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ORIGINAL CONTRIBUTION

Antioxidant and inflammatory response following high-fat meal consumption in overweight subjects

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Abstract
Purpose Postprandial metabolic stress as a consequence of ingestion of high-energy meals is recognized as an important risk factor for cardiovascular disease. The objective of this study was to evaluate the inflammatory and antioxidant response of the body to the acute ingestion of a high-fat meal (HFM).
Methods Fifteen healthy overweight subjects were recruited for the study. After HFM consumption, plasma glucose, insulin, uric acid (UA), triglycerides (TG), total cholesterol (TC), thiols (SH), inflammatory cytokines (IL-6 and TNF-α) and dietary antioxidants were measured at 0, 0, 5, 1, 2, 4, 6 and 8 h points from ingestion.
Results The ingestion of HFM induced significant increases in both TG and TC, with peaks at 4 h ($p < 0.001$) and 8 h ($p < 0.01$), respectively. IL-6 and TNF-α significantly increased postprandially, reaching maximum concentrations 8 h after meal consumption ($p < 0.001$). Whereas plasma concentrations of vitamins and carotenoids were not changed by HFM, SH and UA increased, peaking 2–4 h postingestion ($p < 0.001$ and 0.01, respectively). Increments of SH and UA were positively correlated with AUC for TG (Pearson coefficient 0.888, $p < 0.001$ and 0.923, $p < 0.001$, respectively).
Conclusions Present results indicate that as a consequence of an excess of dietary fat, the body responds through an inflammatory reaction, which is accompanied by an increment of endogenous antioxidant defenses, mediated by UA and SH, but not by vitamins C and E and carotenoids. Although further studies are needed, results of the current investigation represent novel findings on endogenous strategies of redox defense from fat overloads.

Keywords High-fat meal · Postprandial stress · Inflammation · Endogenous antioxidants

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Introduction

The hypothesis that atherosclerosis is a postprandial phenomenon [1] has being gaining consensus among the scientific community. Indeed, the consumption of nutritionally unbalanced meals may result in a variety of postprandial dysmetabolism, including hyperlipidemia, hyperglycemia and hyperinsulinemia potentially detrimental for cardiovascular function [2, 3]. Recent evidence suggests that postprandial, but not fasting, hyperglycemia represents one of the very important pathophysiological states contributing to vascular failure [3]. Similarly, non-fasting high triglyceride levels have been shown to be more predictive than fasting levels for coronary heart disease incidence [4]. Moreover, clinical studies have shown that acute postprandial increments in lipids are associated with endothelium dysfunction and increased risk for atherosclerosis, in healthy subjects [5, 6]. The link between acute fat over-ingestion and vascular impairment involves the generation of excess free radicals, leading to postprandial oxidative stress [7, 8] and the release into the circulation of

pro-inflammatory cytokines [9]. It is not known which tissues are involved in this inflammatory response; however, the observation that the release of pro-inflammatory cytokines is body fat dependent seems to indicate that adipose tissue is an important source in the postprandial period [10]. Under these conditions, the activation of endogenous antioxidant mechanisms of protection may play a pivotal role in the early defense of the body from postprandial stress.

Uric acid (UA), a by-product of purine catabolism, at physiological concentrations is an active antioxidant, accounting for approximately half of the endogenous non-enzymatic antioxidant capacity of human plasma (NEAC) [11]. Nonetheless, the biological role of urate in case of increments in its concentration above physiological levels is still not fully understood. While some authors have reported a significant association between increased UA levels and the development of inflammation-related diseases [12, 13], a protective role in healthy conditions has also been suggested [14].

In addition to UA, thiols (SH), including non-protein forms (NPSH) such as glutathione (GSH) and free cysteine (cys), and protein SH groups (PSH) play a major role in the maintenance of both intra- and extracellular redox homeostasis [15]. In the presence of free radicals, NPSH and PSH combine to form oxidized disulfides, a defense reaction to prevent proteins from oxidation [16]. Normally, the redox state of SH/disulfide couples is finely regulated and an increase in oxidized SH forms is recognized to play a role in the development of early atherosclerosis in healthy people [17, 18].

Though endogenous antioxidant defenses may hamper the radical production induced by postprandial stress, their role has not been investigated as yet. The objective of this study was to evaluate the acute inflammatory and antioxidant response to the ingestion of an HFM in healthy overweight subjects.

