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Changes in serum triglyceride and cholesterol levels independently of free fatty acid after lipolysis inhibitors

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THE AVAILABILITY of compounds that can effectively reduce the serum free fatty acid (FFA) level by inhibiting lipolysis in adipose tissue made it possible to investigate the consequences of such pharmacologically induced changes. For the possible use of lipolysis inhibitors as hypolipidemic agents studying their effects on serum and tissue lipids is significant.

One way to elucidate the role of lipolysis inhibition in the determination of serum lipid levels is to compare the effect of structurally different lipolysis inhibitors.

This paper reports the effect of several lipolysis inhibitors on serum triglyceride and cholesterol levels in a condition with high serum FFA, namely fasting, and in one with low FFA level, namely carbohydrate-induced lipemia. Furthermore, the incorporation of ^{14}C-palmitic acid into serum and liver lipids was studied in fasting state.

Male Wistar Ch. B. rats weighing 190 ± 20 g maintained on a standard laboratory diet were used. The experimental conditions were: (a) 24 hr of fasting, but water given *ad lib.*, (b) carbohydrate-induced lipemia by giving rats 10% fructose in drinking water for 24 hr according to Nikkila.[2] This latter group of rats was fed until exsanguination. The compounds tested were suspended in distilled water by 3% v/v Tween 80 and made up to a volume of 0·5 ml/kg. The total doses, as indicated in the tables were administered orally in two equal portions, 4 hr apart. Four hours after the second treatment the rats were exsanguinated and the sera separated. In experiments where the duration of effect was studied the total dose was administered in one portion.

From the sera total cholesterol,[3] triglyceride[4] and FFA[5] concentrations were determined.

1-^{14}C-palmitic acid incorporation in vivo. Rats were fasted for 24 hr as described previously and treated with nicotinic acid (300 mg/kg) or 3-methylpyrazole-5 carboxylic acid (30 mg/kg) orally. One hour after the administration of drugs 25 μc/kg albumin-bound 1-^{14}C-palmitic acid was injected intravenously and 30 min later the animals were exsanguinated. The 1-hr interval between drug treatments and isotope administration was chosen since our preliminary experiments have shown that up to this time the serum and liver lipid components, except FFA were not significantly altered.

One ml of serum and a portion of liver was extracted in chloroform–methanol and washed according to Carlson.[6] The chloroform phase containing lipids was concentrated by evaporating chloroform and separated by thin-layer chromatography. The chromatoplates were developed in di-isopropyl ether–acetic acid (96:4) and petroleum ether–diethyl ether–acetic acid (90:10:1) successively, according to Skipski *et al.*[6, 7] The dried chromatograms were sprayed with 2% liquid scintillation solution (0·3 g POPOP, 5·0 g PPO and 50 ml methanol and 1000 ml toluol) in absolute ethanol and viewed under u.v. light (355 mμ) according to Boberg.[8] For the measurement of radioactivity the visualized bands of separate lipid classes were directly scraped off into counting vials and the activity was detected in a Packard Tri-Carb liquid scintillation spectrometer (Model 3380). Quenching corrections were made using an internal standard. The activity of phospholipids was measured after prior elution with methanol.[8] The recovery of activities after TLC separation was found to be 92–96 per cent. The activities of mono- and diglyceride fractions due to the low dpm counts were omitted from the table.

Compounds. The following compounds were tested:

I. nicotinic acid as its Na-salt (Serva Lab.),
II. 3-methylpyrazole-5 carboxylic acid,
III. pyrrole-3 carboxylic acid,
IV. chinoline-3 carboxylic acid,
V. pyrazine-carboxylic acid,
VI. pyrazine-carboxamide (Chinoin).

Compounds II–V were supplied by L. Farkas, O. Fehér and I. Toth from our Chemical Department. 1-^{14}C-palmitic acid with specific activity of 1·16 mc/m-mole was prepared by O. Banffy, its purity

checked by TLC and autoradiography was of 96–98 per cent. Lyophilized human albumin in a 5% (w/v) solution with 0·9% NaCl was used to bind labelled palmitic acid.

The statistical evaluation of results was accomplished according to the standard *t*-test. The difference between treated and control groups was tested for significance separately in each experiment.

