, and the interactions between diet, TNF-α methylation and the main features of MetS.

Subjects and Methods

Subjects

This cross-sectional study included 40 normal-weight healthy women, university students of Pamplona (Navarra, Spain), with a mean age of 21 ± 3 (range: 18–28 years) and a mean body mass index (BMI) of 21.0 ± 1.7 (range: 18.5–24.9). Initial enrollment screening evaluations consisted of a medical history, physical examination, and fasting blood biochemistry, to exclude subjects with evidence of any chronic inflammatory, heart, or respiratory diseases, as detailed elsewhere [22]. Other exclusion criteria were hormonal treatment or drug prescription affecting glucose metabolism, alcohol and drug dependence, history of recent diet for weight loss, or unstable weight in the last 6 months. All followed procedures were in accordance with the ethical requirements of the responsible committee on human experimentation (Investigation Ethics Committee of the Clínica Universidad de Navarra, No. 79/2005), and with the Helsinki Declaration of 1975, as revised in 2000 and later years. Informed consent was obtained from all patients for being included in the study.

Anthropometry and Body Fat Composition

Anthropometric measurements were conducted according to previously described procedures [22]. BMI was calculated as the ratio between weight (kg) and height (m^2). Total body fat (%) was estimated by the equations of Durnin and Womersley [23], using four skinfold thicknesses (biceps, triceps, subscapular, and suprailiac).

Dietary Intake Assessment

Dietary intake was assessed with a semiquantitative food frequency questionnaire (136 food items) validated for Spanish people [24]. Nutrient intake was estimated using an ad hoc computer program specifically developed for this purpose, in which syntaxes that considered food frequency, serving size and food composition (amount per 100 g) were set, including the latest available information included in the food composition tables for Spain [25, 26]. Daily energy and nutrient intake were calculated as frequency nutrient composition of each portion size for each consumed food item, where frequencies were measured in nine frequency categories (6+/day, 4–6/day, 2–3/day, 1/day, 5–6/week, 2–4/week, 1/week, 1–3/month, never or almost never) for each food item. All nutrients were adjusted by total energy intake (kcal), using the residual method. The daily intake of micronutrients does not include the intake of supplements, which was analyzed as a qualitative variable. The participants were assigned to two groups (low and high fruit intake) according to the median of fruit intake (293.4 g/day). The median cutoff criteria have been previously applied [27] based on a valid and reliable method to assign two groups of risk in epidemiological studies [28]. Mediterranean dietary pattern [29] and Healthy Eating Index (HEI-2005) scores [30] were calculated to evaluate the quality of diet. HEI is a measure of diet quality based on the food group recommendations, such as fruit, vegetables, grains, dairy foods, meat and beans, oils, saturated fat, sodium and calories of fats, alcohol and added sugar [30].

Blood Pressure and Biochemical Assessments

Systolic and diastolic blood pressures were measured following the WHO criteria [31]. Venous blood samples were drawn after a 12-hour overnight fast. EDTA (ethylenediamine tetraacetic acid) plasma and WBC were separated from whole blood by centrifugation at 3,500 rpm, at 5°C for 15 min (model 5804R; Eppendorf, Germany), and immediately frozen at –80°C until assay (WBC in buffy coat). Serum concentrations of triglycerides, total cholesterol, high-density lipoprotein cholesterol, glucose and insulin were measured by standard methods as previously described [32]. The plasma low-density lipoprotein cholesterol data were calculated using the Friedewald equation [33]. Insulin resistance was estimated by the HOMA-IR (homeostatic model assessment – insulin resistance), through the following calculations: HOMA-IR = [fasting glucose (mmol/l) × fasting insulin (lU/ml)]/22.5, as described elsewhere [34]. Plasma concentrations of high-sensitive TNF-α were measured using enzyme immunoassay-based kits (R&D Systems, Minneapolis, Minn., USA) by means of an automated analyzer system (Triturus; Grifols, Barcelona, Spain).

DNA Isolation and Methylation Assays

DNA from WBC was isolated by using the Master Pure kit (Epicenter, Madison, Wis., USA). DNA quality was assessed with PicoGreen dsDNA Quantitation Reagent (Invitrogen, Carlsbad, Calif., USA) and treated with sodium bisulfite (EZ DNA methylation kit; Zymo Research, Orange, Calif., USA) following the manufacturer's protocols.

The quantitative analysis of the 5-methylcytosine levels of the *TNF-α* gene promoter was performed with Sequenom EpiTyper (Sequenom, San Diego, Calif., USA), which relies on base-specific cleavage followed by MALDI-TOF mass spectrometry, as described elsewhere [35]. Bisulfite-treated genomic DNA was amplified using two pairs of primers: 5′-GGGTATTTTTGATGTTTGTGTGTTT-3′ (forward) and 5′-AAAAATCTCCCTTCTC-CACTCACAA-3′ (reverse), designed to amplify 20 CpG sites located between nucleotides –170 to +359 of the *TNF-α* gene [36].

