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Vegetarian Diet Affects Genes of Oxidative Metabolism and Collagen Synthesis

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Key Words
Vegetarian diet · mRNA expression · Metabolism · Collagen · Omnivores

Abstract
Background/Aim: A vegetarian diet is known to prevent a series of diseases but may influence the balance of carbohydrate and fat metabolism as well as collagen synthesis. This study compares expression patterns of relevant genes in oral mucosa of omnivores and vegetarians. ***Methods:*** Quantitative reverse transcriptase polymerase chain reaction was applied for analysis of mRNA levels from carnitine transporter OCTN2, hepatic CPT1A and nonhepatic CPT1B isoforms of carnitine palmitoyltransferase and collagen (CCOL2A1) in oral mucosa. ***Results:*** Compared with volunteers with traditional eating habits, carbohydrate consumption was significantly higher (+22%) in vegetarians. This was associated with a significant stimulation of CPT1A (+50%) and OCTN2 (+10%) and a lowered collagen synthesis (–10%). ***Conclusion:*** These novel findings provide further insight into the association of a changed fat metabolism and reduced collagen synthesis in vegetarians, which could also play a role in the aging process.

Introduction

It is well established that a vegetarian diet may lower the risk of cancer (e.g., colon cancer) and age-associated diseases [for a review, see ref. 1]. A vegetarian diet may also shift the macronutrient balance (fat and proteins vs. carbohydrate) in favor of carbohydrates [2, 3]. As a higher protein intake is known to optimize gains in muscle mass [4] and stimulates fat metabolism [5], a carbohydrate-rich diet could stimulate a downregulation of key enzymes of fat metabolism.

There is evidence that the transcription of about 25,000 genes is regulated by nutrition, exercise and hormones [6–8]. However, the transcription of 3 fat metabolism-associated genes, OCTN2 (organic cation transporter), CPT1A and CPT1B (hepatic and muscular isoform of carnitine palmitoyltransferase), is regulated in response to diet, exercise and aging [9, 10].

In addition, nutritional factors are associated with buildup of musculoskeletal mass [for a review, see e.g. ref. 11]. As there is a close correlation of protein synthesis with mRNA expression for collagen (CCOL2A1) [10, 12], the latter provides a suitable model for evaluating effects on the expression of relevant proteins.

Our assumption that many metabolism-associated genes are regulated in a systemic manner results from a

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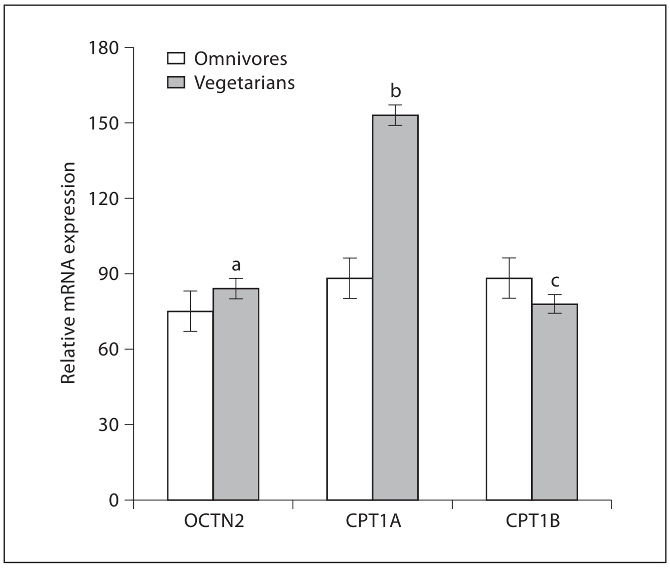

Fig. 1. Gene expression of fat metabolism-associated genes. Compared with omnivores, the gene expression rate (percentage of mRNA in relation to the standard gene G6PD) of OCTN2 (responsible for carnitine uptake) is significantly higher in vegetarians (^{a} $p \leq 0.05$). Considering mitochondrial carnitine palmitoyltransferases, CPT1A is significantly upregulated (^{b} $p \leq 0.001$) and CPT1B is significantly downregulated in vegetarians (^{c} $p \leq 0.05$). There was a 1:1 ratio between CPT1A and CPT1B in omnivores and a 2:1 ratio between CPT1A and CPT1B in vegetarians.

larger comparative study using white blood cells and muscle [13] as well as from preliminary experiments for this study comparing white blood cells and oral mucosa. Thus, oral mucosa obtained from mouth swabs was chosen to evaluate possible differences in gene regulation between probands with traditional eating habits and vegetarians.

