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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 ± 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 glycating 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 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, rood 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.

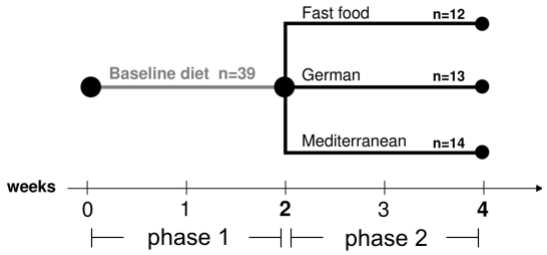

Fig. 1. Schematic timeline of the study and randomization of n = 39 participants in three groups with different dietary portfolio.

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 α -oxoaldehyde derived from triose phosphate intermediates of glycolysis, was studied. Methylglyoxal is a potent glycating agent and is believed to be of main importance in aging and

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.

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 jet. 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^2 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_6 (mg)	1.5	3.3	3.6	1.5
Vitamin B_1 (mg)	1.5	2.6	2	1.2
Vitamin C (mg)	68	242	380	100
Vitamin B_2 (mg)	1.7	2.2	2	1.4
Vitamin B_{12} (μg)	14	11.5	6.8	3
Vitamin B_3 (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)	One way ANOVA			
									P-value	Multiple Comparison Test (q)		
										F vs. G	F vs. M	G vs. M
Urea	a		29 ± 6.396[1)]	37.08 ± 4.209	29 ± 5.583	27.08 ± 5.722	31 ± 4.243	34.79 ± 10.8	0.0057**	4.624[†]	1.080	3.702[†]
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[†]
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
Vitamn 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_1	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_6	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[k]	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™ II analyzer, c) Hemoglobin Analyzer Variant 2 BioRad, d) CA7000 Siemens, e) ADVIA Centauer 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 malondialdehyde (MDA) Adduct ELISA Kit (BioCat), k) 8-hydroxy-2-deoxyguanosine, l) 8-hydroxy-2-deoxyguanosine ELISA (Trevigen), m) As previously published [40].

* $P < 0.05$, ** $P < 0.01$, [†] 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/Leistungsverzeichnis.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 (± 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 µg 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_1 and B_6) 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 multicompound 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-κB 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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REFERENCES

1. Prentice AM, Jebb SA. Fast foods, energy density and obesity: a possible mechanistic link. Obes Rev 2003;4:187-94.
2. Urpi-Sarda M, Casas R, Chiva-Blanch G, Romero-Mamani ES, Valderas-Martínez P, Salas-Salvadó J, Covas MI, Toledo E, Andres-Lacueva C, Llorach R, García-Arellano A, Bulló M, Ruiz-Gutierrez V, Lamuela-Raventos RM, Estruch R. The Mediterranean diet pattern and its main components are associated with lower plasma concentrations of tumor necrosis factor receptor 60 in patients at high risk for cardiovascular disease. J Nutr 2012;142:1019-25.
3. Bédard A, Riverin M, Dodin S, Corneau L, Lemieux S. Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. Br J Nutr 2012;108:1428-34.
4. de Lorgeril M, Salen P. Mediterranean diet in secondary prevention of CHD. Public Health Nutr 2011;14:2333-7.
5. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 2010;92: 1189-96.
6. Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Effectiveness of the Mediterranean diet: can it help delay or prevent Alzheimer's disease? J Alzheimers Dis 2010;20:795-801.
7. Giacosa A, Barale R, Bavaresco L, Gatenby P, Gerbi V, Janssens J, Johnston B, Kas K, La Vecchia C, Mainguet P, Morazzoni P, Negri E, Pelucchi C, Pezzotti M, Rondanelli M. Cancer prevention in Europe: the Mediterranean diet as a protective choice. Eur J Cancer Prev 2013;22:90-5.
8. Ben-Avraham S, Harman-Boehm I, Schwarzfuchs D, Shai I. Dietary strategies for patients with type 2 diabetes in the era of multi-approaches; review and results from the Dietary Intervention Randomized Controlled Trial (DIRECT). Diabetes Res Clin Pract 2009;86 Suppl 1:S41-8.
9. Pérez-Martínez P, García-Ríos A, Delgado-Lista J, Pérez-Jiménez F, López-Miranda J. Mediterranean diet rich in olive oil and obesity, metabolic syndrome and diabetes mellitus. Curr Pharm Des 2011;17:769-77.
10. Vincent-Baudry S, Defoort C, Gerber M, Bernard MC, Verger P, Helal O, Portugal H, Planells R, Grolier P, Amiot-Carlin MJ, Vague P, Lairon D. The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. Am J Clin Nutr 2005;82:964-71.
11. Martínez-González MA, de la Fuente-Arrillaga C, Nunez-Cordoba JM, Basterra-Gortari FJ, Beunza JJ, Vazquez Z, Benito S, Tortosa A, Bes-Rastrollo M. Adherence to Mediterranean diet and risk of developing diabetes: prospective cohort study. BMJ 2008;336: 1348-51.
12. Morrison AC, Ness RB. Sodium intake and cardiovascular disease. Annu Rev Public Health 2011;32:71-90.
13. Wexler R, Pleister A, Raman SV, Borchers JR. Therapeutic lifestyle changes for cardiovascular disease. Phys Sportsmed 2012;40:109-15.
14. Wheeler ML, Dunbar SA, Jaacks LM, Karmally W, Mayer-Davis EJ, Wylie-Rosett J, Yancy WS Jr. Macronutrients, food groups, and eating patterns in the management of diabetes: a systematic review of the literature, 2010. Diabetes Care 2012;35:434-45.
15. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA; PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 2013;368:1279-90.
16. Hoermann R, Grossmann M. Mediterranean diet for primary prevention of cardiovascular disease. N Engl J Med 2013;369:674.
17. Kopel E, Sidi Y, Kivity S. Mediterranean diet for primary prevention of cardiovascular disease. N Engl J Med 2013;369:672.
18. Pereira MA, Kartashov AI, Ebbeling CB, Van Horn L, Slattery ML, Jacobs DR Jr, Ludwig DS. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet 2005;365:36-42.
19. Krebs JD, Elley CR, Parry-Strong A, Lunt H, Drury PL, Bell DA, Robinson E, Moyes SA, Mann JI. The Diabetes Excess Weight Loss (DEWL) Trial: a randomised controlled trial of high-protein versus high-carbohydrate diets over 2 years in type 2 diabetes. Diabetologia 2012;55:905-14.
20. TODAY Study Group, Zeitler P, Hirst K, Pyle L, Linder B, Copeland K, Arslanian S, Cuttler L, Nathan DM, Tollefsen S, Wilfley D, Kaufman F. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med 2012;366:2247-56.
21. Black RN, Spence M, McMahon RO, Cuskelly GJ, Ennis CN, McCance DR, Young IS, Bell PM, Hunter SJ. Effect of eucaloric high- and low-sucrose diets with identical macronutrient profile on insulin resistance and vascular risk: a randomized controlled trial. Diabetes