RNA Extraction and Expression Analysis

Total RNA from WBC was extracted with Trizol reagent (Invitrogen) and subsequently treated with DNase (DNA-free kit; Ambion/Applied Biosystems, Austin, Tex., USA) as previously described [37]. Quantitative real-time PCR was performed in an ABI PRISM 7000 HT Sequence Detection System (Applied Biosystems, Foster City, Calif., USA). Taqman probes for *TNF-α* were also supplied by Applied Biosystems. Gene expression levels were normalized by using 18s rRNA as internal control and calculated with the $2^{-\Delta\Delta Ct}$ method [38].

Table 1. Sample characterization according to median of fruit intake per day and associations between fruit intake and supplementation and physical activity

Variables	Low intake (≤293.4 g/day)	High intake (>293.4 g/day)	p value
Age, years	20.7±2.5	20.7±2.3	0.948
BMI	21.2±1.6	20.8±1.9	0.433
Body fat, %	22.2±4.7	20.7±5.1	0.344
Waist circumference, cm	68.3±4.6	67.8±4.9	0.717
Glucose, mg/dl	80.9±7.3	78.4±5.7	0.237
Insulin, μU/l	8.36±2.72	6.01±3.39	0.020*
HOMA-IR index	1.69±0.60	1.16±0.68	0.014*
TC, mg/dl	178±25	183±28	0.618
LDL cholesterol, mg/dl	102±19	106±25	0.536
HDL cholesterol, mg/dl	64±13	63±12	0.830
TC/HDL ratio	2.85±0.54	2.96±0.59	0.545
Triglycerides, mg/dl	62.9±23	65.9±23.4	0.687
NEFA, mmol/l	0.48±0.25	0.42±0.18	0.382
Systolic BP, mm Hg	113.2±10.0	107.2±9.8	0.063
Diastolic BP, mm Hg	63.5±6.3	64.5±8.7	0.680
TNF-α, pg/ml	2.36±3.90	2.37±2.71	0.992
TNF-α mRNA (RE)	1.87±0.53	1.81±0.56	0.712
Vitamin/mineral suppl. (yes)	5	11	0.053
Physical activity (yes)	8	13	0.113

NEFA = Nonesterified fatty acids; TC = total cholesterol; BP = blood pressure; RE = relative expression; suppl. = supplementation. Results are shown as mean ± SD or frequency (n) of occurrence. p values from Student's t tests for means tests and from the χ^2 test for associations. * $p < 0.05$.

Statistical Analysis

Results are reported as mean ± SD, and the normality condition was determined by the Shapiro-Wilk test. Statistical comparisons between groups were performed by Student's t test or the Mann-Whitney U test, as appropriate. Pearson and Spearman correlations were fitted to evaluate the potential correlations of *TNF-α* promoter methylation with anthropometric or metabolic features and dietary factors. The χ^2 test was used to evaluate the association between low/high intake of fruits and categorical variables of interest (vitamin and mineral supplementation and physical activity). Subjects were also categorized according to the tertiles of orange/tangerine intake. One-way ANOVA was performed to determine means differences, and polynomial contrasts for trend analyses. Statistical analyses were performed with the SPSS 15.0 software (SPSS Inc., Chicago, Ill., USA). A p value < 0.05 was considered as statistically significant.

Results

Subject's features below or above the median of fruit intake per day were similar in relation to age, vitamin and mineral supplementation and physical activity practice. However, those with a higher FV intake showed lower fasting insulin concentration and HOMA-IR. There was no difference in *TNF-α* expression in relation to fruit intake or another group of foods (table 1).

Subjects with a higher intake of fruit showed lower total methylation percentage of the *TNF-α* gene and CpG5 of the region analyzed (fig. 1). A trend towards significance ($p < 0.1$) was also observed in CpGs 14 and 19. *TNF-α* total methylation (*TNF-α* total) was related to higher systolic blood pressure, and CpG5 methylation was associated with higher BMI values (fig. 1).