Materials and Methods

Study Population

A group of 116 volunteers participated in this study: 86 omnivores (43 females/43 males, mean age 24 years, range 18–31) were compared with 30 vegetarians (15 females/15 males, mean age 23 years, range 19–26). Nutritional questionnaires based on a previous European study [14] were given to all participants. Only subjects with a normal body mass index (BMI 18.5–24.9) were included.

Noninvasive Sampling

Buccal cells were collected on sterile cotton sticks by twirling at least 10 times on each of the inner cheeks. Then the cells were released into 1 ml storage buffer for nucleic acid preparation (commercially obtained from Roche Diagnostics).

Analysis of mRNA Expression

Isolation of mRNA and preparation of cDNA was carried out according to standard procedures. Quantitative reverse transcriptase polymerase chain reaction (RTQ-PCR) was carried out using a LightCycler™ System (Roche) or a Rotorgene 6000, which allows amplification and detection (by fluorescence) in the same tube, using a kinetic approach. Quantitative PCR was done using a real-time PCR system (RTQ-PCR) which was also applied for analysis of mRNA expression using commercially available TaqMan primers and probes (Applied Biosystems). As a control, to confirm the intra-individual stability of gene expression, samples of 1 volunteer obtained on 5 different days were analyzed showing identical gene expression patterns (SD <1%).

Statistics

Evaluation of results was done using the StatView 5.01 statistics program using ANOVA to find out significant differences between the groups. All tests were done double sided at a significance level of 5% ($p < 0.05$).

Results

Results of Nutritional Questionnaires

Evaluation of nutritional questionnaires indicated that nutritional habits of vegetarians differed mainly with respect to the refusal of meat consumption and a relatively higher intake of milk products (6–10 meals/week) in the majority of vegetarians (55%, SD 10%) compared with 3–6 meals/week in the majority of the omnivorous group (60%, SD 15%). In addition, one fish meal was reported in the omnivorous group (85%, SD 17%) but not in vegetarians. Thirty percent (SD 10%) of volunteers from each group reported an intake of supplements (vitamins, 2–3 times/week).

Expression of Fat Metabolism-Associated Genes

Transcript levels of OCTN2, CPT1A and CPT1B are shown in figure 1.

The mean expression rate of organic cation transporter OCTN2 in buccal mucosa from omnivores was 75% (SD 7%) of the standard gene (glucose 6-phosphatase, G6PD). OCTN2 expression was significantly higher in vegetarians (84% of G6PD, SD 3%; $p \leq 0.05$).

In omnivores, the mean expression rate of CPT1A was 88% of G6PD (SD 8%), which was significantly higher in vegetarians (153% of G6PD, SD 8%; $p \leq 0.001$). In omnivores, the mean expression rate of CPT1B was 88% of G6PD (SD 8%) compared with 78% of G6PD (SD 8%; $p < 0.05$) in vegetarians. Interestingly, there was a 1:1 ratio between CPT1A and CPT1B in omnivores but a 2:1 ratio between CPT1A and CPT1B in vegetarians.

Table 1. Primers for mRNA analyses

Gene	Sequence	Reference
OCTN2 (246 bp)	OCTN2-F: 5′-TCCAAGTCACACAAGGATG-3′ OCTN2-R: 5′-CATGACGAACATGGGGGCATC-3′	Karlic et al. [9] 2003
CPT1A (117 bp)	CPT1A-F: 5′-GTCCCGGCTGTCAAAGACA-3′ CPT1A-R: 5′-CCGACAGCAAAATCTTGAGCA-3′	Razeghi et al. [20], 2001
CPT1B (85 bp)	CPT1B-F: 5′-CAGGCGAGAACACGATCTTC-3′ CPT1B-R: 5′-GCGGATGTGGTTTCCAAAG-3′	Razeghi et al. [20], 2001
CCOL2A1 (172 bp)	CCOL2A1-F: 5′-GAGGCTGGCAGCTGTGTGCAGGATG-3′ CCOL2A1-R: 5′-CCAGGTTCTCCATCTCTGCCACGAG-3′	This study
G6PD (339 bp)	G6PD-F: 5′-CCGCATCGACCACTACCTGGGCAAG-3′ G6PD-R: 5′-GTTCCCCACGTACTGGCCCAGGACCA-3′	Hochhaus et al. [21], 1996

