

# No short-term effects of calorie-controlled Mediterranean or fast food dietary interventions on established biomarkers of vascular or metabolic risk in healthy individuals

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**BACKGROUND/OBJECTIVES:** This study addressed the question whether the composition of supposedly 'healthy' or 'unhealthy' dietary regimes has a calorie-independent short-term effect on biomarkers of metabolic stress and vascular risk in healthy individuals.

**SUBJECTS/METHODS:** Healthy male volunteers (age  $29.5 \pm 5.9$  years,  $n = 39$ ) were given a standardized baseline diet for two weeks before randomization into three groups of different dietary regimes: fast food, Mediterranean and German cooking style. Importantly, the amount of calories consumed per day was identical in all three groups. Blood samples were analyzed for biomarkers of cardiovascular risk and metabolic stress after two weeks of the baseline diet and after two weeks of the assigned dietary regime.

**RESULTS:** No dietary intervention affected the metabolic or cardiovascular risk profile when compared in-between groups or compared to baseline. Subjects applied to the Mediterranean diet showed a statistically significant increase of uric acid compared to baseline and compared to the German diet group. Plasma concentrations of urea were significantly higher in both the fast food group and the Mediterranean group, when compared to baseline and compared to the German diet group. No significant differences were detected for the levels of vitamins, trace elements or metabolic stress markers (8-hydroxy-2-deoxyguanosine, malondialdehyde and methylglyoxal, a potent glycation agent). Established parameters of vascular risk (e.g. LDL-cholesterol, lipoprotein(a), homocysteine) were not significantly changed in-between groups or compared to baseline during the intervention period.

**CONCLUSIONS:** The calorie-controlled dietary intervention caused neither protective nor harmful short-term effects regarding established biomarkers of vascular or metabolic risk. When avoiding the noxious effects of overfeeding, healthy individuals can possess the metabolic capacity to compensate for a potentially disadvantageous composition of a certain diet.

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## INTRODUCTION

Fast food is commonly accepted to be a health threatening, "atherogenic" diet, regardless of the amount of caloric intake or the mode of preparation. Fast food is characterized by high amounts of saturated fat and a high caloric density. Several studies demonstrated the association with weight gain, poor dietary indicators, insulin resistance and obesity [1]. In contrast, epidemiological studies, but not intervention studies, pointed to a lower prevalence of various diseases in populations on the Mediterranean diet. Lower incidence rates were demonstrated for cardiovascular diseases [2-5], chronic neurodegenerative

diseases [6], malignomas [5,7], obesity and Diabetes mellitus type 2 [8-11]. There is no doubt that diet in general is an important part of the therapeutic regime in metabolic diseases and that certain eating habits are associated with development and progression of metabolic disease [12-14].

In a recent intervention study, Estruch *et al.* [15] reported that a Mediterranean diet supplemented with either extra virgin olive oil or nuts has beneficial effects in the primary prevention of cardiovascular disease in a high risk population. It is of importance to realize that there was no significant effect on death from any cause or myocardial infarction, the only statistically significant protective effect reported was for stroke. The

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margins of risk were very small and the authors' interpretation of the data provoked substantial critique: The benefit reported for stroke was apparent only in the first study year and vanished during further follow-up, while apparent differences in the baseline characteristics between the study groups might have contributed to the minor, short-term differences in absolute risk [16,17]. Furthermore, the results presented by Estruch *et al.* are only statistically significant as long as calculated as a projection for 1,000 person years. Therefore we calculated the data for the entire follow-up period without applying a projection for 1,000 patient years and found the primary end point to be no longer statistically significant; neither for the comparison between the nut-based Mediterranean diet (83 events in 2,454 individuals) and control condition (109 events in 2,450 individuals),  $P = 0.054$  (2-tailed), nor for the comparison between extra virgin olive oil Mediterranean diet (96 events in 2,543 individuals) and control condition (109 events in 2,450 individuals),  $P = 0.23$ . The discrepancy between the interpretation as presented by the authors and the more objective event based calculation used above, indicates that an effect of diet in the primary prevention of cardiovascular disease in high risk populations might be minor. Thus we need to learn more about determinants of atherosclerosis potentially applicable to medical or lifestyle intervention.