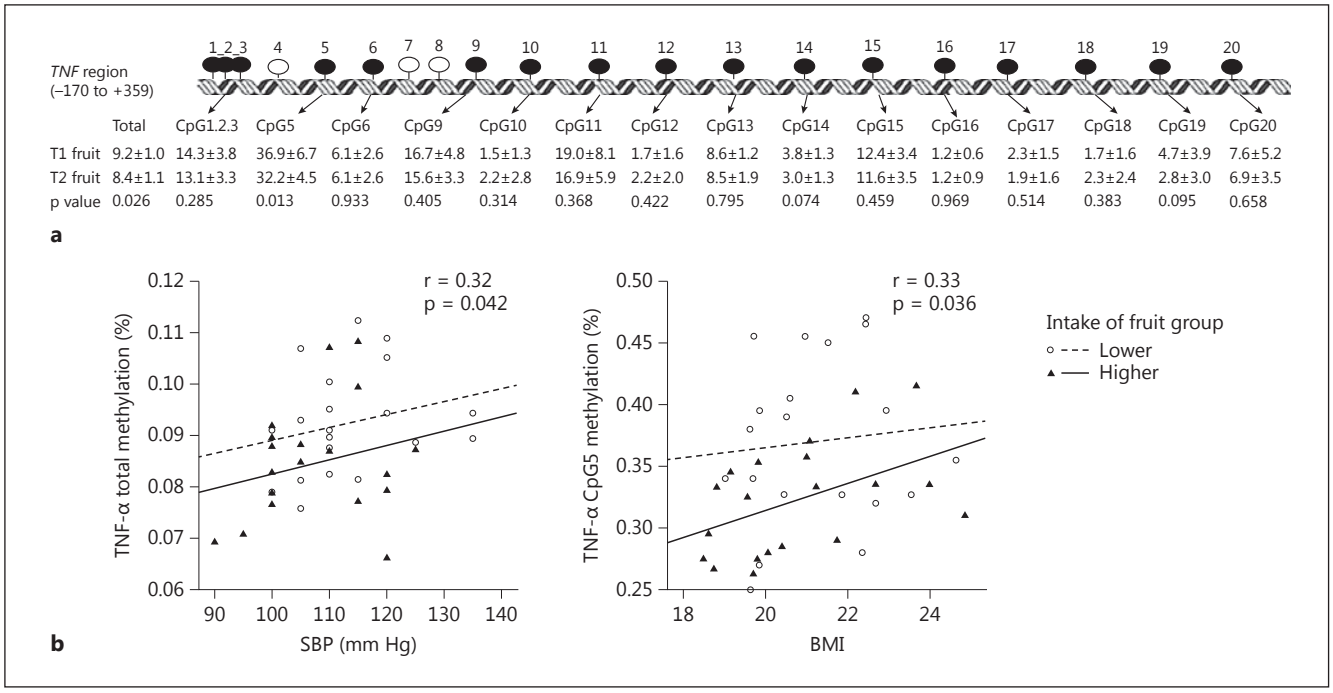

Fig. 1. Methylation (%) of 17 CpGs sites located in the *TNF-α* gene. **a** DNA methylation according to the median of fruit intake (293.4 g/day). **b** Correlation of the methylation levels of different CpGs and anthropometric/metabolic features. SBP = Systolic blood pressure.

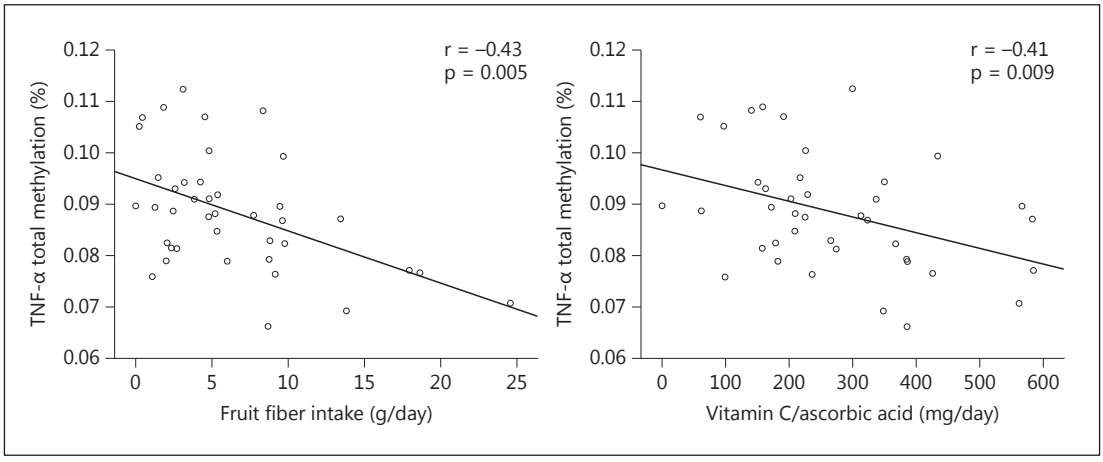

Fig. 2. Correlations between *TNF-α* total methylation and some nutrients present in fruits.

TNF-α total methylation was inversely correlated with fruit fiber and vitamin C (p = 0.005 and 0.009, respectively) (fig. 2), while the methylation levels of CpG5 were correlated with fiber (r = –0.32; p = 0.041).

Other food groups and nutrients did not correlate with CpG5 and *TNF-α* total methylation, except for HEI-2005, which was negatively associated with the methylation levels of both regions, and whole cereals, which showed a trend towards significance (table 2). Orange/tangerine and apple intakes were particularly closely associated with both CpG5 methylation

Table 2. Correlations between healthy food groups and CpG5 and *TNF-α* total methylation

Food group intake	CpG5 methylation (%)		Total methylation (%)	
	R	p	R	p
Vegetables (g/day)	–0.143	0.379	–0.216	0.180
Legumes (g/day)	–0.245	0.128	–0.137	0.400
Nuts (g/day)	–0.104	0.523	–0.014	0.932
Natural juice (g/day)	–0.037	0.822	–0.030	0.855
Cereals (g/day)	–0.022	0.894	–0.116	0.477
Whole cereals (g/day)	–0.195	0.227	–0.276	0.084
Mediterranean dietary pattern	–0.162	0.318	–0.227	0.159
HEI-2005	–0.460	0.003*	–0.355	0.026*

p values from Spearman or Pearson coefficient correlations, as appropriate. * $p < 0.05$.