2006;55:3566-72.
22. Akesson A, Andersen LF, Kristjánsdóttir AG, Roos E, Trolle E, Voutilainen E, Wirfält E. Health effects associated with foods characteristic of the Nordic diet: a systematic literature review. Food Nutr Res 2013;57.
23. Lindeberg S, Jönsson T, Granfeldt Y, Borgstrand E, Soffman J, Sjöström K, Ahrén B. A Palaeolithic diet improves glucose tolerance more than a Mediterranean-like diet in individuals with ischaemic heart disease. Diabetologia 2007;50:1795-807.
24. Ambring A, Friberg P, Axelsen M, Laffrenzen M, Taskinen MR, Basu S, Johansson M. Effects of a Mediterranean-inspired diet on blood lipids, vascular function and oxidative stress in healthy subjects. Clin Sci (Lond) 2004;106:519-25.
25. Athyros VG, Kakafika AI, Papageorgiou AA, Tziomalos K, Peletidou A, Vosikis C, Karagiannis A, Mikhailidis DP. Effect of a plant stanol ester-containing spread, placebo spread, or Mediterranean diet on estimated cardiovascular risk and lipid, inflammatory and haemostatic factors. Nutr Metab Cardiovasc Dis 2011;21:213-21.
26. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, Fiol M, Gómez-Gracia E, López-Sabater MC, Vinyoles E, Arós F, Conde M, Lahoz C, Lapetra J, Sáez G, Ros E; PREDIMED Study Investigators. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 2006;145:1-11.
27. Rees K, Hartley L, Flowers N, Clarke A, Hooper L, Thorogood M, Stranges S. 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2013;8: CD009825.
28. Fitó M, Guxens M, Corella D, Sáez G, Estruch R, de la Torre R, Francés F, Cabezas C, López-Sabater Mdel C, Marrugat J, García-Arellano A, Arós F, Ruiz-Gutierrez V, Ros E, Salas-Salvadó J, Fiol M, Solá R, Covas MI; PREDIMED Study Investigators. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. Arch Intern Med 2007;167:1195-203.
29. Kim JY, Yang YJ, Yang YK, Oh SY, Hong YC, Lee EK, Kwon O. Diet quality scores and oxidative stress in Korean adults. Eur J Clin Nutr 2011;65:1271-8.
30. Urquiaga I, Strobel P, Perez D, Martinez C, Cuevas A, Castillo O, Marshall G, Rozowski J, Leighton F. Mediterranean diet and red wine protect against oxidative damage in young volunteers. Atherosclerosis 2010;211:694-9.
31. Bierhaus A, Fleming T, Stoyanov S, Leffler A, Babes A, Neacsu C, Sauer SK, Eberhardt M, Schnölzer M, Lasitschka F, Neuhuber WL, Kichko TI, Konrade I, Elvert R, Mier W, Pirags V, Lukic IK, Morcos M, Dehmer T, Rabbani N, Thornalley PJ, Edelstein D, Nau C, Forbes J, Humpert PM, Schwaninger M, Ziegler D, Stern DM, Cooper ME, Haberkorn U, Brownlee M, Reeh PW, Nawroth PP. Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. Nat Med 2012;18: 926-33.
32. Keys A, Anderson JT, Grande F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet 1957;273: 959-66.
33. Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C, Christou DD. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. J Nutr 2003;133:411-7.
34. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987;1:122-5.
35. Müller H, Jordal O, Kierulf P, Kirkhus B, Pedersen JI. Replacement of partially hydrogenated soybean oil by palm oil in margarine without unfavorable effects on serum lipoproteins. Lipids 1998;33: 879-87.
36. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr 2005;81:1298-306.
37. Beisswenger PJ, Howell SK, O'Dell RM, Wood ME, Touchette AD, Szwergold BS. alpha-Dicarbonyls increase in the postprandial period and reflect the degree of hyperglycemia. Diabetes Care 2001;24: 726-32.
38. Gregersen S, Samocha-Bonet D, Heilbronn LK, Campbell LV. Inflammatory and oxidative stress responses to high-carbohydrate and high-fat meals in healthy humans. J Nutr Metab 2012;2012: 238056.
39. Vetrani C, Costabile G, Di Marino L, Rivellese AA. Nutrition and oxidative stress: a systematic review of human studies. Int J Food Sci Nutr 2013;64:312-26.
40. Deutsche Gesellschaft für Ernährung; Österreichische Gesellschaft für Ernährung; Schweizerische Gesellschaft für Ernährungsforschung; Schweizerische Vereinigung für Ernährung. Referenzwerte für die Nährstoffzufuhr. 1. Auflage: 4. Korrigierter Nachdruck. Neustadt an der Weinstraße: Neuer Umschau Buchverlag; 2012.
41. Baker EM, Saari JC, Tolbert BM. Ascorbic acid metabolism in man. Am J Clin Nutr 1966;19:371-8.
42. Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999;69:1086-107.
43. Ohrvik VE, Witthoft CM. Human folate bioavailability. Nutrients 2011;3:475-90.
44. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC. Folate requirement and metabolism in nonpregnant women. Am J Clin Nutr 1987;46:1016-28.
45. Sui H, Wang W, Wang PH, Liu LS. Effect of glutathione peroxidase mimic ebselen (PZ51) on endothelium and vascular structure of stroke-prone spontaneously hypertensive rats. Blood Press 2005;14: 366-72.
46. Richard JP. Mechanism for the formation of methylglyoxal from triosephosphates. Biochem Soc Trans 1993;21:549-53.
47. Prochaska LJ, Nguyen XT, Donat N, Piekutowski WV. Effects of food processing on the thermodynamic and nutritive value of foods: literature and database survey. Med Hypotheses 2000;54:254-62.
48. Rosenheck R. Fast food consumption and increased caloric intake: a systematic review of a trajectory towards weight gain and obesity risk. Obes Rev 2008;9:535-47.
49. Desai KM, Chang T, Wang H, Banigesh A, Dhar A, Liu J, Untereiner A, Wu L. Oxidative stress and aging: is methylglyoxal the hidden enemy? Can J Physiol Pharmacol 2010;88:273-84.
50. Fleming TH, Humpert PM, Nawroth PP, Bierhaus A. Reactive metabolites and AGE/RAGE-mediated cellular dysfunction affect the aging process: a mini-review. Gerontology 2011;57:435-43.
51. Sarri K, Bertsias G, Linardakis M, Tsibinos G, Tzanakis N, Kafatos A. The effect of periodic vegetarianism on serum retinol and alpha-tocopherol levels. Int J Vitam Nutr Res 2009;79:271-80.

