A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases[1–4]

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ABSTRACT

Background: The modern Western lifestyle is characterized by the consumption of high-heat-treated foods because of their characteristic taste and flavor. However, it has been shown that treating food at high temperatures can generate potentially harmful compounds that promote inflammation and cardiovascular disease in subjects with diabetes.

Objective: The aim of this study was to determine whether high-heat-treated foods also pose a risk for healthy subjects.

Design: A randomized, crossover, diet-controlled intervention trial with 62 volunteers was designed to compare the potential metabolic effects of 2 diets, one that was based on mild steam cooking and another that was based on high-temperature cooking. These 2 diets differed mainly in their contents of Maillard reaction products (MRPs). MRPs were assessed in the diet and in subjects' feces, blood, and urine samples, with N^{ε}-carboxymethyllysine as an indicator of MRPs. Biological indicators of glucose and lipid metabolism as well as oxidative stress were analyzed in subjects after 1 mo on each diet.

Results: In comparison with the steamed diet, 1 mo of consuming the high-heat-treated diet induced significantly lower insulin sensitivity and plasma concentrations of long-chain n−3 (omega-3) fatty acids and vitamins C and E [−17% ($P < 0.002$), −13% ($P < 0.0001$), and −8% ($P < 0.01$), respectively]. However, concentrations of plasma cholesterol and triglycerides increased [+5% ($P < 0.01$) and +9% ($P < 0.01$), respectively].

Conclusions: A diet that is based on high-heat-treated foods increases markers associated with an enhanced risk of type 2 diabetes and cardiovascular diseases in healthy people. Replacing high-heat-treatment techniques by mild cooking techniques may help to positively modulate biomarkers associated with an increased risk of diabetes mellitus and cardiovascular diseases. *Am J Clin Nutr* 2010;91:1220–6.

INTRODUCTION

Upon heat treatment of foods, a characteristic browning and taste compounds are generated by the so-called Maillard reaction. The Maillard reaction is any reaction between a reducing carbohydrate and an amino acid (1) and occurs in foods during storage and heat treatment, with the rate and diversity of chemical reactions accelerating as the temperature increases (2). Maillard reaction products (MRPs) are chemically highly diverse and comprise taste- and flavor-active molecules (3) and health-beneficial compounds (4, 5). In contrast, potent carcinogens are also generated (6), such as acrylamide (7) or heterocyclic amines (8).

The chronic intake of high-heat-treated foods was shown to accelerate cardiovascular complications in animals and humans with diabetes (9, 10) and to favor type 1 and 2 diabetes development in mice (11, 12). It was recently shown that a typical Western diet characterized by a significantly higher frequency of processed food consumption, such as processed meat, pizza, or snacks, compared with a healthier diet characterized by, eg, a higher intake of vegetables and significantly reduced amounts of processed foods is associated with an increased risk of insulin resistance and metabolic syndrome (13). Therefore, interest has focused on the question of whether dietary MRPs can pose a risk of the development of certain diseases, such as type 2 diabetes or cardiovascular diseases. The progression of both diseases has been associated with the accumulation of one of the MRPs, N^{ε}-carboxymethyllysine (CML) (14, 15). About 30% of dietary CML is absorbed into circulation (16), which poses the concern whether dietary CML as a key indicator compound of MRPs formed in heat-treated foods promotes risk factors for type 2 diabetes or cardiovascular diseases.

The few human intervention trials (10, 17, 18) that reported on health effects of dietary MRPs have all focused on patients with diabetes or renal failure. In one (10) of these studies, a 15-d administration of a diet composed of high-heat-treated foods that contained ≈5 times more CML than the same diet that was based on mildly cooked food increased the formation of proinflammatory cytokines.

Thus, we conducted a randomized, crossover, intervention trial to clarify whether a habitual diet containing high-heat-treated foods, such as deep-fried potatoes, cookies, brown crusted bread, or fried meat, could promote risk factors of type 2 diabetes or cardiovascular diseases in healthy people.

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Although various putatively harmful compounds may be formed on high-heat treatment of food, we decided to specifically quantify CML, as a well-accepted MRP indicator, in the diet and in subjects' feces, plasma, and urine samples after dietary intervention. CML data were correlated with biomarkers of glucose and lipid metabolism as well as biomarkers of oxidative stress.