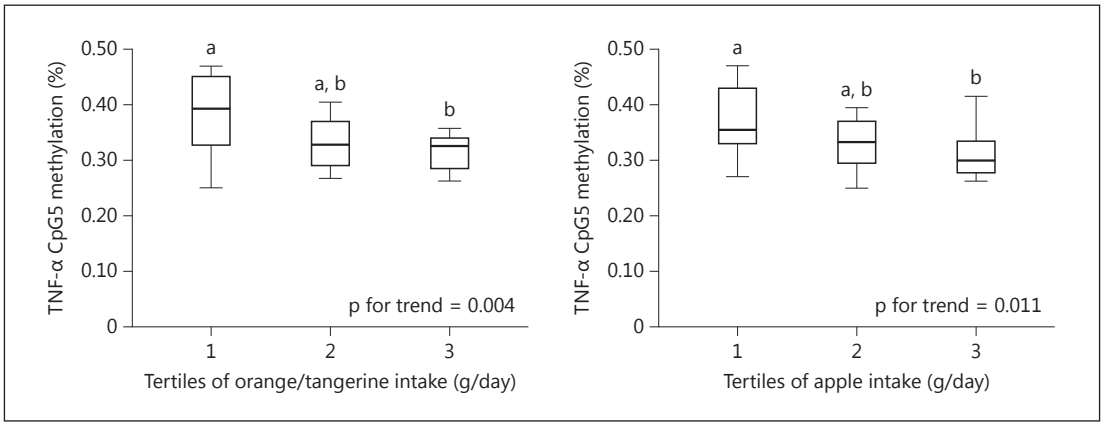

Fig. 3. CpG5 and *TNF-α* total methylation in relation to the tertiles of orange/tangerine daily intake. Different letter indicates statistical differences between the tertiles of intake. p for trend was determined by ANOVA with polynomial contrasts.

(fig. 3) and *TNF-α* total methylation (p for trend: 0.000 and 0.002, respectively). In fact, orange/tangerine and apple were the most consumed fruits by these participants (83.1 and 74.1 g/day, respectively).

Discussion

The encouragement to consume more FV is not a new strategy for health promotion [39]. In this sense, our results reveal that the higher intake of fruits was related to lower fasting insulin levels and HOMA-IR values, which means a better glucose tolerance in these healthy subjects. FV intake has been previously related to lower insulin resistance and MetS [40], and several biomarkers of FV intake, such as carotenoids and vitamin C, have been negatively associated with glycemia, serum insulin concentrations and glycosylated hemoglobin (HbA 1c) levels [41, 42].

As low-grade inflammation is involved in the development of insulin resistance, the effect of changing dietary habits (particularly higher consumption of fruits) on chronic

inflammation may be one of the protective mechanisms regarding metabolic disorders [43]. Hotamisligil [44] reported that the insulin receptor is an important target for TNF-α, and that this cytokine may be involved in the switch of tyrosine to serine phosphorylation. In this context, a beneficial effect of FV, in particular fruits, has been already described in relation to oxidative stress [5, 45] and inflammation [6, 46]. For example, it has been reported that a high FV intake reduces interleukin-6 and TNF-α concentrations and is associated with higher antioxidant capacity in plasma [46–48]. These effects are mainly attributed to antioxidants and bioactive compounds found in FV, especially vitamin C and fiber [49], which corroborates our results.

The beneficial effect of FV consumption on inflammation can be observed even at the molecular level, for example with a reduced expression of inflammatory markers [6], and could be regulated by epigenetic factors. For example, changes in dietary habits including fruit intake have been positively associated with epigenetic modifications, such as changes in LINE-1 DNA methylation [50] or a lower prevalence of DNA hypomethylation, an association that was dose dependent [51]. Changes in the methylation levels of specific gene promoters may regulate the expression of genes by modifying the interaction of transcription factors and methyl-DNA-binding proteins [52]. In this sense, our results reveal that *TNF-α* promoter methylation is related to healthy dietary patterns, especially to higher fruit intake. Citrus fruits and apples, the main fruits in our study, are rich in fiber and vitamin C. Dietary fiber content is considered to have a beneficial effect on inflammation [53]. Krishnamurthy et al. [54] found that, for each 10 g/day increase in total fiber intake, the odds of elevated serum C-reactive protein (CRP) levels were decreased by 11 and 38% in those subjects without and with chronic kidney disease, respectively. Fiber intake was negatively associated with visceral adipose tissue, CRP and fibrinogen, and positively associated with adiponectin in adolescents [55]. Fiber intake can even reverse the side effects of a high-fat, high-carbohydrate meal on inflammatory markers, endotoxemia and oxidative stress in normal-weight subjects [56]. However, no other food groups also rich in dietary fiber, such as vegetables, legumes, nuts and whole cereals, were associated with *TNF-α* methylation, showing a specific effect of fruit intake, and maybe, an interaction between nutrients in the food matrix. Several dietary components found in vegetables, such as sulforaphane and flavonoids, have been reported to influence DNA methylation levels [57, 58]. On the other hand, some polyphenols like curcumin, resveratrol and catechin can modulate NF-κB action and chromatin remodeling and, hence, the inflammatory response through DNA methyltransferase (DNMT) action [59].