Methods

Subjects and study design

Approval for the study was obtained from the ethics committee for Non-Clinical Research of San Camillo Forlanini, and all procedures involving human subjects complied with the Declaration of Helsinki as revised in 2000.

Fifteen overweight men and women were recruited for the study. All the subjects completed an informed consent form and provided their written consent prior to the study. Eligible participants were healthy adults, aged between 30 and 60 years, with a BMI comprised between 25 and 30 kg/m^2, non-smoking and not taking drugs, medications nor supplements that would interfere with the study endpoints (i.e., lipid-lowering medications, anti-inflammatory drugs and vitamin or antioxidant dietary supplements). For 2 days prior to the intervention (wash out), the subjects were asked to follow a low-antioxidant diet, by avoiding fresh fruit, vegetables, fruit juices, wine, tea, coffee and chocolate. Compliance to dietary instructions was evaluated through dietary questionnaire and interviews.

The effect of dietary intervention was assessed by tracking the acute postprandial responses to ingestion of the HFM over an 8-h period. On the day of the study, after an overnight fast, venous blood samples were collected for baseline determinations (0 h). After the ingestion of the test meal, further blood withdraws were conducted at different time points (0.5, 1, 2, 4, 6, 8 h).

Composition and preparation of HFM

The HFM was composed of fat-rich meal and drink, purposely characterized by low-purine content (52.8 mg) to minimize exogenous UA formation. The meal comprised fried potatoes (212 g), fried eggs (108 g), Emmenthal cheese (90 g) and Italian rose-shaped dinner rolls (90 g). The drink was a 500 ml beverage with a low sugar content of 13 g of sucrose, 3.15 g of glucose and 3.2 g of fructose and free from either fruit extracts or vitamins. Total energy content of the HFM was 1416 kcal (5,929 kJ), providing 82 g of fats (52 % of energy; saturated fats, 36.9 g), 123 g of carbohydrates (34 % of energy; sugars, 30 g) and 51 g of proteins (14 % of energy).

Dietary variability was reduced to a minimum by purchasing all food items in the same place and/or from the same company and by using a standardized protocol for each meal preparation. Two average size eggs were fried in 15 g of sunflower oil pre-heated for 3 min in a frying pan. The eggs were served in the same frying pan that was used for preparation in order to allow complete consumption of the food without any loss from plate delivering. Potatoes were pre-fried and pre-sliced frozen potatoes. They were deep fried for 13 min with 120 g of sunflower oil every 100 g of potatoes. The oil was pre-heated for 10 min in a large frying pan. Prior to consumption, the potatoes have been drained and put in a flat plate lined with absorbent paper. Amount of oil absorbed was calculated for difference between the amount of oil present in the pan and on the paper.

Chemicals

The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,4,6-tripyridyl-s-triazine (TPTZ) were

purchased from Fluka (Italy). R-Phycoerythrin (R-PE) was purchased from Europa Bioproducts Ltd.; 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako CHFMical (Germany). Phosphate-buffered saline tablets (PBS), sodium acetate tri-hydrate, 5,5′-dithiobis(2-nitro-benzoic acid) (DTNB), glutathione (GSH) and ethylene-di-amine-tetra-acetic acid were purchased from Sigma (St. Louis, MO USA). Potassium di-hydrogen phosphate (KH_2PO_4) was purchased from Carlo Erba (Milan, Italy); iron(III) chloride 6-hydrate ($FeCl_3.6H_2O$) and di-sodium hydrogen orthophosphate 12-hydrate ($Na_2HPO_4.12H_2O$) were purchased from BDH (Poole, England). Ferrous sulfate 7-hydrate ($FeSO_4.7H_2O$) was purchased from Merck (Darmstadt, Germany). All solvents used were HPLC grade and purchased from Carlo Erba (Milan, Italy). High-purity water was obtained in the laboratory by using an Alpha-Q system (Millipore, Marlborough, MA).

Analytical methods

Blood was collected in EDTA and/or Heparin tubes and centrifuged immediately at 1,300×*g* at 4 °C for 15 min. The resulting plasma samples were stored at −80 °C until analyses were performed.

UA was determined by means of colorimetric kits, provided by Sentinel CH. SpA, Milan (Italy), while the determination of SH was performed using DTNB [19].

Plasma levels of TG, TC and glucose were quantified enzymatically by using colorimetric kits (Sentinel CH. SpA, Milan, Italy). Plasma insulin was measured with DRG International Inc enzyme immunoassay (EIA) kit (TEMA ricerca S.r.l., Bologna, Italy).