SUBJECTS AND METHODS

Subjects

The study was carried out in January 2006 at the LaSalle Beauvais Polytechnical Institute (Beauvais, France), under the responsibility of the promoter, the Nutrition Department, Pasteur Institute (Lille, France). Volunteers were recruited in December 2005 among students of the LaSalle Institute who were living on campus. Inclusion criteria were as follows: overall health, $\geq$18 y of age, and the willingness to take all meals exclusively under supervision at the university's cafeteria. Exclusion criteria were as follows: any kind of medication, tobacco and alcohol consumption, a body mass index (BMI; in kg/m^2) $>$30, pregnancy, digestive diseases, bowel inflammatory diseases, and any other diagnosed pathology, including diabetes and chronic renal insufficiency.

In total, 64 volunteers (18–24 y old; mean age: 19 y; 32 women and 32 men; BMI: 18–26.9, mean BMI: 21.8) signed a written consent and participated in the study. The intervention trial was approved by the Human Research Committee of the University Hospital of Lille (Lille, France) and was in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study started on 3 January 2006.

Diet

The experimental design was a randomized crossover trial. Two successive diets were planned: a standard diet (STD, rich in MRPs) containing habitually consumed food and a steamed diet (STMD, low in MRPs). Each of the experimental diets was designed by a nutritionist to meet the French dietary allowances and was administered for 4 wk. The menus were changed every day but were repeated weekly. Both diets were designed to contain comparable amounts of energy and nutrients (53% of energy from carbohydrates, 15% of energy from proteins, and 32% of energy from lipids). The STD was prepared by using conventional techniques such as grilling, frying, and roasting and contained industrial food known to be highly cooked, such as extruded corn flakes, coffee, dry cookies, and well-baked bread with brown crust. In contrast, the STMD comprised some raw food and foods that were cooked with steam techniques only. In addition, convenience products were chosen according to the minimal process applied (ie, steamed corn flakes, tea, sponge cakes, and mildly baked bread) as detailed elsewhere (19). Breakfast cereals and fruit juices were offered each day independent from the 2 diets to meet the French dietary allowances for thiamine and vitamin C. According to the crossover design, each 4-wk experimental period was preceded by a 10-d washout period during which the subjects were asked not to change their dietary habits. Subjects were allowed to leave the campus for 1 or 2 weekends. Any participant who did not have his or her meal on campus was provided with meals that were previously prepared according to the respective diet group (STD or STMD) and was asked to note precisely the proportion of the food they ate. Within each period, all food ingested during a complete week, including breakfast and snacks, was recorded by double weighing. Mean nutritional intakes were quantified on the basis of weekly intake data by using the French food-composition table (CIQUAL, Paris, France) and corrected for the cooking effect. Chemical analyses of the nutrients that were expected to be strongly influenced by the cooking process, such as water, proteins, lipids, fatty acids, and vitamins C and E, were performed on the whole mixed daily meals (double-portion analysis), and the corresponding values of the food-composition table were adjusted accordingly (19). In addition, the mean daily intake in CML was assessed.

Medical care and biological sampling

Medical supervision and biological sampling were conducted at the Beauvais Hospital under the responsibility of the main investigator at the Pasteur Institute of Lille.

A first run-in visit involving blood drawing, morning urine collection, and biological and medical exams allowed for the selection of 64 individuals from 80 volunteers who initially agreed to participate. Shortly after the start of the dietary intervention, 2 volunteers withdrew from the study, one because of being sick and the other because of insufficient compliance with the diet. The other 62 volunteers successfully completed the study.

In total, 4 visits had to be completed by the study participants: one visit at the beginning and one visit at the end of each of the 2 dietary interventions, with medical supervision and biological sampling of 42 mL blood, 12-h overnight urines, and morning feces.

Analytic methods

Food analyses

Chemical analyses of nutrients strongly influenced by the cooking process, such as water, proteins, lipids, fatty acids, and vitamin C and E concentrations, were performed on the mixed whole daily meals (except drinks) by using reference methods. Vitamin C and E concentrations were quantified by HPLC-fluorescence with methods slightly adapted from Gliguem and Birlouez-Aragon (20) and Cheikhousman et al (21). The content in Maillard products was quantified by gas chromatography–mass spectrometry (GC-MS)/MS assessment of the indicator CML adapted from Charissou et al (22). After acid hydrolysis of a sample aliquot, methylation and acylation of the carboxylic and amino groups of the dried hydrolysate were performed. The amino acid derivative was injected onto a Thermo scientific Focus GC gas chromatograph (Courtaboeuf, France) and analyzed by MS-MS with an ion trap mass spectrometer.