Our hypothesis was that the higher intake of 'healthy food', like FV, could result in a hypermethylation of the *TNF-α* promoter, and hence, in a lower expression of this cytokine. However, we found the opposite outcome, since the higher intake of fruit was related to positive metabolic effects but no changes in circulating TNF-α and *TNF-α* mRNA levels, and *TNF-α* promoter hypomethylation in WBC. It is not known whether these mechanisms are similar in patients with established metabolic disorders and healthy subjects. Moreover, some studies have also reported that, although DNA methylation of the cytosine in the CpG dinucleotide is typically associated with gene silencing, CpG promoters can be both methylated and transcriptionally active due to an increase in the binding of transcription factors in a methylation-dependent manner [60, 61], and that promoter sequence and gene function are major predictors of promoter methylation states [62]. In this sense, a study that evaluated the effect of Roux-en-Y gastric bypass on *TNF-α* methylation reported a decrease in *TNF-α* promoter methylation in whole blood that was accompanied by a reduction in TNF-α plasma levels, with no significant correlation between both features [63]. A bioinformatic analysis [64] revealed that the CpG5 site in the promoter of *TNF-α* comprises the binding sequences of STAT4, which regulates the differentiation of T CD4+ cells, c-ETS that controls the expression of cytokines and chemokines, and ELK, an ETS family member.

Confirming these findings, there was no difference in *TNF-α* mRNA and TNF-α plasma concentration between the groups, and neither fruit intake nor DNA methylation status were related to *TNF-α* expression, showing that *TNF-α* promoter hypomethylation was not followed by an increase in *TNF-α* expression in WBC, and that other regulatory mechanisms could be involved, such as histone modifications [65], noncoding RNA, enhancer function [66], polymorphisms [67] and others. In fact, several investigations reported that there is not a simple association between DNA methylation and gene expression [68, 69] and that dietary influence on DNA methylation, and hence on metabolic features, can be unexpected [70]. In fact, two possible DNA methylation regulatory mechanisms with opposite modes of gene expression regulation have been proposed [71]: (1) tissue-specific differentially methylated regions are negatively correlated with the expression of their associated genes, and (2) the occurrence of negative regulators, such as transcriptional repressors that exhibit specific binding to methylated DNA motifs, causes a positive correlation between gene methylation and gene expression. Moreover, just as different mechanisms may influence the expression of a gene, these same mechanisms may influence the methylation itself [72]. This type of interaction can also be observed in relation to the nutrients. Several phytochemicals related to the reduction of inflammatory processes also appear to influence the expression of DNMT genes, interfering with the target gene de novo methylation. For example, in a study with human breast cancer cells, epigallocatechin gallate, genistein, withaferin A, curcumin, resveratrol and guggulsterone resulted in a significant decrease in DNMT1, DNMT3a and DNMT3b transcripts [73]. A similar effect was found with other polyphenols [74] and sulforaphane [75]. Thus, FV intake would be influencing the methylation of the *TNF-α* gene promoter (as well as other genes), and not necessarily, the circulating levels of this protein.

Specific foods and nutrients found in FV can also influence DNA methylation [20]. In the current study, promising effects were found in relation to the consumption of apples and the orange/tangerine/grapefruit group. In this context, it has been described that apple polyphenol dietary supplementation in rats inhibited adipocyte hypertrophy and enhanced lipolytic response through the regulation of genes involved in adipogenesis, lipolysis and fatty acid oxidation, which could be mediated, in part, by changes in DNA methylation [76]. The anti-inflammatory effects of orange juice were reviewed by Coelho et al. [77], who reported that the modulation of inflammatory markers by orange juice consumption can be due to bioactive compounds, such as the flavonoids hesperidin and naringenin. On the other hand, mice supplemented with grapefruit extract (rich in naringenin and kaempferol) showed a significant decrease in fasting glucose levels, which was accompanied by a lower mRNA expression of some proinflammatory genes (monocyte chemoattractant protein-1 – *MCP-1*, *TNF-α*, cyclooxygenase-2 – *COX-2*, *nuclear factor-κB* – *NF-κB*) in the liver and epididymal adipose tissue [70]. In that study, the CpG3 site of *TNF-α* showed higher methylation in the grapefruit group compared with the nontreated group, suggesting that DNA methylation changes in *TNF-α* in adipose tissue might help reduce the inflammation associated with diabetes and obesity [70], which corroborates our findings.

The present study has some limitations, such as the sample size, the lack of data about WBC distribution (granulocytes, monocytes and lymphocytes) and the cross-sectional design, but it is very conclusive in determining the positive effects of fruit intake even in apparently healthy normal-weight subjects and the occurrence of epigenetic changes in proinflammatory genes that could be related to these beneficial effects. However, this relationship between fruit intake and DNA methylation in inflammation-related genes should also be investigated in longitudinal and clinical studies.