Plasma human tumor necrosis factor alpha (TNF-α) was measured using a commercially available enzyme-linked immune-sorbent assay (ELISA, Endogen, TEMA ricerca S.r.l., Bologna, Italy). Human interleukin 6 (IL-6) was measured with a commercially available ELISA assay (ALPCO), provided by Prodotti Gianni, Milan, Italy. Finally, vitamin C [20], vitamin E, retinol and carotenoids [21, 22] were measured by HPLC.

Statistics

Statistical analysis was carried out by means of one-way repeated measures ANOVA, followed by Bonferroni post hoc test, in order to isolate single treatment effects at different time points, with respect to baseline. Incremental area under the curve (AUC) was calculated using the trapezoid rule and represents the increase in area following the response of the test meal above baseline concentrations. Pearson product-moment correlation was used for testing variable associations. All statistical evaluations were performed using Sigmastat software (Jandel Scientific Inc., San Rafael, CA).

Table 1 Physical characteristics and biomarkers baseline profile of the subjects[a]

Biomarkers	Subjects
Age (years)	45 ± 8
BMI (Kg/m^2)	26.7 ± 1.9
Glucose (mg/dl)	86 ± 16
Insulin (mU/l)	7.5 ± 6.2
Triglycerides (TG) (mg/dl)	89 ± 51
Total cholesterol (TC) (mg/dl)	172 ± 31
Uric acid (μM)	345 ± 66
Thiol (SH) (μM)	499 ± 49
Vitamin C (mg/dl)	0.7 ± 0.3
α-tocopherol (mg/dl)	1.1 ± 0.3
Cryptoxanthin (μg/dl)	11.5 ± 4.8
Lycopene (μg/dl)	47.0 ± 7.7
α-carotene (μg/dl)	4.8 ± 4.9
β-carotene (μg/dl)	34.6 ± 31.5
TNF-α (pg/ml)	29.1 ± 29.0
IL-6 (pg/ml)	0.3 ± 0.3

[a] Data are expressed as means ± SD, $N = 15$

Results

Thirteen males and 2 females passed eligible criteria and were included in the study. Physical characteristics and baseline clinical values of the study group are shown in Table 1. Subject age ranged from 30 to 56 years, and mean BMI was 26.7 ± 1.9. As compared to standard reference values (glucose 60–110 mg/dl, TG <180 mg/dl and TC <200 mg/dl), 1 subject had a moderate fasting hypoglycemia (54 mg/dl) and 1 a moderate hyperglycemia (114 mg/dl). With respect to lipid levels, 1 subject had high fasting TG levels (253 mg/dl) and 2 were slightly hypercholesterolemic (223 and 237 mg/dl). UA and SH fasting ranges were 158–432 and 417–618 μM, respectively. Plasma levels of ascorbic acid and α-tocopherol were within the normal reference values for all subjects (0.5–1.5 and 0.8–1.5 mg/dl for vitamins C and E, respectively).

Fasting cytokine levels varied widely among subjects, TNF-α ranging from 2 to 110 pg/ml and IL-6 from 0 to 1.28 pg/ml.

Metabolic responses to HFM

The effect of HFM on glucose and lipid metabolism is described in Fig. 1. The glucose and insulin time courses reflected the glycemic postprandial load for healthy people. Both glucose and insulin levels peaked 30 min after meal