Biological samples

Plasma and urine indicators of glucose and lipid metabolism were quantified by immunologic (insulin) and colorimetric tests (glucose, total and LDL cholesterol, and triglycerides) (Cobas analyzer; Roche Diagnostics, Meylan, France). The plasma lipid profile was analyzed after methylation by GC analysis. Markers of oxidative stress (vitamins C and E, glutathione, and

ubiquinone) were assessed by HPLC with fluorescence and electrochemical detection (23), whereas malondialdehyde, a lipid peroxidation product, was assessed by HPLC-ultraviolet detection (24). The plasma resistance to oxidative stress was quantified as the time needed to hemolyze 50% of the red blood cells exposed to a controlled free-radical attack, according to the KRL test (SPIRAL, Courtenon, France). Plasma CML was analyzed by GC-MS/MS with stable isotopic dilution (25), and urinary and fecal CML were quantified by the method adapted from Charissou et al (22). Before hydrolysis, plasma proteins were isolated by using Folch's extraction procedure and tricarboxylic acid (5%). Urine was purified and derivatized without applying acid hydrolysis of proteins. Plasma concentrations of fructosyllysine were quantified by stable-isotope dilution analysis after enzymatic hydrolysis of the samples and liquid chromatography–MS/MS analysis according to Vinale et al (26).

Statistical analyses

A multivariate regression analysis (analysis of variance; PROCMIXED procedure, SAS version 8; SAS Institute, Chicago, IL) was applied on the data obtained at the end of each dietary intervention, taking into account possible confounding factors such as the time of the sampling and succession of the 2 diets. The specific effect of each diet, standard or steamed, was analyzed. In the case of a nonnormal distribution of the variables, a log transformation was applied to allow normalization of the variable. The adjusted mean and SD were calculated and are presented for each variable. No interaction between time and diet was observed for any of the variables studied.

RESULTS

Dietary intake of selected macro- and micronutrients

Although the offered meals were comparable in their macro- and micronutrient compositions (19), quantitative analyses of the ingested foods revealed some significant differences between the intakes of volunteers in the STD group compared with those in the STMD group (**Table 1**). A 10% higher caloric intake was evidenced in the STD group as result of a higher overall food consumption and higher energy density, with a significantly higher intake of carbohydrates and fats, but the dietary fatty acid profile was not significantly different, and the ratio of omega 3 (n−3) to omega 6 (n−6) fatty acids was similar in both diets. Vitamin C intake was significantly lower with the STD than with the STMD, whereas vitamin E intake was not significantly different.

Mean exposure to dietary CML

CML, an indicator of dietary advanced Maillard products, was quantified in the complete, mixed daily meals and in selected individual in situ prepared or ready-to-eat foods. As a result, the mean (±SD) total CML intake was 5.4 ± 2.3 and 2.2 ± 0.9 mg CML/d for subjects consuming the STD and STMD, respectively. In the STD group, bread and dough were the major CML sources, whereas in the STMD group, cookies delivered most of the dietary CML (**Table 2**).

TABLE 1
Comparison of the nutritional intakes of the volunteers consuming the standard diet (STD) and steam diet (STMD)[1]

	STD	STMD	*P*
Daily intake (g)	1186 ± 237[2]	1366 ± 267	0.006
Energy intake (kcal)	2549 ± 481	2284 ± 448	0.002
Energy density (kcal/g)	2.1[3]	1.7	0.0001
Protein (g)	89.4 ± 16.8	92.3 ± 16.0	NS
Protein (%)	14	16.2	NS
Carbohydrates (g)	338.6 ± 77.9	301.2 ± 71.9	0.005
Carbohydrates (%)	52.8	52.3	NS
Fat (g)	92.0 ± 15.5	78.9 ± 13.5	0.001
Fat (%)	33.2	31.5	NS
SFA (g/d)	40.0 ± 6.7	34.7 ± 5.9	NS
MUFA (g/d)	37.2 ± 6.2	29.2 ± 5.0	NS
PUFA (g/d)	13.9 ± 2.3	12.6 ± 2.2	NS
n−6 PUFA (g)	11.6 ± 1.9	10.2 ± 1.8	NS
n−3 PUFA (g)	1.2 ± 0.2	1.1 ± 0.2	NS
n−6/n−3	10	10	NS
trans FA (g)	1.9 ± 0.3	1.2 ± 0.2	NS
Vitamin C (mg/d)	60.7	86.1	0.003
Vitamin E (mg/d)	9.1 ± 1.7	7.3 ± 1.3	NS

[1] FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values were compared by using ANOVA as explained in Subjects and Methods.
[2] Mean ± SD (all such values).
[3] Mean (all such values).