In conclusion, a healthy dietary pattern and higher daily fruit intake are related to better glucose tolerance and lower methylation of *TNF-α* in WBCs, and fiber and vitamin C from fruits are putatively involved in this relationship.

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Disclosure Statement

The authors declare no conflict of interest.

References

1 Slavin JL, Lloyd B: Health benefits of fruits and vegetables. Adv Nutr 2012;3:506–516.
2 Casiglia E, Tikhonoff V, Caffi S, Boschetti G, Grasselli C, Saugo M, et al: High dietary fiber intake prevents stroke at a population level. Clin Nutr 2013;32:811–818.
3 Radhika G, Sudha V, Mohan Sathya R, Ganesan A, Mohan V: Association of fruit and vegetable intake with cardiovascular risk factors in urban south Indians. Br J Nutr 2008;99:398–405.
4 Yuan L, Zhang L, Ma W, Zhou X, Ji J, Li N, et al: Glutathione S-transferase M1 and T1 gene polymorphisms with consumption of high fruit-juice and vegetable diet affect antioxidant capacity in healthy adults. Nutrition 2013;29:965–971.
5 Cocate PG, Natali AJ, de Oliveira A, Longo GZ, Alfenas RdCG, Peluzio MDCG, et al: Fruit and vegetable intake and related nutrients are associated with oxidative stress markers in middle-aged men. Nutrition 2014;30:660–665.
6 Hermsdorff H, Zulet M, Puchau B, Alfredo Martínez J: Fruit and vegetable consumption and proinflammatory gene expression from peripheral blood mononuclear cells in young adults: a translational study. Nutr Metab 2010;7:42.
7 Hong S, Song Y, Lee KH, Lee HS, Lee M, Jee SH, et al: A fruit and dairy dietary pattern is associated with a reduced risk of metabolic syndrome. Metabolism 2012;61:883–890.
8 Hornick BA, Weiss L: Comparative nutrient analysis of commonly consumed vegetables: support for recommending a nutrition education approach emphasizing specific vegetables to improve nutrient intakes. Nutr Today 2011;46:130–137.
9 Saito I, Iso H, Kokubo Y, Inoue M, Tsugane S: Metabolic syndrome and all-cause and cardiovascular disease mortality: Japan Public Health Center-Based Prospective (JPHC) Study. Circ J 2009;73:878–884.
10 Unger RH, Clark GO, Scherer PE, Orci L: Lipid homeostasis, lipotoxicity and the metabolic syndrome. Biochim Biophys Acta 2010;1801:209–214.
11 Ramírez-Vargas E, Arnaud-Viñas MdR, Delisle H: Prevalence of the metabolic syndrome and associated lifestyles in adult males from Oaxaca, Mexico. Salud Publica Mex 2007;49:94–102.
12 Penckofer S, Schwertz D, Florczak K: Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and pro-oxidants. J Cardiovasc Nurs 2002;16:68–85.
13 Pollex RL, Hegele RA: Genetic determinants of the metabolic syndrome. Nat Clin Pract Cardiovasc Med 2006;3:482–489.
14 Milagro FI, Gómez-Abellán P, Campión J, Martínez JA, Ordovás JM, Garaulet M: CLOCK, PER2 and BMAL1 DNA methylation: association with obesity and metabolic syndrome characteristics and monounsaturated fat intake. Chronobiol Int 2012;29:1180–1194.
15 Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, et al: Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. J Physiol 2009;587:4963–4976.
16 Zulet MA, Puchau B, Navarro C, Martí A, Martínez JA: Biomarcadores del estado inflamatorio: nexo de unión con la obesidad y complicaciones asociadas. Nutr Hosp 2007;22:511–527.
17 Fantuzzi G: Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911–919.
18 Xydakis AM, Case CC, Jones PH, Hoogeveen RC, Liu M-Y, Smith EOB, et al: Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid weight loss through caloric restriction. J Clin Endocrinol Metab 2004;89:2697–2703.
19 Wieser V, Moschen A, Tilg H: Inflammation, cytokines and insulin resistance: a clinical perspective. Arch Immunol Ther Exp 2013;61:119–125.
20 Remely M, Lovrecic L, Garza A, Migliore L, Peterlin B, Milagro F, et al: Therapeutic perspectives of epigenetically active nutrients. Br J Pharmacol 2015;172:2756–2768.
21 Turcot V, Tchernof A, Deshaies Y, Pérusse L, Bélisle A, Marceau S, et al: LINE-1 methylation in visceral adipose tissue of severely obese individuals is associated with metabolic syndrome status and related phenotypes. Clin Epigenet 2012;4:10.