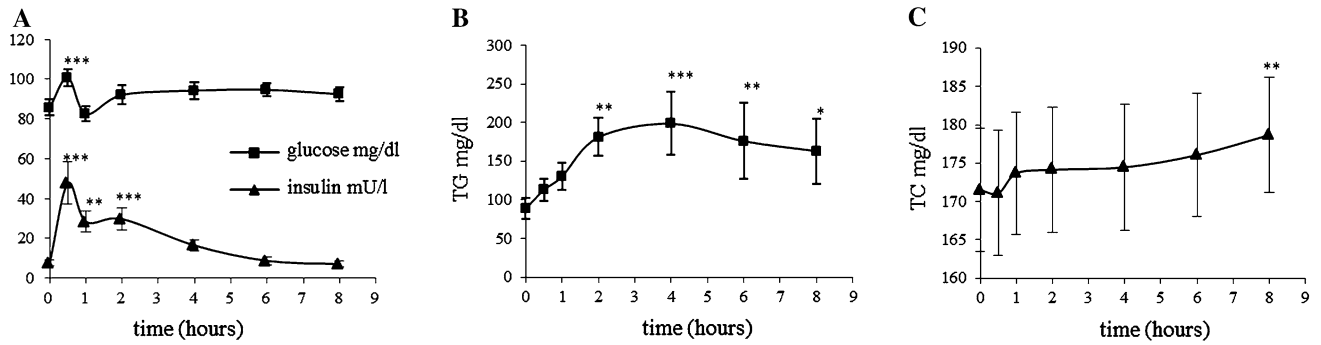

Fig. 1 Effect of high-fat meal [HFM] on glucose and lipid metabolism. *Line plots* showing the changes as means ± SE in plasma glucose and insulin (**a**), triglycerides (TG; **b**) and total cholesterol (TC; **c**), following HFM intake [$n = 15$]. One-way repeated measures ANOVA followed by Bonferroni post hoc analysis: $^{*}p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$ single time points versus T0

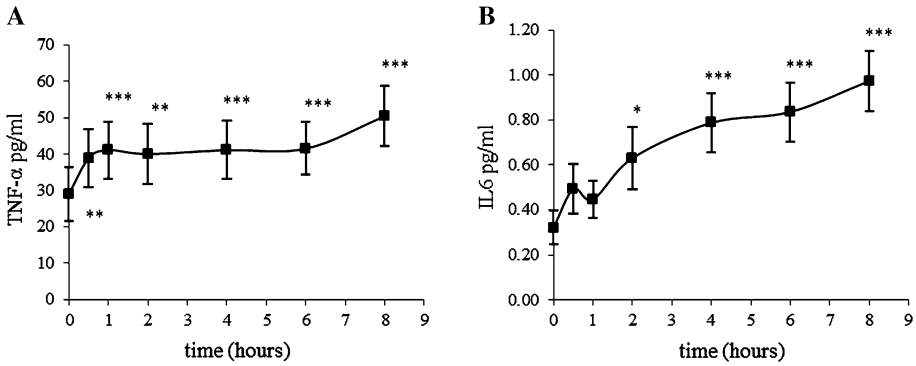

Fig. 2 Effect of high-fat meal [HFM] on inflammatory cytokines. *Line plots* showing the changes as means ± SE in plasma TNF-α (**a**) and IL-6 (**b**) following HFM intake [$n = 15$]. One-way repeated measures ANOVA followed by Bonferroni post hoc analysis: $^{*}p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$ single time points versus T0

ingestion (glucose 101 ± 16 mg/dl, $p < 0.001$; insulin 48 ± 41 mU/l, $p < 0.001$) (Fig. 1a). Glucose levels returned to baseline values within 1 h, whereas insulin remained significantly above pre-ingestion values for 2 h ($p < 0.001$), returning to baseline levels after 4 h.

With respect to lipid metabolism, the test meal induced hypertriglyceridemia for the entire study period: plasma TG concentration rose 0.5–4 h postmeal ingestion, reaching a peak of 199 ± 158 mg/dl (+110 mg/dl, $p < 0.001$) at the 4 h point (Fig. 1b). Although a slight decrease was recorded, TG concentrations remained significantly higher than fasting levels for 8 h (+74 mg/dl, $p < 0.05$). Also, TC levels increased 0-8 h after the intervention, reaching statistical significance at the 8 h point (+7 mg/dl; $p < 0.01$), as shown in Fig. 1c.

Effect of HFM on circulating cytokines

The effect of HFM on circulating cytokines is described in Fig. 2. Both plasma TNF-α and IL-6 were significantly increased after HFM consumption (Fig. 2a, b, respectively). TNF-α levels increased for 1 h up to 41.1 ± 30.3 pg/ml (+12 pg/ml, $p < 0.001$) and remained significantly above fasting values within the entire study period, peaking to 50.5 ± 32.3 after 8 h (+21 pg/ml, $p < 0.001$) (Fig. 2a). IL-6 constantly increased after intervention, doubling its initial value after 2 h (+0.3 pg/ml, $p < 0.05$) and reaching a maximum concentration of 0.97 ± 0.52 pg/ml ($p < 0.001$) after 8 h.