CML concentrations in plasma, urine, and feces

CML contents of plasma proteins were significantly higher in volunteers consuming the STD than in subjects consuming the STMD (0.0979 ± 0.0002 μmol CML/mol lysine compared with 0.0918 ± 0.0002 μmol CML/mol lysine, respectively; $P < 0.002$). The urinary CML concentration corrected for creatinine was also higher for the STD group than for the STMD group (0.5039 ± 0.0025 ng CML/mmol creatinine compared with 0.3623 ± 0.0025 ng CML/mmol creatinine, respectively; $P <$

TABLE 2
Contribution of specific food groups to the total N^{ε}-carboxymethyllysine (CML) content of the steam diet (STMD) and standard diet (STD)[1]

	STMD	STD
	%	%
Vegetables	1.7	15
Cookies	48.3	17.2
Breakfast cereals	5.0	11.7
Bread and dough	16.7	26.9
Meat and fish	9.8	15.7
Dairy products	1.5	13.8
Total	83.0	86.7

[1] Results were obtained from a single aliquot of the pooled food offered within each dietary regimen per week and analyzed in duplicate. CML contents of selected foods were also analyzed and used to calculate their contribution to the total CML intake per week. Average CML contents of the food offered daily by each dietary regimen were 5.4 ± 2.3 mg CML/d and 2.2 ± 0.9 mg CML/d with the STD and the STMD, respectively; 13% and 17% of the total CML in the STD and STMD, respectively, were not recovered from the individual foodstuffs, which are grouped in the table into main food groups. Details on cooking procedures and heat processing of ready-to-eat food for both diets are provided in reference 19.

0.002). The protein-adjusted CML concentration of feces did not differ between the groups (STD: 88.9 ± 6.7 μg CML/mg nitrogen; STMD: 88.1 ± 6.8 μg CML/mg nitrogen).

Plasma lipid profile

At the end of the dietary interventions, a 5% ($P < 0.0005$) lower plasma total cholesterol and a 10% lower HDL content ($P < 0.0001$) were found in subjects consuming the STMD compared with subjects consuming the STD (**Table 3**). LDL cholesterol was not significantly different, whereas total plasma triglycerides were lower by 9% ($P < 0.01$) after consumption of the STMD compared with after consumption of the STD. Furthermore, substantial differences in the plasma fatty acid profile with higher plasma concentrations of long-chain n−3 fatty acids (20:5n−3, 22:6n−3) and lower concentrations of n−6 fatty acids (18:2n−6, 20:4n−6) were analyzed in the STMD group compared with in the STD group.

Plasma glucose, insulin, and markers of glycosylation

Fasting glycemia was not different between the experimental groups, but insulinemia was significantly lower in subjects after administration of the STMD compared with after administration of the STD, which resulted in a 17% lower homeostatic model assessment (HOMA) index in the STMD group (**Table 4**). In contrast, no differences were observed for glycosylated hemoglobin and fructosyllysine contents (Table 4).

Indicators of oxidative stress in plasma

For the major antioxidant vitamins in the plasma, vitamin C and E, higher concentrations were analyzed in subjects on the STMD (**Table 5**). In contrast, concentrations of total and reduced (ubiquinol) coenzyme Q10 were increased in the STMD group compared with in the STD group, whereas concentrations of reduced glutathione remained unchanged. No diet-dependent changes in the lipid peroxidation marker malondialdehyde or in the resistance of plasma to free radicals were analyzed.

