the neuronal network in the eye cup preparation is not expected to vary to such an extent, the possibility remains that the effects of retinal DA mediated by DA_i and DA_e receptors differ in their time course. The effects mediated by the DA_i receptors are thought to develop faster. A participation of the DA-ergic neurons in a negative feed back circuit between the on-type bipolar cells (BCs) and the ACs seems plausible. This is in agreement with the finding that exogenous DA induces a hyperpolarization of the on-type BCs[4]. Such a possibility is also compatible with some models[19,20] suggesting that the on-type BCs activity underlies the K^+ fluxes responsible for b-wave generation. In the case of HAL probably a more complex mechanism is involved. Recent data showing that exogenous DA penetrates the nuclei of the retinal neurons[21] indicate that retinal DA may participate in the control of long-term events such as RNA synthesis and protein metabolism. We presume that the delayed effects of HAL on ERG partially depend on its influencing these metabolic events. As HAL inhibits the DA effect on the DA-sensitive adenyl cyclase in retinal homogenates[16-18] one may assume that the effects of HAL on ERG may be mediated by its influence on some adenyl cyclase systems coupled to the DA_e receptors.

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Changes in the lipoproteins of rabbits on a high-fat, cholesterol-free diet; preventive action of metformin

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Summary. Endogenous hypercholesterolemia induced by a cholesterol-free, high-fat diet corresponds to an increase in the level of low density lipoproteins and their enrichment in cholesterol esters. Metformin has no effect on the rise in plasma cholesterol but completely prevents the appearance of cholesterol-rich low-density lipoprotein.

It has been clearly established that cholesterol-induced atherosclerosis, in rabbits, is accompanied by characteristic changes in circulating very low density lipoprotein (VLDL) and low density lipoprotein (LDL)[1-4]. Lipoproteins modifications were also seen with other atherogenic diets[5-7].

In order to confirm the relationship between the lipoprotein composition changes and the higher incidence of atherosclerosis, investigations into the influence of other factors are of particular interest. The present work reports the influence of metformin (N,N-dimethylbiguanide) on lipoprotein changes induced by a high-fat, cholesterol-free diet. Metformin is a drug used in the treatment of diabetes which is well known for its preventive effects on cholesterol-induced atherosclerosis[8,9].

Techniques. Animals and diets. Fauve de Bourgogne male rabbits, weighing on average 2.5 kg at the start of the 3-month experiment, were divided into 3 groups of 10 and received the following treatments: chow diet, high-fat diet, high-fat diet and a daily dose of 120 mg/kg of metformin in 2 treatments per day. The composition of the high-fat diet used was: 10% coconut oil; 10% butter, 16% protein; 44% carbohydrate; 12.5% cellulose, 7.5% salt and vitamin mixture.

Lipid and lipoprotein analysis. Following an 18-h fast, the animals were killed and the blood collected over EDTA (1 mg/ml). Total plasma cholesterol[10] and triglycerides[11] were measured. Lipoprotein fractions: VLDL ($d < 1.006$), LDL ($1.019 < d < 1.063$), and high density lipoprotein (HDL) ($1.063 < d < 1.21$) were separated by ultracentrifugation on a KBr density gradient[12] in a Beckman SW 41 rotor. The composition of each class of lipoprotein was determined by measuring the levels of protein[13], triglycerides[11], total and free cholesterol[10], and phosphorus[14]. Phosphorus was converted to phospholipids by multiplying by 25. The amount of cholesteryl esters was calculated as the difference between total and free cholesterol and multiplied by 1.67. Quantitative analysis was carried out as previously described[15]. Plasma lipoprotein levels were calculated using the proportion of cholesterol obtained in the qualitative analysis. The liver cholesterol content[16] and total fatty acids[17] were determined after extraction with a chloroform-methanol (2:1, v/v) mixture.

Results. Fat-fed rabbits showed an increase in their plasma cholesterol while triglycerides were not significantly modified. Metformin had no effect on the hypercholesterolemia induced by the fatty diet and it increased plasma triglycerides. Lipid overload of the liver was also observed with the fatty diet. Metformin completely prevented accumulation of cholesterol in the liver (table 1).

Results concerning the lipoprotein pattern are presented in

Table 1. Lipid analysis of the plasma and liver

	Plasma Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)	Liver Cholesterol (mg/100 g)	Free fatty acids (g/100 g)
Control	31 ± 5.4[a]	54 ± 5.2[a]	290 ± 14.6[a]	3.79 ± 0.161[a]
Fat	71 ± 7.7[b]	54 ± 3.3[a]	410 ± 31.5[b]	4.23 ± 0.165[b]
Fat + metformin	90 ± 7.4[b]	107 ± 17.6[b]	293 ± 6.08[a]	4.60 ± 0.172[b]

Means ± SEM of 10 rabbits. Means not sharing a common superscript are significantly different ($p < 0.05$).

