Vol. 180, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1991 Pages 162-168

HEPATIC EXPRESSION OF APOLIPOPROTEIN A-I GENE IN RATS IS UPREGULATED BY MONOUNSATURATED FATTY ACID DIET

Jesus Osada[1], Antonio Fernandez-Sanchez, Jose Luis Diaz-Morillo, Hortensia Aylagas, Maria Jesus Miro-Obradors, Jose Maria Ordovas[1] and Evangelina Palacios-Alaiz[#]

[1]Lipid Metabolism Laboratory, U.S.D.A. Human Nutrition Center on Aging at Tufts University, Boston, MA 02111

Instituto de Bioquimica. (Centro mixto CSIC-UCM), Facultad de Farmacia, Ciudad Universitaria. 28040 Madrid, Spain

Received August 14, 1991

SUMMARY. The effect of the degree of dietary fat saturation on the hepatic expression of apolipoprotein A-I mRNA was studied in male rats. Animals were maintained for two months on a high fat diet (40% w/w) containing 0.1% cholesterol. Two groups of control animals received either chow diet or chow plus 0.1% cholesterol, while experimental groups received their fat supplement as coconut, corn or olive oil respectively. Dietary cholesterol did not affect apolipoprotein A-I mRNA levels as compared to control animals. Corn oil fed animals had significantly higher levels of hepatic apolipoprotein A-I mRNA than those receiving cholesterol, or coconut oil plus cholesterol. Olive oil fed animals had significantly higher levels of hepatic apolipoprotein A-I mRNA when compared to all other dietary groups. Our data indicate that monounsaturated fatty acids supplied as olive oil play a major role in regulating the hepatic expression of apolipoprotein A-I in male rats.

Apolipoprotein A-I (apoA-I) is the major protein component of high density lipoproteins (HDL) (1), and several "in vitro" studies have shown that apoA-I is an essential component of HDL particles (2, 3). This essential role of apolipoprotein A-I in the HDL particle has been reinforced by the finding of homozygote individuals lacking this protein and whose levels of HDL-cholesterol were almost non-detectable (5,6). Experiments in transgenic mice and rats have also confirmed this function (7,8). In

[#]To whom correspondence should be addressed.

0006-291X/91 $1.50

addition to this structural role, ApoA-I serves as a cofactor for lecithin: cholesterol acyltransferase (E.C. 2.3.1.43) (9). Apolipoprotein A-I is synthesized in liver and intestine (10). We and others have demonstrated that levels of hepatic apoA-I mRNA correlate with serum levels of apo A-I in non-human primates and are influenced by nutritional stimuli (11-14).

Recent studies have shown an effect of monounsaturated fatty acids (oleic acid) in lowering serum levels of low-density lipoprotein cholesterol without modification of HDL-cholesterol levels when substituted for saturated fatty acids in human and non-human primates (15-17). We have previously shown that rat undergoes significant changes in lipid parameters when fed a diet containing 40% (w/w) as fat as compared to chow diet (18). The present work was design to investigate the effect of type of fat on the hepatic apoA-I mRNA levels.

MATERIALS AND METHODS

Materials. Standard rat chow was provided by Sander S.A. (Barcelona). Cholesterol was obtained from Sigma Chemical Co. (St.Louis, MO). Starch was provided by Cofares S.A. (Madrid). Olive oil was obtained from Mora S.A. (Alcolea), coconut oil was obtained from Cofares and corn oil from Koipe (Andujar). Molecular biology reagents were purchased from BRL (Bethesda, MD) and Sigma. Restriction enzymes were purchased from Pharmacia (Piscataway, NJ). [α-^{32}P]-dATP was obtained from New England Nuclear (Boston, MA).

Animals and diets. Inbred male Wistar rats (200-250 g, aged two months) were kept under different experimental conditions. Group 1. Control animals were fed a standard commercial diet. Group 2. Cholesterol group. Rats were fed a 0.1% (w/w) cholesterol in diet. Groups 3 to 5. Rats were fed 0.1% (w/w) cholesterol in diet and fat as 40% (w/w). All diets contained a 1% starch used as agglutinant (Table 1). Three different types of fat were used: coconut oil, corn oil and olive oil. As shown in table 2, a wide range

Table 1. Percentage composition of the diets

	Control diet	Cholesterol diet	Fat diets
Carbohydrates	60	60	36
Added starch	1	1	1
Lipids	3.5	3.5	40
Added Cholesterol	-	0.1	0.1
Protein	19	19	12

Data are expressed as g/ 100.