TABLE 3
Plasma lipids and plasma fatty acid profiles after a 1-mo intake of a standard diet (STD) or steam diet (STMD)[1]

Variable	STD (n = 62)	STMD (n = 62)	P
Total cholesterol (mmol/L)	4.05 ± 0.08	3.83 ± 0.08	0.0005
HDL cholesterol (mmol/L)	1.56 ± 0.04	1.40 ± 0.04	0.0001
LDL cholesterol (mmol/L)	2.49 ± 0.08	2.43 ± 0.08	NS
TG (mmol/L)	0.79 ± 0.04	0.72 ± 0.04	0.01
Total n−3 FA (%)	3.90 ± 0.09	4.39 ± 0.09	0.0001
18:3n−3 (%)	0.38 ± 0.01	0.39 ± 0.01	NS
20:5n−3 (%)	0.54 ± 0.03	0.67 ± 0.03	0.0001
22:6n−3 (%)	2.52 ± 0.07	2.80 ± 0.07	0.0001
Total n−6 FA (%)	34.1 ± 0.3	33.6 ± 0.3	NS
18:2n−6 (%)	24.7 ± 0.3	23.5 ± 0.3	0.0003
20:4n−6 (%)	6.62 ± 0.13	7.11 ± 0.13	0.0003

[1] All values are means ± SDs, which were calculated for the 62 volunteers after each diet period. TG, triacylglycerol; FA, fatty acid. Statistical analyses were performed by using ANOVA after normalization of the variables as explained in Subjects and Methods.

TABLE 4
Plasma concentrations of glucose metabolism variables after a 1-mo intake of a standard diet (STD) or steam diet (STMD)[1]

Variables	STD (n = 62)	STMD (n = 62)	P
Fasting glucose (mmol/L)	4.36 ± 0.04	4.29 ± 0.04	0.08
Fasting insulinemia (mU/L)	7.63 ± 0.40	6.52 ± 0.40	0.01
HOMA ($\mu mol \cdot mU^{-1} \cdot L^{-1}$)	1.35 ± 0.06	1.12 ± 0.06	0.002
Plasma protein fructosyllysine (mmol/L)	3.42 ± 0.43	3.40 ± 0.46	NS
Red blood cell Hb A_{1c} (%)	5.11 ± 0.26	5.09 ± 0.26	NS

[1] All values are means ± SDs. HOMA, homeostatic model of assessment; Hb A_{1c}, glycosylated hemoglobin. Values were compared by using ANOVA as explained in Subjects and Methods.

Pearson's correlations between dietary intake, CML concentrations in biological samples, and biomarkers of risk factors for diabetes mellitus and cardiovascular diseases

Pearson's correlations were calculated between variables, especially between the dietary variables and biological markers, to prove that the slight changes observed in macronutrient and vitamin C intakes were not associated with the metabolic changes observed. With the exception of 22:6n−3 concentrations, there was an absence of significant correlations in plasma with energy intake, total fat, saturated fatty acids, and carbohydrates (**Table 6**). Plasma CML concentrations were significantly correlated with the plasma total and HDL cholesterol, triglycerides, eicosapentaenoic acid (20:5n−3), and ubiquinol.

DISCUSSION

Modern lifestyles commonly embrace new cooking techniques and food choices, which allows for a decreasing amount of time need for food preparation. Ready-to-eat food such as breakfast cereals, cookies, and snacks are produced by high-temperature processes and rapid cooking techniques, such as grilling and frying. Although such techniques have been known for a long time, a high frequency of use characterizes most current lifestyles. The wide range of MRPs generated by cooking foods at high temperatures also contributes to the consumer preference for such tasty food. However, among the many compounds formed, some are of concern because of their possible role in

TABLE 5
Plasma concentrations of oxidative stress indicators after a 1-mo intake of the standard diet (STD) or steam diet (STMD)[1]

Variables	STD (n = 62)	STMD (n = 62)	P
Vitamin C (μmol/L)	65.3 ± 1.73	69.6 ± 1.73	0.01
Vitamin E (μmol/mg cholesterol)	3.05 ± 0.05	3.25 ± 0.05	0.0001
Ubiquinol + ubiquinone (nmol/L)	773 ± 28	710 ± 28	0.025
Ubiquinol (nmol/L)	685 ± 25	616 ± 25	0.025
Reduced glutathione (μmol/L)	1885 ± 48	1889 ± 48	NS
Malondialdehyde (mmol/L)	1.65 ± 0.50	1.59 ± 0.50	NS
AOP (Eq μmol/L Trolox[2])	3580 ± 552	3591 ± 552	NS

[1] All values are means ± SDs. AOP, antioxidant power of the plasma; Eq, equivalents. Values were compared by using ANOVA as explained in Subjects and Methods.