22 Hermsdorff HHM, Zulet MÁ, Puchau B, Bressan J, Martínez JA: Association of retinol-binding protein-4 with dietary selenium intake and other lifestyle features in young healthy women. Nutrition 2009;25:392–399.
23 Durnin JVGA, Womersley J: Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
24 de la Fuente-Arrillaga C, Ruiz Z, Bes-Rastrollo M, Sampson L, Martinez-Gonzalez M: Reproducibility of an FFQ validated in Spain. Public Health Nutr 2010;13:1364–1372.
25 Mataix J: Tabla de composición de alimentos, ed 5. Granada, Universidad de Granada, 2009.
26 Moreiras O, Carbajal A, Cabrera L, Cuadrado C: Tabla de composición de alimentos, ed 13. Madrid, Ediciones Pirámide, 2010.
27 Hermsdorff H, Zulet MÁ, Abete I, Martínez JA: Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects. Endocrine 2009;36:445–451.
28 Willett WC: Nutritional Epidemiology, ed 2. New York, Oxford University Press, 1998.
29 Trichopoulou A, Costacou T, Bamia C, Trichopoulos D: Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599–2608.
30 Guenther PM, Reedy J, Krebs-Smith SM: Development of the Healthy Eating Index-2005. J Am Diet Assoc 2008; 108:1896–1901.
31 Whitworth JA, Chalmers J: World Health Organisation-International Society of Hypertension (WHO/ISH) hypertension guidelines. Clin Exp Hypertens 2004;26:747–752.
32 Hermsdorff H, Zulet MÁ, Puchau B, Martínez JA: Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. Inflammation 2011;34:161–170.
33 Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
34 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419.
35 Campión J, Milagro FI, Goyenechea E, Martínez JA: TNF-α promoter methylation as a predictive biomarker for weight-loss response. Obesity 2009;17:1293–7129.
36 Hermsdorff HH, Mansego ML, Campión J, Milagro FI, Zulet MA, Martínez JA: TNF-alpha promoter methylation in peripheral white blood cells: relationship with circulating TNFα, truncal fat and n-6 PUFA intake in young women. Cytokine 2013;64:265–271.
37 Puchau B, Hermsdorff HHM, Zulet M, Martínez JA: DDAH2 mRNA expression is inversely associated with some cardiovascular risk-related features in healthy young adults. Dis Mark 2009;27:37–44.
38 Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001;25:402–408.
39 World Health Organization: Diet, Nutrition and the Prevention of Chronic Diseases. Geneva, World Health Organization, 2003.
40 Salas-Salvadó J, Martinez-González MÁ, Bulló M, Ros E: The role of diet in the prevention of type 2 diabetes. Nutr Metab Cardiovasc Dis 2011;21(suppl 2):B32–B48.
41 Ylönen K, Alfthan G, Groop L, Saloranta C, Aro A, Virtanen SM, et al: Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. Am J Clin Nutr 2003;77:1434–1441.
42 Carter P, Gray LJ, Talbot D, Morris DH, Khunti K, Davies MJ: Fruit and vegetable intake and the association with glucose parameters: a cross-sectional analysis of the Let's Prevent Diabetes Study. Eur J Clin Nutr 2013;67: 12–17.
43 Martínez-González MA, García-Arellano A, Toledo E, Salas-Salvadó J, Buil-Cosiales P, Corella D, et al: A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS One 2012;7:e43134.
44 Hotamisligil GS: Mechanisms of TNF-alpha-induced insulin resistance. Exp Clin Endocrinol Diabetes 1999;107: 119–125.
45 Cocate P, Natali A, Alfenas R, de Oliveira A, Dos Santos E, Hermsdorff H: Carotenoid consumption is related to lower lipid oxidation and DNA damage in middle-aged men. Br J Nutr 2015;114:257.
46 Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, et al: Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. J Am Diet Assoc 2009;109:414–421.
47 Jiang Y, Wu S-H, Shu X-O, Xiang Y-B, Ji B-T, Milne GL, et al: Cruciferous vegetable intake is inversely correlated with circulating levels of proinflammatory markers in women. J Acad Nutr Diet 2014;114:700–708.e2.
48 Root MM, McGinn MC, Nieman DC, Henson DA, Heinz SA, Shanely RA, et al: Combined fruit and vegetable intake is correlated with improved inflammatory and oxidant status from a cross-sectional study in a community setting. Nutrients 2012;4:29–41.
49 Hermsdorff HHM, Barbosa KBF, Volp ACP, Puchau B, Bressan J, Zulet MÁ, et al: Vitamin C and fibre consumption from fruits and vegetables improves oxidative stress markers in healthy young adults. Br J Nutr 2012;107:1119–1127.
50 Delgado-Cruzata L, Zhang W, McDonald JA, Tsai WY, Valdovinos C, Falci L, et al: Dietary modifications, weight loss, and changes in metabolic markers affect global DNA methylation in Hispanic, African American, and Afro-Caribbean breast cancer survivors. J Nutr 2015;145:783–790.