Table 2. Fatty acid composition of the different diets

Fatty acid	Fat diet		
	Coconut oil	Corn oil	Olive oil
C8:0	8.03	-	-
C10:0	6.02	-	-
C12:0	47.56	-	-
C14:0	18.18	-	-
C16:0	9.09	10.48	11.27
C18:0	2.53	2.51	2.50
C20:0	-	0.52	0.43
C16:1	-	0.52	1.56
C18:1(ω9)	7.08	32.60	74.87
C18:2(ω6)	1.48	52.41	8.35
C18:3(ω3)	-	0.94	0.99
Σsats	91.41	13.51	14.20
Σmonounsat	7.08	33.12	76.43
Σpolyunsats	1.48	53.35	9.34
P/S	0.016	3.95	0.65

Abbreviations used: Σsats, sum of saturated fatty acids; Σmonounsats, sum of monounsaturated fatty acids; Σpolyunsats, sum of polyunsaturated fatty acids. P/S, polyunsaturated to saturated fatty acid ratio. Data are expressed as % (w/w of total fatty acids).

of polyunsaturated to saturated fatty acid ratio (P/S) was covered (0.016-3.948) and a wide range of monounsaturated fatty acids was also utilized (7-76%). All groups were fed ad libitum for two months. Before killing, rats were kept on a fasting regimen with free access to water for 18 hr. Animals used in this study were handled and killed always observing criteria from the European Community for care and use of animal laboratory in research.

RNA preparation. A liver biopsy was flash frozen in liquid nitrogen and stored -70 C until use. Liver fragments were homogenized in guanidinium isothiocyanate and applied to a cushion of cesium chloride as described by Kingston (19).

Preparation of DNA Probes. A 0.6 kb Rsa I fragment of human apoA-I cDNA (20) was purified by gel electrophoresis and electroelution. A 1.6 kb Pst I/Sst I insert of rat β-tubulin was used as internal standard. Purified DNA inserts were labelled with [α-^{32}P]-dATP by a random primer protocol (21) to a specific activity of 10^9 dpm/μg.

Analysis of mRNA. Northern analysis. Denaturated RNA was run in a formaldehyde agarose gel, blotted into nylon membranes, hybridized to radiolabelled probe, washed and exposed to film following current procedures (22).
Slot-blot analysis. Several amounts of denaturated total RNA (1-10 μg) were applied to nylon filters using a slot-blot apparatus (Schleider and Schuell). Films were scanned by laser densitometry using an LKB 2202 densitometer and a LKB 2400 gel scan XL software. mRNA abundance was measured in absorbance units. Blots were stripped and reprobed with β-tubulin, exposed to film and scanned as described. The relative abundance of apo A-I message was normalized to values of β-tubulin as an internal standard.

Statistical analysis. Results are presented as means and their standard deviations. A two-tailed unpaired Student's t-test was used to evaluate differences between groups (23).

RESULTS AND DISCUSSION

Figure 1 shows that the human apoA-I cDNA probe hybridized to a single electrophoretic mRNA species of the appropriate molecular size (1100 bases)(24). Animals fed diets with different content of saturated, monounsaturated and polyunsaturated fatty acids had significant variations in the hepatic expression of apoA-I mRNA. No effect was noticed on the mRNA size. In contrast to previous reports in other animal species (12-14), no significant effect was observed due to the addition of cholesterol to the diet (Table 3). When compared to chow fed animals, expression of apoA-I mRNA in coconut oil fed animals was not significantly affected. Conversely, both corn oil and olive oil fed animals experienced significant increases in apoA-I expression, especially in those animals on the olive oil diet. These results are in contrast with results observed in non human primates, in which the major expression of apoA-I mRNA is associated with saturated diets. However, the comparison was exclusively done between highly polyunsaturated and highly saturated diets (12-14). To our