Results

Effect in fasting rats. In fasting rats all compounds were found to have a consistent FFA lowering effect (Table 1) 2 hr after administration. The minimal effective dose of the compounds was similar to that of nicotinic acid (I). Thus all compounds were less active than the previously investigated pyrazole derivatives,[9–12] which presented activity at doses of as low as 1 mg/kg. Concerning the duration of activity at high dose levels compounds I, V and VI were able to decrease serum FFA level for over 6 hr after administration.

TABLE 1. EFFECT OF LIPOLYSIS INHIBITORS ON SERUM FFA LEVELS IN FASTING RATS

Compound	Dose mg/kg orally	Time in hours after administration 2	6
I	10	133·0*	121·0*
	30	88·0	115·0
	100		71·6*
	300	52·1*	45·2*
III	10	124·0	
	30	60·9*	107·0
	100	61·9*	102·0
	300	63·0*	121·0
IV	30	85·0	94·0
	100	62·8*	93·3
	300	64·5*	93·3
V	10	101·2	
	30	60·0*	144·0*
	100	57·7*	131·2
	300	66·0*	70·0*
VI	30	115·0	115·2
	100	61·6*	101·2
	300	64·0*	60·0*

The difference between control and treated groups was evaluated for significance in each experiment separately and the mean of control group was taken as 100. Statistically significant difference (P 0·05) is indicated by *. For further details see text.

The serum FFA levels in untreated (control) fasting rats were 980 ± 55 μequiv./1000 ml ($n = 20$).

At repeated administration (Table 2) effect on serum cholesterol was exhibited only by nicotinic acid (I). The reference compound could induce a significant decrease of about 20 per cent, which in the course of repeated experiments proved to be highly reproducible. None of the other compounds were effective in this respect. On the other hand the serum FFA level was decreased by all compounds at appropriate doses.

Effect in carbohydrate-induced lipemia (Table 3). The elevated triglyceride levels in fructose-lipemic rats (120·1 ± 12·9 mg% compared to 60·5 ± 4·7 mg% in normally fed rats of the same weight and sex) were significantly reduced by all tested compounds. The low serum FFA levels in fructose-lipemic rats (374 ± 33 μequiv./1000 ml) remained either unchanged or were elevated. The serum cholesterol was not significantly influenced by either of the compounds, including nicotinic acid in this test.

TABLE 2. EFFECT OF LIPOLYSIS INHIBITORS ON SERUM CHOLESTEROL AND FFA LEVELS IN FASTING RATS AFTER REPEATED ADMINISTRATION

Compound	Dose total mg/kg orally	Cholesterol	FFA
I	100	83·4*	115·0
	300	78·5*	33·0*
III	100	92·0	38·0*
	300	109·0	
IV	100	101·0	42·5*
	300	107·0	
V	100	94·2	124·0
	300	100·0	70·0*
VI	100	96·5	75·0*
	300	99·1	49·0*

For details see Table 1.
The serum cholesterol levels in untreated (control) fasting rats were 114·1 ± 2·4 mg % (n = 30).

TABLE 3. EFFECT OF LIPOLYSIS INHIBITORS ON SERUM LIPIDS IN FRUCTOSE-LIPEMIC RATS AFTER REPEATED ADMINISTRATION

Compound	Dose total mg/kg orally	Triglyceride	Cholesterol	FFA
I	30	104·0	95·0	83·0
	100	63·5*	91·0	104·0
	300	50·8*	92·2	80·0
II	1	98·0	96·2	96·8
	3	85·5	93·0	100·0
	10	60·0*	94·0	122·1
	30	37·7*	95·0	
	100	36·7*	90·7	
IV	300	39·0*	97·4	224·0*
V	30	116·0	99·8	
	100	46·3*	96·6	
	300	39·0*	111·3	130·0
VI	30	63·9*	100·8	
	100	56·6*	92·5	
	300	37·1*	102·3	90·0

For details see Table 1.
The serum lipid levels in untreated (control) fructose-lipemic rats were: triglyceride 120·1 ± 12·9 mg% (n = 19), cholesterol 115·5 ± 2·4 mg% (n = 30), FFA 374 ± 33 μequiv./1000 ml (n = 25).