Numerous studies addressed the issue of protective effects of "healthy food" for healthy people, but prospective randomized trials proving efficiency of e.g. low fat, high fiber intake in healthy people with respect to hard endpoints are not yet available. Thus the question arises whether healthy people can become even healthier by a specific diet or whether fast food per se has a negative impact on a healthy person's individual

**Table 1.** Subject characteristics: body weight and body mass index (BMI) measured before and after two weeks of dietary intervention

	Fast food	German	Mediterranean
Age (yrs)	27.36 ± 5.73	29.14 ± 5.83	31.88 ± 6.25
Height (m)	1.81 ± 0.04	1.82 ± 0.06	1.84 ± 0.05
Body weight (kg) before	78.54 ± 6.13	81.0 ± 9.0	81.78 ± 8.38
after	79.54 ± 7.04	79.1 ± 8.28**	80.50 ± 7.68
BMI before	23.95 ± 1.95	24.54 ± 2.27	24.01 ± 1.53
after	24.25 ± 2.46	23.97 ± 2.26**	23.67 ± 1.50

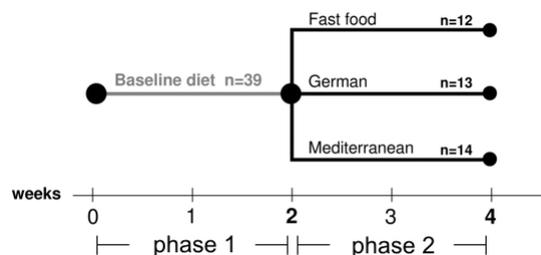
All the values are given as mean ± SD.

Body weight: \*\*  $P < 0.01$  (paired t-test)

Body mass index (BMI): \*\*  $P < 0.01$  (paired t-test)

**Table 2.** Main features of the three diet groups

Diet group	Diet characteristics
Fast food	High amounts of red meat and sausages (3-4 portions a day), white bread, French fries and eggs. Moderate to high amounts of sweet foods and drinks. Low amounts of fish and poultry. Very low amounts of fruits, vegetables and dairy products.
Mediterranean	High amounts of pasta, rice, couscous and potatoes. High amounts of fruits (3-4 pieces a day), nuts, legumes and vegetables (5 different varieties). High amounts of olive oil. Moderate amounts of wine, grilled and steamed fish, dairy products and poultry. Low amounts of red meat and eggs.
German	High amounts of meat (pork), sausages and butter. Moderate amounts of whole-meal bread, potatoes, fruit and vegetables. Moderate amounts of dairy products, fish and eggs. Moderate to high amounts of beer, sweet foods and sugar.



**Fig. 1.** Schematic timeline of the study and randomization of  $n = 39$  participants in three groups with different dietary portfolio.

risk profile independent of caloric intake [18]. It was recently shown that there is no beneficial effect of a low fat diet compared to either increased protein or carbohydrate intake, with respect to weight loss in type 2 diabetic patients [19]. Another study demonstrated the lack of favorable effects of lifestyle intervention in young patients with type 2 diabetes [20]. While guidelines for healthy dietary regimes recommend restriction of sucrose intake, a weight-maintaining diet with high sucrose intake had no effect on insulin sensitivity, glycemic profiles or measures of vascular compliance in healthy, non diabetic subjects [21]. In a recent systemic review, only the consumption of whole grain products showed moderate-grade evidence for protection against type 2 diabetes and cardiovascular disease [22]. In addition, the Palaeolithic ("old stone age") diet, which is rich in meats, fish, fruits, vegetables, root vegetables, eggs and nuts, improved glucose tolerance in patients with heart disease and either glucose intolerance or type 2 diabetes, more than the Mediterranean diet [23,24]. These data indicate that the concept of healthy vs. unhealthy diet for healthy individuals should be viewed more critically.

In order to address the question of short-term effects of dietary regimes in healthy individuals, we conducted a two-week intervention study in healthy male volunteers (Table 1), comparing three dietary regimes (Table 2) after a standardized run-in period of two weeks (Fig. 1). The Mediterranean diet as an example of a supposedly health improving diet was compared to fast food, and the German cooking style - reflecting the traditional local eating regime - served as a reference group. Importantly, all groups received equal amounts of calories per day.