[2] Manufactured by Sigma, St Quentin Fallavier, France.

TABLE 6
Pearson's correlations (r) between plasma variables and N^{ε}-carboxymethyllysine (CML) concentrations in biological media or dietary intake of energy, macronutrients, and vitamin C[1]

	CML		Nutritional intakes					
	Plasma	Urine	Fat	SFA	Sugar	Protein	Energy	Vitamin C
HDL cholesterol	0.321[2]	0.015	0.149	0.138	−0.043	−0.061	0.008	−0.134
Total cholesterol	0.285[2]	−0.161	−0.03	−0.038	0.004	−0.095	−0.02	−0.066
Triglycerides	0.220[3]	0.007	−0.009	−0.013	0.039	−0.058	0.015	−0.087
Insulinemia	0.036	0.092	0.109	0.113	−0.012	−0.005	0.024	0.111
HOMA	−0.007	0.086	0.121	0.100	0.022	0.023	0.053	0.132
Plasma vitamin C	0.068	0.038	−0.089	−0.080	−0.053	0.002	−0.06	0.146
Plasma vitamin E	−0.065	0.054	0.1	0.102	0.149	0.159	0.147	0.11
Ubiquinol	0.292[2]	0.02	0.002	−0.007	−0.037	−0.095	−0.036	−0.139
18:2n−6	0.005	0.081	−0.057	−0.052	0.092	0.022	0.045	0.059
20:4n−6	−0.128	−0.081	0.016	0.031	−0.043	0.107	−0.008	0.026
20:5n−3	−0.194[3]	−0.16	−0.044	−0.026	−0.051	0.066	−0.037	−0.019
22:6n−3	0.033	−0.129	−0.215[3]	−0.203[3]	−0.219[3]	−0.094	−0.218[3]	0.034

[1] HOMA, homeostatic model of assessment; SFA, saturated fatty acid.
[2] $P < 0.01$.
[3] $P < 0.05$.

inflammation, insulin resistance, and cardiovascular diseases (27). Several studies (9, 11, 12, 28) performed in animals indicate that a higher dietary intake of MRPs promotes the formation of proinflammatory cytokines and the development of insulin resistance and renal dysfunction and aggravates cardiovascular diseases in normal and diabetic animals. In turn, decreasing the dietary intake of MRPs was shown to improve the pathophysiology (29, 30).

Controlled intervention studies have only been performed in patients with diabetes, comparing the health effects of an STD with those of an equivalent diet that was based on steam cooking (10, 17, 18). The results uniformly show that plasma concentrations of MRPs and proinflammatory cytokines, such as tumor necrosis factor-α, are proportional to the dietary intake of MRPs. These results, suggesting that dietary MRPs may contribute to the increasing risk of diabetes and cardiovascular diseases in populations adopting a modern Western diet, prompted us to perform the current study in healthy volunteers.

Our objective was to test whether a diet prepared by using high-temperature cooking techniques, which results in the high MRP content of foods, has different effects on clinical indicators of the risk of diabetes mellitus and cardiovascular diseases than a diet that is based on low-temperature cooked foods with low contents of MRPs (19). Although numerous neoformed potential contaminants were shown to be higher in the STD than in the STMD, such as hydroxymethylfurfural (40-fold higher) and acrylamide (5-fold higher) (19), we chose CML as a well-accepted indicator of food-borne MRPs (31). In vivo, CML was observed to bind to the receptor of advanced glycation end products, which, in turn, results in an activation of proinflammatory pathways (32).

Although the 2 experimental diets tested in the current study were designed to be comparable in their contents in energy and macronutrients but to contain different amounts of CML (19), the actual intakes of energy, carbohydrates, and fat were higher in the STD group than in the STMD group. The reason for the higher caloric intake (+10%) by subjects on the STD may have been the higher caloric density of the STD (2.14 kcal/g) compared with that of the STMD (1.67 kcal/g). The higher temperatures applied to foods in the STD resulted in a 42% lower vitamin C intake in the STD group. The mean concentration of CML in the daily meals was 2.5 times higher in the STD than in the STMD. The amount of 2.2 mg CML present in the STMD mainly originated from cookies (50%), whereas in the STD, brown baked bread, breakfast cereals, cookies, and dough in pizzas were the primary sources (57%) of CML. Home-cooked vegetables, meat, and dairy preparations contributed <25–30% to the total CML content of the 2 diets.