52. Trepanowski JF, Bloomer RJ. The impact of religious fasting on human health. Nutr J 2010;9:57.
53. Rudolph TK, Ruempler K, Schwedhelm E, Tan-Andresen J, Riederer U, Böger RH, Maas R. Acute effects of various fast-food meals on vascular function and cardiovascular disease risk markers: the Hamburg Burger Trial. Am J Clin Nutr 2007;86:334-40.
54. Papadaki A, Scott JA. Follow-up of a web-based tailored intervention promoting the Mediterranean diet in Scotland. Patient Educ Couns 2008;73:256-63.
55. Hansen AS, Marckmann P, Dragsted LO, Finné Nielsen IL, Nielsen SE, Grønbaek M. Effect of red wine and red grape extract on blood lipids, haemostatic factors, and other risk factors for cardiovascular disease. Eur J Clin Nutr 2005;59:449-55.
56. Wismann J, Willoughby D. Gender differences in carbohydrate metabolism and carbohydrate loading. J Int Soc Sports Nutr 2006;3:28-34.
57. Clausen JO, Borch-Johnsen K, Ibsen H, Bergman RN, Hougaard P, Winther K, Pedersen O. Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young healthy Caucasians. Analysis of the impact of gender, body fat, physical fitness, and life-style factors. J Clin Invest 1996;98:1195-209.
58. Hollenbeck C, Reaven GM. Variations in insulin-stimulated glucose uptake in healthy individuals with normal glucose tolerance. J Clin Endocrinol Metab 1987;64:1169-73.
59. Fielding CJ, Havel RJ, Todd KM, Yeo KE, Schloetter MC, Weinberg V, Frost PH. Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. J Clin Invest 1995;95:611-8.
60. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. Am J Clin Nutr 2005;82:941-8.
61. Norum KR, Blomhoff R. McCollum Award Lecture, 1992: vitamin A absorption, transport, cellular uptake, and storage. Am J Clin Nutr 1992;56:735-44.
62. Talegawkar SA, Johnson EJ, Carithers T, Taylor HA Jr, Bogle ML, Tucker KL. Total alpha-tocopherol intakes are associated with serum alpha-tocopherol concentrations in African American adults. J Nutr 2007;137:2297-303.
63. Levine M, Rumsey SC, Daruwala R, Park JB, Wang Y. Criteria and recommendations for vitamin C intake. JAMA 1999;281:1415-23.
64. Hung J, Abratte CM, Wang W, Li R, Moriarty DJ, Caudill MA. Ethnicity and folate influence choline status in young women consuming controlled nutrient intakes. J Am Coll Nutr 2008;27:253-9.
65. Gregory JF, 3rd, Williamson J, Liao JF, Bailey LB, Toth JP. Kinetic model of folate metabolism in nonpregnant women consuming [2H2]folic acid: isotopic labeling of urinary folate and the catabolite para-acetamidobenzoylglutamate indicates slow, intake-dependent, turnover of folate pools. J Nutr 1998;128:1896-906.
66. Horwitt MK, Elliott WH, Kanjananggulpan P, Fitch CD. Serum concentrations of alpha-tocopherol after ingestion of various vitamin E preparations. Am J Clin Nutr 1984;40:240-5.
67. Kallner A, Hartmann D, Hornig D. Steady-state turnover and body pool of ascorbic acid in man. Am J Clin Nutr 1979;32:530-9.
68. Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, Ferstl R, von Eynatten M, Wendt T, Rudofsky G, Joswig M, Morcos M, Schwaninger M, McEwen B, Kirschbaum C, Nawroth PP. A mechanism converting psychosocial stress into mononuclear cell activation. Proc Natl Acad Sci U S A 2003;100:1920-5.
69. Held C, Iqbal R, Lear SA, Rosengren A, Islam S, Mathew J, Yusuf S. Physical activity levels, ownership of goods promoting sedentary behaviour and risk of myocardial infarction: results of the INTERHEART study. Eur Heart J 2012;33:452-66.
70. van Dam HA, van der Horst FG, Knoops L, Ryckman RM, Crebolder HF, van den Borne BH. Social support in diabetes: a systematic review of controlled intervention studies. Patient Educ Couns 2005;59:1-12.
71. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343-50.
72. Rees K, Dyakova M, Ward K, Thorogood M, Brunner E. Dietary advice for reducing cardiovascular risk. Cochrane Database Syst Rev 2013;3:CD002128.
73. Cascio G, Schiera G, Di Liegro I. Dietary fatty acids in metabolic syndrome, diabetes and cardiovascular diseases. Curr Diabetes Rev 2012;8:2-17.
74. Vessby B, Karlström B, Ohrvall M, Järvi A, Andersson A, Basu S. Diet, nutrition and diabetes mellitus. Ups J Med Sci 2000;105:151-60.
75. Wilkin TJ. Early nutrition and diabetes mellitus. BMJ 1993;306:283-4.