Main parameters to detect changes in the individual risk profile were classical biomarkers for vascular risk such as LDL- and HDL-cholesterol and homocysteine, previously shown to be affected by the Mediterranean diet [10,25-27]. Since oxidative stress has been shown to contribute to vascular disease and aging in general, we included biomarkers of oxidative stress, namely 8-hydroxy-2-deoxyguanosine and malondialdehyde. 8-hydroxy-2-deoxyguanosine derives from deoxyguanosine oxidation and reflects the level of DNA damage by oxidation, while Malondialdehyde is a reactive aldehyde that results from the degradation of polyunsaturated lipids by reactive oxygen species [28-30]. Furthermore methylglyoxal, a highly cytotoxic  $\alpha$ -oxoaldehyde derived from triose phosphate intermediates of glycolysis, was studied. Methylglyoxal is a potent glycation agent and is believed to be of main importance in aging and

degenerative neuronal disease. In experimental settings, methylglyoxal has been shown to mediate vascular disease and pain by modification of amino acids [31]. Thus methylglyoxal is believed to be a major toxic metabolite derived from energy flux and a biomarker for overloaded metabolic detoxification mechanisms, predicting development of pain and endothelial cell dysfunction. The effects of dietary intervention on methylglyoxal as a biomarker for changes in metabolic flux and detoxification processes have not been studied yet. The relatively short intervention phase of two weeks was chosen because numerous studies in healthy subjects demonstrated that e.g. high-fat vs. low-fat interventions or changing the fatty acid composition result in diet-induced changes in the vascular and metabolic risk profile as early as two weeks after intervention, suggesting a significant immediate effect of substantial dietary changes on the individual risk profile [32-39]. Importantly, two intervention studies comparing low-fat/high-protein and low-fat/high-carbohydrate diets with regard to markers of vascular risk report a more pronounced short-term response after 2 or 4 weeks when compared to the effects in the long run [33,36], pointing towards the ability of healthy individuals to metabolically compensate for changes in the dietary composition.

The first objective of this study was to assess whether changes in the food composition and the mode of preparation - reflected by different dietary regimes - have an immediate effect on the individual risk profile, independent of overall energy consumption. The second objective was to study the effect of different

diets on the metabolic marker methylglyoxal, not studied in the context of dietary intervention in healthy subjects before. Thus this study was planned to understand whether calorie-adjusted diets affect biomarkers reflecting the risk of vascular and metabolic disease in healthy individuals, or whether healthy individuals possess the metabolic capacity to balance changes in the dietary composition.

## SUBJECTS AND METHODS

### Subjects

Forty-five males ranging in age from 21 to 40 years were recruited via newspaper advertisement, gave their informed consent and were submitted to physical examination and medical history. Inclusion criteria were male sex, age 20-40 years and good health (absence of acute or chronic disease). We chose a mid-aged study collective in order to minimize a potential bias from inapparent, age-related medical conditions. Females were excluded in order to avoid hormone dependent changes in the parameters determined. The following exclusion criteria were defined in order to exclude an influence of pre-existing conditions known to effect parameters of vascular and metabolic risk: smoking, current medication, consumption of food additives, extensive physical activity, body mass index (BMI) > 30 kg/m<sup>2</sup> and evidence for chronic disease. Subjects included in the study (age 29.5 ± 5.9 years, n = 39, Table 1) were encouraged to maintain their normal life style with average

**Table 3.** Content analysis of the nutrients in all diet group portfolios compared with recommended daily doses according to DACH [40]

Parameter (unit)	Fast food	German	Mediterranean	Recommended daily doses
kcal	2,925	2,871	2,901	2,500
Fat (g)	157	139	121	80
Saturated FA (g)	58	59	32	< 32
MUFA (g)	56	51	56	> 32
PUFA (g)	33	17	17	≤ 32
Protein (g)	135	134	133	95
Carbohydrates (g)	243	271	320	350
Dietary fibers (g)	17	35	40	30
Cholesterol (mg)	736	704	313	< 300
Purine (mg)	188	262	283	< 300
Docosahexaenoic acid (mg)	382	391	759	not available
Arachidonic acid (mg)	494	301	174	not available
Glucose (g)	17.7	12.2	23.4	not available
Saccharose (g)	32	20	13	not available
Magnesium (mg)	230	452	546	350
Folic acid (µg)	96	209	329	400
Calcium (mg)	979	1,020	1,183	1,000
Vitamin A (mg)	1.3	1.8	2.4	1
Vitamin D (µg)	5.6	15	5.8	20
Vitamin E (mg)	24	16.3	26.7	15
Vitamin K (µg)	591	718	772	70
Vitamin B <sub>6</sub> (mg)	1.5	3.3	3.6	1.5
Vitamin B <sub>1</sub> (mg)	1.5	2.6	2	1.2
Vitamin C (mg)	68	242	380	100
Vitamin B <sub>2</sub> (mg)	1.7	2.2	2	1.4
Vitamin B <sub>12</sub> (µg)	14	11.5	6.8	3
Vitamin B <sub>3</sub> (mg)	20.9	26	31.1	16