Table 2. Lipoprotein levels and composition

		Total (mg/100 ml)	Proteins	Triglycerides	Phospholids	Cholesterol esters	Free cholesterol
VLDL	Control	26 ± 2.24[a]	9.5 ± 1.16	45.1 ± 2.88	18.0 ± 1.93	22.6 ± 1.42	5.0 ± 0.36
	Fat	20 ± 2.37[a]	9.4 ± 1.83	43.5 ± 6.23	15.1 ± 1.69	27.2 ± 3.74	4.7 ± 0.67
	Fat + metformin	111 ± 26.3[b]	10.1 ± 1.03	44.4 ± 5.54	15.5 ± 0.80	26.0 ± 4.02	4.2 ± 0.29
LDL	Control	40 ± 12.9[a]	25.2 ± 1.90	19.1 ± 1.97[a]	25.6 ± 2.35	24.9 ± 3.01[a]	5.1 ± 0.76
	Fat	118 ± 18.7[b]	22.9 ± 0.93	11.6 ± 1.55[b]	23.2 ± 1.65	36.9 ± 1.61[b]	5.5 ± 1.14
	Fat + metformin	177 ± 25.1[b]	23.1 ± 1.25	22.5 ± 1.88[a]	23.7 ± 4.11	27.7 ± 3.43[a]	5.2 ± 0.75
HDL	Control	77 ± 12.2[a]	48.8 ± 3.99	10.8 ± 2.05	22.5 ± 3.25	15.6 ± 2.99	2.4 ± 0.69
	Fat	189 ± 24.2[b]	56.2 ± 2.71	6.9 ± 0.72	20.5 ± 1.39	15.4 ± 2.58	1.9 ± 0.23
	Fat + metformin	217 ± 41.6[b]	52.7 ± 1.59	8.9 ± 1.52	23.9 ± 0.98	13.1 ± 1.65	1.4 ± 0.22

Means ± SEM of 6 rabbits. Means not sharing a common superscript are significantly different ($p < 0.05$).

table 2. The increase in total plasma cholesterol observed in fat-fed rabbits corresponds to an increase of the LDL and HDL. In addition LDL presented an enrichment of cholesterol esters at the expense of a decrease in the level of triglycerides. An increase in all lipoprotein fractions was observed in metformin-treated rabbits. However, metformin has a very clear preventive effect on the appearance of abnormal LDL. Therefore during metformin-treatment LDL composition is normalized in spite of an increase in total plasma cholesterol induced by a high intake of triglycerides.

Discussion. The results confirm the importance of nutritional factors, not only is the level of the circulating lipoproteins modified but also their composition. Endogenous hypercholesterolemia induced by a high intake of glycerides is accompanied by an enrichment of the LDL fraction in cholesterol esters. The appearance of such cholesterol-rich lipoproteins has already been reported for semi-synthetic, cholesterol-free diets[5-7] as well as for cholesterol feeding[1-4].

The prevention of changes in the LDL composition by metformin is quite remarkable. This result could well explain the beneficial effect of metformin already observed on experimental atherosclerosis[8,9]. A fundamental role is attributed to LDL in the pathogenesis of atherosclerosis[18]. Moreover it has been suggested that the abnormal VLDL and LDL of cholesterol-fed rabbits are strongly atherogenic. They are trapped more quickly by the arterial wall[19]. In addition LDL from hyperlipoproteinemic animals can stimulate the proliferation of smooth muscle cells[20,21].

A result comparable with ours was obtained in cholesterol-fed rabbits, metformin preventing most of the VLDL modifications induced by the cholesterol[9]. The present study shows that the preventive effect of metformin on lipoprotein alterations appears in the case of endogenous, as well as exogenous, hypercholesterolemia. The results do not yet allow elucidation of the mechanism of metformin action. It would seem that the point of impact does not only concern VLDL synthesis and degradation since an action is also observed on the LDL in the case of fatty, cholesterol-free diets.

In the rabbit, the beneficial effect of metformin on the arterial lesions and fatty liver induced by cholesterol occurs in spite of the persistence of hypercholesterolemia[8,9]; this is in good agreement with our results.

In conclusion, metformin, which is known to have a beneficial action on experimentally-induced atherosclerosis, completely prevents LDL alteration thus confirming the importance of lipoprotein composition in atherosclerosis.

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