Antioxidant response to HFM

Plasma dietary antioxidant vitamins (vitamin C, α-tocopherol and retinol) and carotenoids (cryptoxanthin, lycopene, α-carotene and β-carotene) were not modified by the test meal (Table 2). On the other hand, HFM ingestion caused a significant increase in the endogenous antioxidants UA and SH (Fig. 3a, b). UA started to increase 30 min from intervention and reached a peak of 478 ± 186 μM at 2 h (+133 μM, $p < 0.01$), returning to basal levels after 6 h. A larger increase was recorded for SH, which almost doubled from initial values between 2 and 4 h postingestion (+348 μM, $p < 0.001$) and remained significantly above the fasting value (+241 μM, $p < 0.01$) for 8 h. The area under the curve (AUC) of both UA and SH was significantly correlated with the TG AUC (Pearson coefficient 0.923, $p < 0.001$ and 0.888, $p < 0.001$, respectively).

Table 2 Vitamins and carotenoids plasma levels[a], before (T0) and after[b] high-fat meal (HFM) ingestion

Time (hours)	T0	T0.5	T1	T2	T4	T6	T8
Ascorbic acid (mg/dl)	0.67 ± 0.24	0.65 ± 0.26	0.58 ± 0.25	0.60 ± 0.33	0.61 ± 0.25	0.68 ± 0.41	0.64 ± 0.36
α-tocopherol (mg/dl)	1.13 ± 0.28	1.09 ± 0.31	1.11 ± 0.28	1.08 ± 0.28	1.10 ± 0.30	1.12 ± 0.33	1.17 ± 0.30
Cryptoxanthin (μg/dl)	11.5 ± 4.82	11.4 ± 4.39	13.2 ± 7.61	12.2 ± 5.10	11.3 ± 5.21	12.2 ± 5.37	12.3 ± 4.98
Lycopene (μg/dl)	47.0 ± 7.72	46.1 ± 15.41	47.7 ± 10.35	47.5 ± 15.62	44.2 ± 16.71	47.1 ± 13.20	45.8 ± 20.51
α-carotene (μg/dl)	4.75 ± 4.85	4.94 ± 3.83	5.93 ± 5.88	4.44 ± 4.45	5.46 ± 4.16	5.46 ± 5.38	5.05 ± 5.55
β-carotene (μg/dl)	34.6 ± 31.51	35.6 ± 30.01	40.7 ± 30.66	37.2 ± 28.30	34.5 ± 29.16	37.2 ± 31.82	29.3 ± 17.28

[a] Data are expressed as means ± SD

[b] After 0.5 (T0.5), 1 (T1), 2 (T2), 4 (T4), 6 (T6) and 8 (T8) hours from consumption

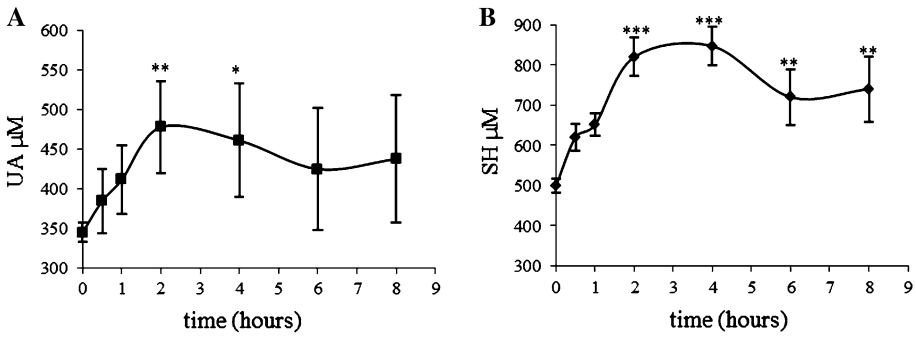

Fig. 3 Effect of high-fat meal [HFM] on markers of endogenous antioxidant status. *Line plots* showing the changes as means ± SE in plasma uric acid, UA (**a**) and sulphydryl groups, SH (**b**). One-way repeated measures ANOVA followed by Bonferroni post hoc analysis: $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ single time points versus T0

Conclusions

To our knowledge, this is the first time that evidence has been provided for an endogenous antioxidant response concomitant to dietary-induced inflammation.