**Table 4.** The parameters of vascular risk analyzed after the baseline diet and after two weeks of different dietary interventions

Parameter / Method	Unit	Baseline	fast food (F)	Baseline	German (G)	Baseline	Mediterranean (M)	P-value	One way ANOVA		
									Multiple Comparison Test (q)		
									F vs. G	F vs. M	G vs. M
Urea	a	29 ± 6.396 <sup>1)</sup>	37.08 ± 4.209	29 ± 5.583	27.08 ± 5.722	31 ± 4.243	34.79 ± 10.8	0.0057**	4.624 <sup>†</sup>	1.080	3.702 <sup>†</sup>
Uric acid	a	5.517 ± 0.979	5.558 ± 0.696	5.831 ± 1.031	5.362 ± 0.8312	5.671 ± 0.9762	6.264 ± 0.997	0.0241*	0.8098	2.956	3.861 <sup>†</sup>
Glucose	a	94.83 ± 5.937	94.5 ± 5.351	94.92 ± 8.76	99.77 ± 7.726	97.29 ± 8.879	94.79 ± 15.2	0.3728	1.759	0.097	1.729
Cholesterol	a	171.3 ± 33.92	180.5 ± 34.8	171.2 ± 47.4	196.8 ± 41.1	167.8 ± 29.21	177.2 ± 27.57	0.3139	1.654	0.340	2.066
Creatinine	a mg/dL	0.8625 ± 0.102	0.9267 ± 0.07596	0.9023 ± 0.107	0.9185 ± 0.08877	0.9 ± 0.122	0.9943 ± 0.11	0.0829	0.310	2.597	2.947
Lipoprotein(a)	b	9.842 ± 9.273	12.1 ± 13.69	10.24 ± 7.629	9.581 ± 7.882	15.56 ± 15.89	13.79 ± 11.75	0.6284	0.787	0.537	1.367
Triglyceride	a	118.1 ± 106	101.9 ± 72.45	80.62 ± 48.17	113.4 ± 97.69	78.07 ± 20.68	84.43 ± 25.06	0.5682	0.572	0.888	1.502
HDL	a	43.83 ± 8.861	53.75 ± 14.37	40.46 ± 8.11	52.62 ± 9.82	44.36 ± 9.145	55.93 ± 10.06	0.7488	0.349	0.682	1.060
LDL	a	103.7 ± 26.86	106.3 ± 27.61	114.6 ± 42.51	121.5 ± 38.32	107.9 ± 25.48	104 ± 27.28	0.3155	1.698	0.266	2.036
Magnesium	a mmol/L	0.8742 ± 0.085	0.8767 ± 0.06415	0.8569 ± 0.065	0.8692 ± 0.08067	0.8529 ± 0.06866	0.8886 ± 0.053	0.7505	0.393	0.641	1.064
HbA1c	c mW%	5.255 ± 0.216	5.2 ± 0.2236	5.218 ± 0.223	5.127 ± 0.2102	5.279 ± 0.2486	5.171 ± 0.216	0.7319	1.113	0.463	0.715
GOT	a	28.83 ± 18.61	21 ± 8.571	26.31 ± 6.909	24.08 ± 6.958	24.43 ± 8.026	23 ± 12.73	0.7343	1.103	0.730	0.401
GPT	a U/L	39.92 ± 40.04	29.42 ± 20.02	28.77 ± 16.43	28.62 ± 21.27	24.07 ± 14.58	22.57 ± 8.093	0.5376	0.164	1.428	1.288