As a consequence of the 2.5-times higher dietary exposure to CML, the urinary CML excretion was 40% higher and fasting plasma CML was 7% higher in subjects on the STD compared with subjects on the STMD. This suggests that dietary CML is absorbed in the intestines and rapidly excreted, which confirms the results obtained in animals (16).

In humans, Vlassara et al (10) reported a higher concentration of LDL-bound CML in subjects exposed to high doses of dietary MRPs than in subjects on diets low in MRPs.

Considering the results of the current study, which showed that a high intake of CML leads to increased plasma concentrations of CML (as a key indicator compound of dietary MRPs), the main aim of this study was to correlate the endogenous CML load with biochemical indicators of diabetes mellitus and cardiovascular risk factors as suggested by results from animal studies (9, 28, 29). Consequently, most of the metabolic indicators of the risk factors selected were significantly altered after the volunteers were administered a high-heat-treated diet compared with the dietary intake of predominantly steamed foods. The ≈10% higher concentrations in plasma triacylglycerol, total and HDL cholesterol, and ubiquinol of subjects after intake of the STD than after intake of the STMD were correlated with serum CML (r between 0.22 and 0.32; $0.01 < P < 0.05$) (Table 6).

No association was shown between any of the risk factors analyzed in the plasma and the dietary intakes of total, energy, fat, saturated fatty acids, carbohydrates, proteins, and vitamin C. However, the plasma docosahexaenoic acid (DHA) content was inversely correlated with the intakes of total energy, fat, and

carbohydrates (Table 6), suggesting that oxidation of DHA during high-temperature cooking applied to foods of the STD. This hypothesis was supported by a trend for higher DHA contents in the STDM compared with the STD (0.36 ± 0.24 compared with 0.16 ± 0.05 g for 100 g total fat; $P > 0.05$).

Lower ratios of plasma vitamin E to total cholesterol ($P < 0.0001$) and decreased plasma vitamin C concentrations ($P < 0.015$) also suggest an increased oxidative stress and lower protection of the plasma lipids against oxidation after the STD. An increased synthesis of ubiquinol probably originating from an adaptive response by the liver, as already evidenced in the scope of metabolic syndrome (33), was significantly correlated with plasma CML concentrations in the current study ($P < 0.025$).

A strong increase (+17%) in the HOMA index after consumption of the STD compared with that of the STMD suggested that insulin was less efficient at normalizing plasma glucose in the case of a higher exposure to cooking-derived MRPs. However, contrary to what was shown in the study of Uribarri et al (34), no association was shown with plasma CML, suggesting that other MRPs should be involved.

As a limitation of the study, we cannot exclude a contribution of the higher fat and carbohydrate intake or the lower vitamin C intake with the STD on plasma lipid and glucose variables, although no statistical correlation for these variables was shown. However, HOMA and fasting insulin concentrations also decreased after STMD intake. This observation is in agreement with the improved insulin sensitivity shown in mice (30) and decreased fasting glucose in humans (10) after a diet low in MRPs. Therefore, the data of the current study support the hypothesis that a diet rich in MRPs modulates biomarkers that indicate an elevated risk of the development of diabetes mellitus.

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The authors' responsibilities were as follows—IB-A: design of the study, development of analytical methods, writing of the manuscript, and scientific coordinator of the project; GS: CML analysis in urine and feces samples and interpretation of the data; FD: vitamin C analysis in plasma and logistical aspects regarding dietary and biological samples and data; AG: ubiquinone, GSH, and vitamin E analysis in plasma and interpretation of the data; LA-T: CML and vitamin E analysis in food samples; C-NN: statistical analysis of the data; NA: analysis of plasma CML and fructosyllysine; VS: development of plasma CML and fructosyllysine analysis and writing of the manuscript; and J-ML: design of the study and clinical follow up. IB-A created Small and Medium Enterprises (SME; SPECTRALYS Innovation, Romainville, France) to develop and commercialize sensors for monitoring of process contaminants in processed food. Studies revealing the possible safety problems associated with those contaminants could promote the need for safety agencies and the food industry to control those contaminants. The authors declared no conflicts of interest.

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