First of all, in agreement with previous studies [23, 24], our results confirm that the acute ingestion of a fat-rich meal induces the onset of a postprandial metabolic stress characterized by high levels of TG and TC. It has long being known that postprandial hyperlipidemia is associated with an increased risk of atherogenesis [1, 25]. Vogel et al. [26] reported that a mean TG increment of 53 mg/dl reached 2 h after consuming 50-g fat meal resulted in a twofold lower flow-mediated brachial artery vasoactivity in healthy volunteers. At the same time point, in another study, the ingestion of a meal containing 90 g of fats induced a mean TG increment of 31 mg/dl and significantly affected vessel resistance in healthy subjects [5]. Ceriello et al. [23] found that a increment of about 45 mg/dl of serum TG measured 1 h after the ingestion of a high-fat–high glucose meal (75 g of fats and 75 g of glucose) was associated with 13 and 30 % higher circulating levels of adhesion molecules, one of the first stages of atherogenic process. Overall, these data suggest that postprandial blood TG increments of about 20–50 mg/dl may induce pro-atherogenic conditions in healthy people. In the present study, we measured a similar TG increment of 42 mg/dl 1 h after test meal ingestion and this value was almost twofold higher 8 h later, suggesting that a Western style meal, composed of eggs with cheese, fried potatoes, bread and a sweet beverage, overall providing 82 g of fats may induce a postprandial pro-atherogenic state, lasting for up to 8 h.

Secondly, the concept that inflammation plays a pivotal role in the causal link between fat overload and vascular diseases is gaining momentum [10]. Our study supports this hypothesis, as we found that both inflammatory cytokines TNF-α and IL-6 were increased by HFM consumption. Accordingly, Nappo and colleagues [6] reported that the ingestion of a fat-rich meal delivering 50 g of fats was able to significantly increase IL-6 and TNF-α levels [6] in healthy adults. Interestingly, the same authors found similar results after a high-carbohydrate meal consumption (70 % of energy from carbohydrates) in diabetic subjects, but not in healthy volunteers [6]. Differently from fats, alterations in the regulation of carbohydrate metabolism rather than steady-state glucose and insulin concentrations are reported to be the main determinant of the pro-inflammatory responses to high-carbohydrate meals [10]. In another work, Lundman et al. [9] found that a 65-g fat meal induced increased inflammatory activity, through an overexpression of IL-6, no matter the magnitude of the postprandial triglyceridemia. Although not statistically significant, a trend for IL-6 and TNF-α increments after a 50-g fat meal was also reported by Devaraj et al. [8] in metabolic syndrome patients, together with a significant

increase in IL-1β. More recently, our group gave evidence that a Western style meal delivering 1,351 kcal, with 55 % as fat energy, induced plasma IL-17, IL-6 and TNF-α increases in overweight subjects [27].

It has been reported that adipose tissue is a likely candidate for the cytokine production in the postprandial state [10]. However, in agreement with previous results [27], we recorded a fast rise of TNF-α at 30 min from HFM consumption. Although studies are needed to clarify this effect, we suggest that this early increment may be due to a dietary-fat-mediated transport of lipopolysaccharides to the gut-associated lymphoid tissue (GALT), where they can activate immune cells to secrete TNF-α [28]. The following stronger inflammatory response recorded at 2–8 h, temporally associated with TG and TC increases, may be attributed to further secretion of both TNF-α and IL-6 by other body districts, including adipose tissue.

Finally, postprandial inflammation caused by nutritionally unbalanced meals has been shown to be associated with an oxidative stress condition [3]. The observation that postprandial inflammation induced by high-fat meals is attenuated by dietary antioxidants further supports the hypothesis of a redox-based mechanism [27, 29]. In the present study, for the first time, we showed that in healthy people, in the absence of exogenous antioxidants, the body responds to postprandial stress by inducing endogenous defenses. This evidence is supported by the fact that the appearance in the blood of UA and SH was driven by a precise timing: initially, between 1 and 2 h postingestion, the body produced a clear increase in plasma UA and only a slight increase in SH levels. Between 2 and 4 h, TG levels reached the peak of increase. By this time, inflammatory cytokine levels were also increased and the antioxidant protection was maximized through a massive release of SH into circulation. Despite the decrease between 6 and 8 h postingestion, SH levels were still significantly above initial values at the end of the observation period, when both TNF-α and IL-6 levels were maximum. Interestingly, dietary antioxidant vitamins and carotenoids were not affected by HFM, suggesting that endogenous antioxidants represent the first line of defense against postprandial stress, sparing vitamins. This pattern of metabolic response indicates that as a consequence of fat overingestion, the body responds through an inflammatory reaction, which is accompanied by an increment in endogenous antioxidant defenses provided by UA and SH.