GGT	a	26.25 ± 18.14	23.83 ± 15.9	22.77 ± 12.92	22.15 ± 11.71	20.71 ± 15.22	19.43 ± 9.346	0.6608	0.477	1.274	0.805
Quick	d %	82.64 ± 9.204	93.6 ± 5.802	78.64 ± 11.02	87.49 ± 10.13	80.79 ± 10.45	88.18 ± 11.92	0.2477	2.204	1.991	0.257
Fibrinogen	d g/L	2.25 ± 0.368	2.683 ± 0.6394	2.585 ± 0.815	2.446 ± 0.3865	2.407 ± 0.4215	2.464 ± 0.465	0.433	1.666	1.566	0.132
PTH	e pmol/L	5.167 ± 2.483	5.908 ± 2.742	4.615 ± 2.539	5.154 ± 2.685	4.386 ± 1.608	5.071 ± 1.975	0.6501	1.078	1.217	0.122
Homocysteine	e	11.89 ± 2.456	8.417 ± 1.468	12.06 ± 2.152	8.777 ± 2.254	12.27 ± 3.076	8.95 ± 2.7	0.8286	0.570	0.858	0.285
Vitamin A	f	1.783 ± 0.482	1.933 ± 0.3393	1.792 ± 0.353	2.054 ± 0.5953	1.793 ± 0.3339	2.293 ± 0.604	0.2271	0.797	2.420	1.643
Vitamin E	f	30.39 ± 9.006	31.68 ± 7.686	28.32 ± 7.518	33.13 ± 7.948	27.6 ± 3.716	31.56 ± 6.48	0.8326	0.698	0.054	0.781
Selen	g µmol/L	1.318 ± 0.498	1.077 ± 0.2916	1.184 ± 0.386	1.122 ± 0.2213	1.366 ± 0.2735	1.118 ± 0.261	0.8915	0.613	0.572	0.052
Vitamin D	h	14.88 ± 7.773	11.64 ± 6.041	14.2 ± 5.35	14 ± 5.151	19.24 ± 9.954	17.89 ± 9.034	0.0853	1.187	3.194	2.029
Zink	g	14.91 ± 2.284	12.58 ± 1.512	16.37 ± 2.905	13.33 ± 1.428	15.29 ± 1.749	12.29 ± 1.656	0.2105	1.716	0.695	2.493
Vitamin B <sub>1</sub>	f	160.8 ± 32.47	144.9 ± 34.2	152.6 ± 19.89	139.4 ± 20.02	163.5 ± 24.75	131.4 ± 21.25	0.4085	0.749	1.893	1.155
Vitamin B <sub>6</sub>	f nmol/L	82.21 ± 32.97	73.69 ± 36.36	80.15 ± 18.09	89.48 ± 48.51	83.6 ± 41.65	90.31 ± 50.41	0.5987	1.216	1.302	0.066
Osteocalcitonin	i ng/mL	22.13 ± 4.102	23.25 ± 5.888	18.54 ± 6.894	23.26 ± 6.063	25.12 ± 5.987	29.07 ± 15.23	0.2541	0.004	2.027	2.066
MDA	j µmol/L	16.61 ± 5.149	9.345 ± 7.849	16.76 ± 8.346	12.91 ± 16.92	13.85 ± 7.13	7.598 ± 5.699	0.462	1.153	0.576	1.750
HT8-oxo-dG <sup>k</sup>	l nmol/L	69.7 ± 12.95	68.49 ± 15.14	70.19 ± 17.96	64.10 ± 11.05	66.21 ± 12.4	66.96 ± 16.0	0.7301	1.109	0.393	0.736
Methylglyoxal	m	466 ± 155.1	290.7 ± 52.50	444.8 ± 155.3	310.7 ± 39.18	409.9 ± 136.6	353.0 ± 107.3	0.0940	0.978	3.099	2.103