Synthesis of Collagen (CCOL2A1)

As shown in figure 2, relative mRNA levels of CCOL2A1 in oral mucosa were higher than the standard gene G6PD in all cases. However, the expression was reduced by 10% in vegetarians (140% of G6PD, SD 5%) as compared with omnivores (157% of G6PD, SD 5%; $p \leq 0.05$).

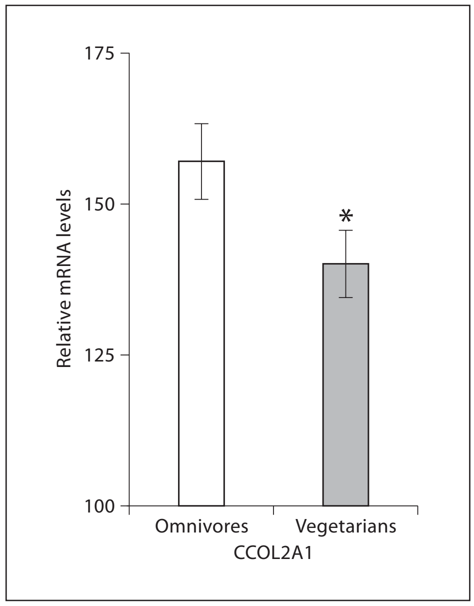

Fig. 2. Gene expression of collagen. As compared with omnivores, the gene expression rate (percentage of mRNA in relation to the standard gene G6PD) of collagen (CCOL2A1) is significantly lower in vegetarians (* $p \leq 0.05$).

Discussion

Our new model system uses noninvasive sampling of oral mucosa for the analysis of mRNA synthesis of metabolism-associated genes which are known to be regulated in a systemic manner. Taken together, we could show that a vegetarian diet has a significant impact on genes regulating essential features of carnitine metabolism, which are also affected in a series of diseases ranging from diabetes to cancer [9].

The stimulation of carnitine uptake as evidenced by an elevated OCTN2 expression which was observed in vegetarians participating in this study may compensate lower carnitine levels which are usually sufficient not to lead to any evident metabolic disturbances. An existing systemic carnitine deficiency resulting for example from inherited mutations of the OCTN2 gene is dramatically worsened by a strictly vegetarian diet [15].

Intracellular carnitine is also essential for the transport of fatty acids into mitochondria as mediated by carnitine palmitoyltransferases [16]. This could support the interpretation of the difference in the CPT1A/CPT1B relation between omnivores (1:1) and vegetarians (2:1), resulting from an upregulation of CPT1A and a downregulation of CPT1B, in the sense that carbohydrate metabolism is favored in vegetarians, whereas in omnivores, there is a more balanced relation between carbohydrate and fat metabolism.

The stimulatory effect of protein supplementation on fat metabolism supports a plethora of previous data [for a review, see e.g. ref. 11, 17] documenting the importance of dietary protein for the maintenance of musculoskeletal mass and confirms our observation indicating reduced synthesis of collagen (CCOL2A1) in vegetarians. In addition, an increased risk of osteoporosis, which is also known to be associated with a lowered collagen synthesis, has been associated in vegetarians with a low cobalamin (vitamin B_{12}) status. Cobalamin is an essential vitamin for DNA synthesis and is primarily present in a protein-bound form in foods of animal origin [18]. Additional evidence for collagen disorders resulting from a low-protein diet is given for a series of cutaneous manifestations [19].

In conclusion, our data show that a vegetarian lifestyle has an impact on fat metabolism causing a remarkable stimulation of carnitine uptake and a reduction in collagen synthesis-associated genes.

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