It is well known that in humans, at physiological concentrations of 160–450 μM, UA works to prevent lipid and protein peroxidation [30], acting at different body districts: reduction in the ferryl-oxo heme oxidant and protection of erythrocyte ghosts, in blood [31]; scavenging of radicals formed from the reaction of per-oxy-nitrite with carbon dioxide and in neural tissue [32]; moreover, UA has been reported to protect against liver injury by affecting nitric oxide metabolism [33]. Altogether, these actions underlie the protective effect of UA against cardiovascular diseases and support the hypothesis that an increase in blood UA could be an adaptive response to persistent exposure to potentially damaging oxidants [34]. However, due to epidemiologic evidence that associate increased UA plasma levels to the development of inflammatory diseases, an intense scientific debate has been raised, whether UA production is a strategy of the body to counteract oxidative stress and inflammation or an inflammatory mechanism itself [11, 35, 36].

UA is produced through purine catabolism, the two important contributors being dietary purine levels and purines released from the DNA and RNA of damaged cells. From our data, it is not possible to state which is the origin of the production of UA. However, the tested HFM was designed to have a negligible purine content. This indicates that the increased UA was more likely to be derived by endogenously produced, rather than dietary-induced urate. In a previous work, Cao and Prior [37] found dietary-induced serum NEAC increments, which were in part accounted for UA rises recorded after consumption of meals with negligible antioxidant content. In agreement with the authors, we suggest that this might be considered an adaptive response to the postprandial stress. Indeed, we observed that the pattern of increase in UA was parallel to those of both inflammatory cytokines and triglycerides, indicating that the body produced UA as an antioxidant strategy to counteract postprandial stress. The present results might have critical physiological implications: if they will be confirmed, it would be more likely that UA is a marker of inflammation, rather than an inflammatory molecule itself. This hypothesis is in agreement with what has been previously suggested by other authors [34, 38].

Apparently in contradiction with our results, Cardona and colleagues [38] reported an association between hypertriglyceridemia and reduced UA plasma concentration. However, it is possible that Cardona missed UA increments, by evaluating only the 3-h time point postmeal ingestion, when UA concentration may have already returned to pre-intervention levels. The fact that we found still significantly high UA concentrations at 4 h from meal consumption may be due to the almost threefold higher caloric overload (1,416 vs. 540 kcal) and higher fat content (82 vs. 60 g) of our test meal with respect to that of the study by Cardona.

The evidence of an endogenous antioxidant reaction to acute inflammatory events caused by HFM consumption is further supported by the SH response, which mirrored the UA pattern of increase, although the peak of concentration was reached slightly later, between the 2 and 4 h from ingestion. While GSH and its oxidized forms represent a

major intracellular antioxidant system, notably, free and protein-bound forms of homo-cysteine (hcys) and cys comprise one of the most important extracellular antioxidant systems [39]. It has been reported that in presence of free radicals, protein cys groups may undergo oxidation, which is prevented by protein thiolation with cys, hcys or GSH, the latter being the dominant ligand [40]. Because cys is more reactive to oxidation and it is present at higher concentration in plasma, increased circulating pro-oxidant molecules preferentially oxidize cys, and redox balance is preserved by the supply of GSH from tissues [41]. In our study, we found increased plasma total SH levels, concomitantly to the TG increase and the onset of an inflammatory state. These results suggest that an acute response possibly aimed at increasing reduced SH groups available for defense from protein oxidation may have occurred. The pattern of postprandial SH increase here reported is in agreement with previous observations by Blanco and colleagues [42], who showed that SH redox state exhibits diurnal variations, with a peak of reduced forms starting 3 h after the beginning of meal time. However, considering that our method cannot discern between protein and non-protein thiol components, we cannot exclude that the great increase in reduced SH forms measured after HFM consumption [almost +350 μM between 2 and 4 h from ingestion] may have derived from the cumulative effect of redox balance mechanisms, dietary protein absorption and increased protein production.

In conclusion, our data indicate that in healthy people specific antioxidant agents, such as UA and SH, may be internally produced by the body to counteract the postprandial metabolic stress induced by pro-oxidant and pro-inflammatory molecules. Although further studies are needed, the results of the current investigation give new insights into the redox strategies activated by the body under stress conditions.

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Conflict of interest The authors declared no conflict of interest.

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