All the values are given as mean ± SD.

a) Siemens ADVIA 2440 analyzer, b) N Latex Lipoprotein (a) reagent, BN<sup>TM</sup> II analyzer, c) Hemoglobin Analyzer Variant 2 BioRad, d) CA7000 Siemens, e) ADVIA Centaur XP Siemens, f) Reagent Kit for HPLC, Chromosystem, g) Standard solutions of Selen and Zink for atomic absorption spectrometry (Merk) on a Varian SpectrAA240Z GTA 120 (Agilent Technologies) h) 25-OH-Vitamin D Total Integral (DiaSorin), i) Immulite 2000 Siemens Platform, j) OxiSelect malonaldehyde (MDA) Adduct ELISA Kit (BioCat), k) 8-hydroxy-2-deoxyguanosine, l) 8-hydroxy-2-deoxyguanosine ELISA (Trevigen), m) As previously published [40].

\*  $P < 0,05$ , \*\*  $P < 0,01$ , <sup>†</sup> turkey's multiple comparison test  $P < 0,05$

physical activities. The study protocol was approved by the Ethical Committee of the University Hospital Heidelberg, all 39 participants completed the study.

### Study design

In phase 1 of the study, all subjects were given a standardized control diet for a period of two weeks (Fig. 1). This baseline diet during phase 1 of the study, based on the German cooking style, was applied in order to equalize the participants' diets on the basis of a familiar eating regime prior to the dietary intervention of phase 2. The baseline diet was continued for the German diet group during the intervention phase 2, serving as a reference group for the comparisons between the Mediterranean diet and fast food. The baseline diet was composed of 30% fat, 15% proteins and 47% of carbohydrates. In order to exclude a bias from overnutrition, the energy amount of 2,500 kcal per day for both phases of the study was calculated on the basis of local recommendation for males aging 25-51 years with a moderate physical activity level [40].

The participants' body weights were measured at baseline and at the end of the study. At the end of phase 1, blood samples were obtained and subjects were randomized in three different dietary intervention groups for phase 2. The composition of the diets was calculated using Prodi Software Version 5.0 (NutriScience) and Bundeslebensmittelschlüssel Version 3.01, the general characteristics and the overall composition of the meals in the different diet groups are presented in Tables 2 and 3, respectively. Importantly, each day three meals per participant were freshly prepared from high quality foods by distinguished chefs and were eaten on site to assure that participants completely ate their dishes. On a few occasions, a take-away package was prepared upon individual request. Beside the study regime diet, only calorie-free drinks were allowed. Regular meetings and supervisions ensured that all participants strictly followed the assigned protocol.

While the energy content of the meals did not differ between the study groups, the caloric density differed considerably: ca. 2 kcal/g for fast food and ca. 1.2 kcal/g for the Mediterranean

and German diet. In addition, calorie-rich drinks and shakes were included in the fast food diet, resulting in a considerably lower quantity of food in the fast food group than in the Mediterranean group (average daily quantities were 940 and 2,060 g, respectively). After a few days, most of the participants of the fast food group suffered from feeling constantly hungry and several participants considered dropping out of the study. We therefore increased the amount of calories to 2,900 kcal per day on the seventh day of phase 2 for all three study groups, maintaining the same proportion of nutrients. This increase of 400 kcal (16%) per day was calculated as a trade-off between meeting the needs of the fast food group for larger quantities of food on the one hand and not generating excessive, inconsumably large quantities of food for the Mediterranean group on the other hand.

#### *Blood samples and analysis*

Venous blood samples were collected after  $\geq 12$  hours of fasting in Monovette® blood collection tubes (Sarstedt, Nümbrecht, Germany) containing either EDTA (1 g/L), citrate (3.8%) or heparin (500 I.U.). A volume of 20 ml whole blood was collected at the end of phase 1 and at the end of phase 2. Plasma and serum was obtained by low-speed centrifugation at 1500 *g* for 15 minutes at 4°C. Plasma and serum samples were kept in aliquots at -20°C and were analyzed in the central laboratory of the Heidelberg University Hospital, applying validated and accredited routine analytical methods. Briefly, total cholesterol, HDL-cholesterol and triglycerides were analyzed via enzymatic reactions followed by photometric quantification on a Siemens ADVIA 2400 analyzer according to the manufacturer's instructions. LDL-cholesterol was calculated according to the Friedewald formula. Lipoprotein(a) was measured immunologically on a Behring Nephelometer analyzer (BN II) and homocysteine on a Siemens ADVIA Centaur analyzer. Serum levels 8-hydroxy-2-deoxyguanosine and malondialdehyde were quantified with ELISA kits purchased from BioCat (Heidelberg, Germany) and Trevigen (Biozol Diagnostica, Eching, Germany), respectively. Methylglyoxal was measured as previously published [31]. A complete list of analytical methods is given in Table 4 and is available online at <http://www.klinikum.uni-heidelberg.de/Leistungverzeichnis.137009.0.html>.

#### *Statistical analysis*

The normality of variables was tested using Shapiro test. The differences in the means of the groups was analyzed by one-way ANOVA, followed by Tukey's Multiple Comparison Test. Significance was assumed for  $P < 0.05$ . Student's *t* test for paired observations was used for the before-after comparison within the same group. All data presented in the text, tables and graphs are mean values (M) followed by the standard deviation of the mean ( $\pm$  SD).