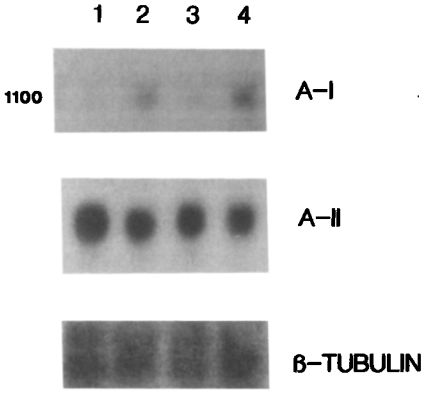

Figure 1. Northern blot analysis reflecting the influence of several diets on apoA-I mRNA levels. 30 μg of each RNA preparation was subjected to electrophoresis in a 1% agarose gel. The RNA was transferred to a nylon membrane and hybridized to radioactive apoA-I, apoA-II and β-Tubulin cDNAs. ApoA-I mRNA size is indicated in bases on the autoradiogram. Lane 1 corresponds to an animal fed control diet, lane 2, coconut oil diet, lane 3, corn oil diet and lane 4, an olive oil diet.

Table 3. Hepatic levels of apolipoprotein A-I mRNA

Experimental condition	Absorbance units/ β-Tubulin
CONTROL (n=5)	1.0 ± 0.3
CHOLESTEROL (n=5)	0.8 ± 0.2
CHOLESTEROL + COCONUT OIL (n=5)	0.9 ± 0.2
CHOLESTEROL + CORN OIL (n=5)	1.2 ± 0.2[b,c]
CHOLESTEROL + OLIVE OIL (n=6)	1.6 ± 0.3[a,b,c]

Data are means with their standard deviation of duplicate analysis of the number of animals indicated into brackets. Statistical analysis was done using unpair two-tails Student's t-test (a, $p<0.05$ vs CONTROL; b, $p<0.001$ vs CHOLESTEROL; c, $p<0.002$ vs CHOLESTEROL+ COCONUT OIL).

knowledge, this is the first report of the effect of monounsaturated fatty acid on hepatic apoA-I mRNA. Recently, it has been reported that monounsaturated fatty acids increase serum apo A-I in monkeys (15) or maintain their levels either following a saturated fatty acid diet or a polyunsaturated fatty acid diet in humans (16,17,25). Considering the correlation between serum apo A-I and hepatic levels of apoA-I mRNA reported by several groups (11-14), we can assume that this hepatic increase in apoA-I message by effect of olive oil may be responsible of the behavior of serum apoA-I. Analyzing the effect of olive oil (75% oleic acid) and the trend observed in corn oil (33% oleic acid), we can conclude that the rat is especially sensitive to oleic acid. Related molecular mechanisms are not yet known, but suggestive speculations may be made taking into consideration that oleic acid controls phosphorylation and dephosphorylation of enzymes implicated in phospholipid metabolism (26) and phosphorylation processes have been found to control gene expression (27).

The conditions used in our study represent a valuable model to more precisely define which mechanisms are involved in this increase in hepatic apoA-I mRNA levels: An increase in transcription rate or a stabilization of mRNA by altering its structure or through an induction of a protein, that modulates apoA-I mRNA half life. All these caveats need an answer in order to know how to control serum HDL levels; and, in this way, to decrease the incidence of coronary artery disease in man. An approach such as the one reported here may also provide basis for further study of nutritional regulation of gene expression.

ACKNOWLEDGMENTS

We would like to thank Dr. P. Mata for his critical reading of this manuscript and Erik Lundin for his help in preparing this manuscript. This work was supported by contract # 53-3K-06-10 from the United States Department of Agriculture Research Services and by grants awarded by DGICYT (SM90-002) and EUROPHARMA. J. Osada and A. Fernandez-Sanchez are recipients of post-doctoral and predoctoral fellowships respectively from the Spanish Ministry of Education.