51 Zhang FF, Morabia A, Carroll J, Gonzalez K, Fulda K, Kaur M, et al: Dietary patterns are associated with levels of global genomic DNA methylation in a cancer-free population. J Nutr 2011;141:1165–1171.
52 Ross SA: Diet and DNA methylation interactions in cancer prevention. Ann NY Acad Sci 2003;983:197–207.
53 Wannamethee SG, Whincup PH, Thomas MC, Sattar N: Associations between dietary fiber and inflammation, hepatic function, and risk of type 2 diabetes in older men: potential mechanisms for the benefits of fiber on diabetes risk. Diabetes Care 2009;32:1823–1825.
54 Krishnamurthy VMR, Wei G, Baird BC, Murtaugh M, Chonchol MB, Raphael KL, et al: High dietary fiber intake is associated with decreased inflammation and all-cause mortality in patients with chronic kidney disease. Kidney Int 2012;81:300–306.
55 Parikh S, Pollock NK, Bhagatwala J, Guo D-H, Gutin B, Zhu H, et al: Adolescent fiber consumption is associated with visceral fat and inflammatory markers. J Clin Endocrinol Metab 2012;97:E1451–E1457.
56 Ghanim H, Dhindsa S, Batra M, Green K, Abuaysheh S, Chaudhuri A, et al: The intake of fiber suppresses the high-fat high-carbohydrate meal-induced endotoxemia, oxidative stress and inflammation. Endocr Abstracts 2012;29:P613.
57 Hsu A, Wong C, Yu Z, Williams D, Dashwood R, Ho E: Promoter de-methylation of cyclin D2 by sulforaphane in prostate cancer cells. Clin Epigenet 2011;3:3.
58 Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, et al: Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 2003;63:7563–7570.
59 Rahman I, Chung S: Dietary polyphenols, deacetylases and chromatin remodeling in inflammation. J Nutrigenet Nutrigenomics 2010;3:220–230.
60 Flower K, Thomas D, Heather J, Ramasubramanyan S, Jones S, Sinclair AJ: Epigenetic control of viral life-cycle by a DNA-methylation dependent transcription factor. PLoS One 2011;6:e25922.
61 Rishi V, Bhattacharya P, Chatterjee R, Rozenberg J, Zhao J, Glass K, et al: CpG methylation of half-CRE sequences creates C/EBPα binding sites that activate some tissue-specific genes. Proc Natl Acad Sci USA 2010;107:20311–20316.
62 Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, et al: Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 2007;39:457–466.
63 Kirchner H, Nylen C, Laber S, Barrès R, Yan J, Krook A, et al: Altered promoter methylation of PDK4, IL1 B, IL6, and TNF after Roux-en Y gastric bypass. Surg Obesity Relat Dis 2014;10:671–678.
64 Messeguer X, Escudero R, Farré D, Nuñez O, Martínez J, Albà M: PROMO: detection of known transcription regulatory elements using species-tailored searches. Bioinformatics 2002;18:333–334.
65 Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33(suppl):245–254.
66 Ong C-T, Corces VG: Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat Rev Genet 2011;12:283–293.
67 Groote D: Tumour necrosis factor (TNF) gene polymorphism influences TNF-α production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998;113:401–406.
68 Saito Y, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S: Expression of mRNA for DNA methyltransferases and methyl-CpG-binding proteins and DNA methylation status on CpG islands and pericentromeric satellite regions during human hepatocarcinogenesis. Hepatology 2001;33:561–568.
69 Ehrlich M, Woods CB, Yu MC, Dubeau L, Yang F, Campan M, et al: Quantitative analysis of associations between DNA hypermethylation, hypomethylation, and DNMT RNA levels in ovarian tumors. Oncogene 2006;25:2636–2645.
70 la Garza ALd, Etxeberria U, Palacios-Ortega S, Haslberger AG, Aumueller E, Milagro FI, et al: Modulation of hyperglycemia and TNFα-mediated inflammation by helichrysum and grapefruit extracts in diabetic db/db mice. Food Funct 2014;5:2120–2128.
71 Wan J, Oliver VF, Wang G, Zhu H, Zack DJ, Merbs SL, et al: Characterization of tissue-specific differential DNA methylation suggests distinct modes of positive and negative gene expression regulation. BMC Genomics 2015;16:49.
72 Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, et al: MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. Nat Struct Mol Biol 2008;15:259–267.
73 Mirza S, Sharma G, Parshad R, Gupta SD, Pandya P, Ralhan R: Expression of DNA methyltransferases in breast cancer patients and to analyze the effect of natural compounds on DNA methyltransferases and associated proteins. J Breast Cancer 2013;16:23–31.
74 Paluszczak J, Krajka-Kuźniak V, Baer-Dubowska W: The effect of dietary polyphenols on the epigenetic regulation of gene expression in MCF7 breast cancer cells. Toxicol Lett 2010;192:119–125.
75 Meeran SM, Patel SN, Tollefsbol TO: Sulforaphane causes epigenetic repression of hTERT expression in human breast cancer cell lines. PLoS One 2010;5:e11457.
76 Boqué N, de la Iglesia R, de la Garza AL, Milagro FI, Olivares M, Bañuelos Ó, et al: Prevention of diet-induced obesity by apple polyphenols in Wistar rats through regulation of adipocyte gene expression and DNA methylation patterns. Mol Nutr Food Res 2013;57:1473–1478.
77 Coelho R, Hermsdorff H, Bressan J: Anti-inflammatory properties of orange juice: possible favorable molecular and metabolic effects. Plant Foods Hum Nutr 2013;68:1–10.