## **RESULTS**

#### *Analysis of dietary composition*

This study investigated the effects of three dietary regimes on parameters of vascular and metabolic risk in healthy individuals, independent of changes in caloric intake (Fig. 1,

Table 1 and 2). The analysis of the content of the nutrients in each of the three study groups is shown in Table 3. The most outstanding differences were found in respect to dietary fibers being lowest in the fast food dishes and cholesterol being lowest in the Mediterranean group. As expected, the total fat content and saturated fatty acids (SFA) were lowest in the Mediterranean dishes, while the relative amount of monounsaturated fatty acids (MUFA) was highest in this diet (MUFA/SFA-quotients being 0.97, 0.86 and 1.75 for fast food, German and Mediterranean diet, respectively). The Mediterranean group obtained more docosahexaenoic acid, less arachidonic acid and the most folic acid. Compared to the recommendations for folic acid and Vitamin C of 400  $\mu$ g per day and 100 mg per day, respectively [40], there was a deficiency concerning these nutrients in the fast food group. However, the currently recommended doses for folic acid and Vitamin C greatly exceed the daily requirement for preventing clinical symptoms and have not been studied with respect to hard endpoints in healthy individuals [41-44].

#### *Clinical and biochemical parameters*

When body weight and BMI after intervention were compared to those at the end of baseline diet, a significant weight lost was only observed in the group receiving the German cooking style diet. The weight and BMI changes in other groups were not statistically significant (Table 1). However it must be noted that the fast food group suffered from feeling constantly hungry after a few days, therefore the calories offered were increased from 2,500 to 2,900 kcal per day after the seventh day of phase 2 for all participants. After two weeks of dietary intervention (phase 2), the investigated parameters of vascular risk did not show any relevant statistical differences neither between the three study groups nor compared to baseline (Table 4). The only statistically significant differences in-between study groups were a lower level of urea in the German diet group when compared to the fast food group and the Mediterranean group. Uric acid was also lower in the German group when compared to Mediterranean. The participants of the Mediterranean group showed an increase in plasma levels of urea and uric acid during the intervention phase ( $P = 0.0241$ ), while subjects on the fast food regime showed an increase over baseline for urea ( $P = 0.0057$ ). Vitamin levels (A, E, D, B<sub>1</sub> and B<sub>6</sub>) and trace elements like selenium and zinc showed no significant oscillations among tested groups (Table 4).

LDL- and HDL-cholesterol, total cholesterol, lipoprotein(a) and homocysteine showed no significant changes between the study groups or compared to baseline levels (Table 4). Markers of oxidative stress such as 8-hydroxy-2-deoxyguanosine and malondialdehyde (MDA) have recently been implicated as mediators of vascular risk [45]. Both 8-hydroxy-2-deoxyguanosine and MDA, as well as the highly cytotoxic glycating agent methylglyoxal [46], did not change significantly during the two-week intervention phase or compared in-between groups. Thus, consuming equal caloric amounts of fast food, Mediterranean or German cooking style, comprising freshly cooked dishes, did not affect the investigated biomarkers of cardiovascular and metabolic risk.

## DISCUSSION

With this pilot study the authors addressed one main question: do changes in the dietary regime affect parameters reflecting aging, vascular risk or metabolic disease in healthy young men independent of differences in caloric intake? This study did not address the already extensively investigated question of diet-induced impacts on health by excessive caloric intake, the effect of diet on metabolically ill patients or the effect of unbalanced nutrition. The short duration of the study is a limitation. However, we chose a two-week intervention period in order to detect potential immediate diet-induced changes in biomarkers of vascular and metabolic risk, previously shown to be affected by a two-week intervention [32-39]. Another limitation is that potential effects of temperature difference in the process of preparation of the dishes were not investigated in this study, also potential effects of cooling and rewarming [47] that occur in fast food restaurants, have been excluded by the study design. Thus this study focused exclusively on the short-term effect of three different diets on markers reflecting potential risk of disease in healthy volunteers. An important feature and strength of this study is the investigation of dietary intervention under stringent control for equal energy consumption in all study groups, assuring the investigation of effects highly specific to the dietary composition itself and not due to differences in calorie consumption coming along with changes in the dietary regime (fast food: high energy density, short-lasting in satisfying peoples' appetite; Mediterranean diet: low energy density, long-lasting satisfaction of appetite). Furthermore, the inclusion of fast reacting biomarkers of oxidative and metabolic stress such as methylglyoxal, which has not been studied in the setting of dietary intervention in healthy individuals before, allowed the detection of potential immediate metabolic effects of the dietary composition.

