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Effect of unsaturated fat intake from Mediterranean diet on rat liver mRNA expression profile: selective modulation of genes involved in lipid metabolism

Davide Eletto, Arturo Leone, Maurizio Bifulco, Mario F. Tecce*

Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, I-84084 Fisciano (SA), Italy

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Summary *Background and aim:* The lipid content of Mediterranean diet is mostly accounted for its disease preventive action. We investigated whether the short term nutritional effect of a fat quota mainly derived from olive and fish oil affects liver mRNA expression profile in rats.
Methods and results: The study was carried out using DNA microarray techniques. The effect was evaluated at liver mRNA expression level to identify genes whose expression was regulated by dietary modifications. Two groups of six rats were alternatively supplied for two weeks with either a control or with an experimental diet. Both diets were semisynthetic and isocaloric, with identical major nutrients composition (protein 20%, carbohydrates 56% and lipids 22% of total energy) being different only in the quality of fats. The lipid quota of the control diet contained exclusively saturated animal fats, derived from butter, while in the experimental diet some unsaturated fats were present, being derived also from olive and fish oil (10% and 6% of total energy, respectively). Out of 26,334 genes analyzed, 11,292 were found expressed in the liver, 72 were induced and 180 were inhibited from the experimental diet. Out of these, 33 of the induced and 59 of the inhibited species have a well known function.
Conclusions: The diet with olive and fish oil modulates several genes related to lipolysis or lipogenesis and newly identified responders from other metabolisms. Some of these genes are also reported to be similarly modulated by the action of fibrates, but without the complete gene activation typical of these PPARα ligands.

* Corresponding author. Tel.: +39 089 962065; fax: +39 089 962828.
E-mail address: tecce@unisa.it (M.F. Tecce).

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Introduction

In the last decades, epidemiological, clinical and molecular studies have reported many relations between nutrition and health. Numerous connections have been established between dietary habits, such as Mediterranean diets, and degenerative diseases, including cardiovascular diseases, diabetes and cancer [1—4]. For this reason, dietary adjustment represents now a major tool for prevention and treatment of several human diseases. The precise definition of Mediterranean diet is difficult since it has to be the sum of several formulations from different countries. Nevertheless, some common major points can be described: one of the most important is the limited intake of total fats and proteins, leaving to carbohydrates the majority of total energy of the diet. The provenience of nutrients from specific foods is also essential: the high abundance of fruit, vegetables, olive oil and fish is also very effective, each likely acting through specific components.

The molecular mechanisms by which diet composition may prevent diseases are only partly known, nevertheless their detailed knowledge could both allow to further develop these effects, improving food composition, and also to make new applications in therapy and diagnosis, on the basis of relevant metabolic processes resulting useful as pharmacological targets and/or biomarkers. A possible approach in these studies may start from individual food components and metabolic processes to evaluate their importance in the effect of complete diet on the whole organism. For example, numerous studies have demonstrated the beneficial effects of polyunsaturated (n-3) fatty acids for lowering serum lipids as possible basis for the preventive effect of dietary fish oil [5,6]. Nevertheless, a search limited to candidate substances and processes excludes the possibility that other components either totally unknown or unknown for a specific role in this case, may be involved: the nutritional effect of fish products might depend from substances other than (n-3) fatty acids, or their most important effect might not regard lipid metabolism. Therefore approaches not starting from specific factors or processes might also be useful to collect additional information.

Differential analysis of gene expression have the objective to identify which mRNA species changes quantitatively between two certain tissue or cellular conditions. DNA microarray techniques allow differential gene expression analysis almost on a total scale [7,8]. Since nutrition has wide metabolic effects, it is likely that these include the modulation of a specific mRNA expression profile. Gene patterns affected by dietary docosahexaenoic acid [9] and protein quality and quantity [10] were recently reported.

Our goal is targeted to study specifically the effect of the saturation of the fat quota of diet. One of the most typical features of Mediterranean diet is, in fact, the significant presence of olive oil and fish products, making the lipid intake rich in mono- and polyunsaturated fatty acids. The presence of this kind of lipids has been clearly shown extremely effective in preventive nutrition [11—13]. To evaluate at the molecular level this specific aspect and to identify which mRNA are quantitatively affected, the liver expression pattern of a control diet with an optimal distribution of major nutrients was then compared to an experimental one differing only in the saturation of lipids.

Methods

Animal feeding and diets

The care and use of animals were approved by the Animal Care and Ethic Committee of INRAN (Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy), which kindly hosted this part of the work in its animal facility. Female pathogen-free Fischer-344 rats (*Rattus norvegicus*, CDF (F-344) CrlBR (Charles River, Italy), with a mean body weight of 120 g were individually housed in wire-bottom stainless-steel cages in pathogen-free environment under constant temperature/light conditions (room temperature 22 ± 1 °C, 12 h light—dark cycle). They were acclimated for two weeks before treatment and then were randomly assigned to two groups containing six animals each. One group was fed with control and the other with the experimental diet. Rats had ad libitum access to food and water during the study.

The two diets were prepared starting from diet #110801 obtained from Dyets, Inc., Bethlehem, PA. This is a modified AIN-93G [14] purified rodent diet. This preparation, which is fat free and contains vitamin free casein, dextrose, cellulose, salt and vitamin mix, L-cystine and choline bitartrate, was mixed by a blender with other components (additional cellulose and dextrose, butter, olive and fish oil) to obtain two diets differing in fat saturation but giving the same amount of energy per kg and containing similar amounts by weight percentage of total fats. Fish (Menhaden) oil, dextrose and cellulose were also obtained from Dyets Inc., while