REFERENCES

1. Nestel, P.J. (1987) Am. Heart J. 113, 518-521.
2. Lamon-Fava, S., Ordovas, J.M., Mandel, G., Forte, T.M., Goodman, R.H. and Schaefer, E.J.(1987) J. Biol. Chem. 262, 8944-8947.
3. Fennewald, S.M., Hamilton, R.L. and Gordon, J.I. (1988) J. Biol. Chem. 263, 15568- 15577.
5. Ordovas, J.M., Cassidy, D.K., Civeira, F., Bisgaier, C.L. and Schaefer, E.J. (1989) J. Biol. Chem. 264, 16339- 16342.
6. Karathanasis, S.K., Ferris, E. and Haddad, I.A. (1987) Proc. Natl. Acad. Sci. USA 84, 7198- 7202.
7. Walsh, A., Ito, Y. and Breslow, J.L. (1989) J. Biol. Chem. 264, 6488- 6494.
8. Hughes,T.E., Nottage, B.F., Mone, M.D. & Paterniti, J.R. (1990) Arteriosclerosis 10, 774a.
9. Fielding, C.J., Shore, V.G. and Fielding, P.D(1972) Biochem. Biophys. Res. Commun. 46, 1943- 1949.
10. Zannis, V.I., Karathanasis, S.K., Kentmann, H., Goldberber, G. and Breslow, J.L. (1983) Proc. Natl. Acad. Sci. USA 80, 2574- 2578.
11. Stucchi, A.F., Hennessy, L.K., Vespa, D.B., Weiner, E.J., Osada, J., Ordovas, J.M., Schaefer, E.J. and Nicolosi, R.N. (1991) Arteriosclerosis. In press.
12. Osada, J., Hennessy, L., Ordovas, J.M., Stucchi,A.F., Nicolosi, R.N. and Schaefer, E.J. (1989) Circulation 80, II-621.
13. Sorci-Thomas, M., Prack, M.M., Dashti, N., Johnson, F., Rudel, L.L. & Williams, D.L. (1988) J. Biol.Chem. 263, 5183-5189.
14. Sorci-Thomas, M., Prack, M.M., Dashti, N., Johnson, F., Rudel, L.L. & Williams, D.L. (1989) J. Lipid Res. 30, 1397-1403.
15. Rudel, L.L., Haines, J.L. and Sawyer, J.K. (1990) J. Lipid Res. 31, 1873-1882.
16. Grundy, S.M. and Denke, M.A. (1990) J. Lipid Res. 31, 1149-1172.
17. Mattson, F.H. and Grundy, S.M. (1985) J. Lipid Res. 26, 194-202.
18. Osada, J. Aylagas, H., Miro-Obradors, M.J., Cao,G. and Palacios-Alaiz, E. (1989) Br. J. Nutr. 62, 343-348.
19. Kingston, R.E.(1987) in Current Protocols in Molecular Biology (Asubel, F.M., Brent, R., Kingston, R.E., Moore, D.O., Seldman, J.G., Smith, J.A. and Struhl, K., eds.), Greene Publishing and Wiley-Interscience, New York.

20. Seilhamer, J.J., Protter, A.A., Frossard, P. and Levy-Wilson, B. (1984) DNA 3, 309- 317.
21. Feinberg, A.P. & Vogelstein, B. (1983) Anal. Biochem. 132, 6- 13.
22. Sambrock, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A laboratory manual, second edition, pp 2.6- 2.8, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N Y.
23. Sohal, R.R. and Rohlf, F.J. (1981) in: The Principles and Practice of Statistics in Biological Research (Cotter, S.), 2nd ed. W.H. Freeman and Co., New York.
24. Haddad, I.A., Ordovas, J.M., Fitzpatrick, T. and Karathanasis, S.K. (1986) J. Biol. Chem. 261, 13268-13277.
25. Mata, P., Alvarez-Sala, L., Nuno, J. and De Oya, M. (1991) Am. J. Clin. Nutr. In press.
26. Sanghera, J.S. and Vance, D.E. (1989) J. Biol. Chem. 264, 1215-1223.
27. Denner, L.A., Weigel, N.L., Maxwell, B.L., Schrader, W.T. and O'Malley, B.W. (1990) Science 250, 1740-1743.