In this study, biomarkers of vascular and metabolic risk showed no difference between study groups or compared to baseline in the setting of freshly prepared dishes with equal amounts of calories during a two-week intervention period. The minor, but statistically significant increase in uric acid in the Mediterranean group (+0.59 mg/dl when compared to baseline) could, at least in part, be explained by the relatively high amount of purines (Table 3) and is unlikely to be of significant clinical relevance in healthy individuals. The same is true for the increases over baseline for urea observed in the fast food and in the Mediterranean group, since the levels remained well within the reference value of < 45 mg/dl. But we cannot completely rule out the possibility that changing from the traditional German baseline diet to Mediterranean or fast food might have caused slight changes in protein metabolism. It is noteworthy, but not the focus of the study, that the participants in the fast food group complained about feeling constantly hungry when consuming 2,500 kcal per day and demanded increased amounts of food, while participants on the German or Mediterranean diet did not. The observation of feeling malnourished by fast food is in accordance with other studies proving that fast food is less long-lasting in satisfying peoples' appetite, thus leading towards excessive caloric intake, obesity and metabolic disorders in the long run [48].

In our approach with study groups controlled for equal energy consumption, significant effects were observed neither for classical biomarkers of cardiovascular risk nor for the sensitive and fast reacting biomarkers of oxidative and metabolic stress 8-hydroxy-2-deoxyguanosine, malondialdehyde and especially the toxic reactive carbonyl methylglyoxal [49,50], which has not been studied in such a setting before. Even in studies with longer intervention periods [51,52] or in studies focused on very early acute effects of different diets [53], no significant effects were observed previously. Additionally, studies using multicomponent diet interventions comparing Mediterranean diet with other diets [54] or dietary interventions with red wine [55] did not show significant benefits on total cholesterol, LDL-cholesterol or triglyceride levels in healthy subjects. Certainly there were some differences with respect to the content of individual nutrients in the three cooking styles tested, but for most components the contents of the three diets were not as different as generally believed (Table 3). One should keep in mind that the differences between individuals, even healthy individuals, with respect to carbohydrate metabolism [56], insulin secretion and sensitivity [57,58], lipid [59,60] and vitamin metabolism [61-67] are by far greater than the differences between the nutrient contents in the three groups tested. Thus healthy individuals have the capacity to balance the differences in the nutrient composition of common diets as long as changes in the caloric uptake are avoided. Therefore, the question arises whether recommended daily doses for certain nutrients are applicable and valid for each and every healthy person, since these recommendations neglect the huge metabolic variability between individuals and most importantly are not based on intervention studies in healthy people. Concerning nutrition counseling, our data indicate that short-term noncompliance to a recommended "healthy" dietary regime is unlikely to substantially increase a healthy person's individual risk profile. Therefore, preventive nutrition counseling should take into account that extrinsically motivated changes in a person's eating habits might induce psychic and social stress, while an occasional return to the previous dietary regime, excluding increased energy consumption, could be a feasible way to avoid those stresses. Others and we have shown recently that psychosocial stress significantly affects signaling events mediated by reactive oxygen species (ROS), culminating in activation of the ROS-sensitive transcription factor NF- $\kappa$ B and finally in atherosclerosis [68]. These data in human and mice are consistent with the data of the "Interheart" Study [69], demonstrating an almost nine fold stronger effect of psychic stress compared to nutrition. The role of psychosocial factors has been highlighted recently in social intervention studies, demonstrating that social intervention can reduce the incidence of obesity and diabetes [70,71]. The psychosocial effect was stronger and resulted in a lower number needed to treat compared to published nutritional intervention studies in obesity or diabetes [71]. Therefore it is not surprising that the recently published PREDIMED study [15], while assuming a potential effect of Mediterranean cooking on stroke, had no effect on death or even myocardial infarction in the high risk population studied. While on the one hand the issue of healthy food turning healthy individuals even healthier is questioned by the authors of this

study, there is on the other hand no doubt that wrong nutrition does contribute to disease. Thus further studies are needed to investigate the differences in energy and nutrient metabolism in healthy people, with the perspective of identifying new surrogate markers for clinically relevant end points. Having such studies at hand, it would become easier to give valid nutrition counseling to healthy individuals [72].

In summary, there were neither diet-specific negative effects of fast food on the parameters studied compatible with a threat to health, nor a beneficial effect of Mediterranean food in healthy young men when consuming the same amount of calories. We conclude that, while there is no doubt about the health threatening effects of overnutrition, undernutrition or malnutrition, there is no solid evidence that the cooking style itself impacts on the health of young healthy men studied here and in other cohorts [73-75]. Thus healthy individuals can, at least for two weeks, compensate for the relatively small differences in nutrient composition of supposedly 'unhealthy' dietary regimes. It remains to be studied how social factors and enzymatically controlled pathways of energy metabolism cooperate and might have the capability to affect the results of this study.

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