Incorporation of ^{14}C-palmitic acid into serum and liver of fasting rats. Both lipolysis inhibitors, namely nicotinic acid (I) and 3-methylpyrazole-5 carboxylic acid (II) could markedly reduce the incorporation of ^{14}C-palmitic acid into total lipids of serum (Table 4). The decrease of radioactivity did not effect equally all lipid components of serum, but was restricted solely to the triglyceride and FFA fractions. The incorporation of labelled palmitic acid into cholesterol, cholesterol esters and phospholipids was not significantly changed.

There was also a trend of decrease in liver total lipid radioactivity, though after nicotinic acid (I) it did not reach the level of significance. However, the individual lipid components of liver were similarly

TABLE 4. INCORPORATION *IN VIVO* OF 1-^{14}C-PALMITIC ACID INTO SERUM AND LIVER LIPIDS IN FASTING RATS

	Experiment I					
	Serum (10^3 × dpm/ml)			Liver (10^3 × dpm/g liver)		
	Control	Nicotinic acid	P	Control	Nicotinic acid	P
Total lipid	10·98 ± 1·05	6·50 ± 0·44	0·01	137·00 ± 5·94	117·40 ± 6·51	n.s.
Cholesterol esters	0·24 ± 0·02	0·22 ± 0·02	n.s.	4·72 ± 0·38	5·41 ± 0·93	n.s.
Triglycerides	3·80 ± 0·60	1·62 ± 0·33	0·05	49·16 ± 5·12	26·06 ± 4·70	0·02
FFA	4·26 ± 0·56	2·64 ± 0·37	0·05	9·73 ± 0·75	11·52 ± 2·37	n.s.
Cholesterol	0·51 ± 0·05	0·42 ± 0·03	n.s.	3·89 ± 0·28	3·97 ± 0·53	n.s.
Phospholipids	0·59 ± 0·08	0·51 ± 0·05	n.s.	61·52 ± 4·52	65·35 ± 3·09	n.s.
	Experiment II					
	Control	3-methyl-pyrazole-5 carboxylic acid		Control	3-methyl-pyrazole-5 carboxylic acid	
Total lipid	11·57 ± 1·25	5·85 ± 0·25	0·02	236·10 ± 10·60	184·70 ± 23·90	0·05
Cholesterol esters	0·31 ± 0·02	0·32 ± 0·02	n.s.	5·71 ± 0·66	5·83 ± 0·63	n.s.
Triglycerides	5·29 ± 1·15	1·93 ± 0·27	0·05	63·58 ± 8·52	20·30 ± 4·29	0·01
FFA	3·60 ± 0·48	1·43 ± 0·10	0·05	8·25 ± 0·40	6·97 ± 1·01	n.s.
Cholesterol	0·77 ± 0·07	0·73 ± 0·07	n.s.	4·50 ± 0·54	3·81 ± 0·16	n.s.
Phospholipids	0·77 ± 0·11	0·61 ± 0·10	n.s.	143·20 ± 10·40	138·00 ± 22·27	n.s.

affected as in serum; the triglycerides decreased, while the cholesterol, cholesterol esters and phospholipids remained essentially unchanged.

Discussion

Up till now the most intensively studied lipolysis inhibitor has been nicotinic acid.[1, 21] The relationship between its lipolysis inhibitory and well-established serum cholesterol- and triglyceride-lowering effect is, however, far from being elucidated. In humans the effect on serum FFA and triglyceride is manifested after a single dose,[13] while the cholesterol becomes affected after several days of treatment.[14] In fasting rats the reduction of FFA and triglycerides was followed by a small but significant decrease of cholesterol after a single dose.[15] A dose-dependent effect on serum cholesterol was observed in fasting rats after repeated doses of nicotinic acid,[12] as well as its precursor beta-pyridylcarbinol.[16]

On the other hand, very potent lipolysis inhibitors of pyrazole structure were found to have no effect on serum cholesterol either in rats[11, 12, 17] or in humans[18] in fasting state. Similarly the lipolysis inhibitors with pyrazine and chinoline structures (compounds IV, V and VI), as reported above were also without effect on serum cholesterol in fasting rats. The ability of lipolysis inhibitors with pyridine structure (nicotinic acid, beta-pyridylcarbinol) to reduce serum cholesterol in fasting rats thus seems to be independent of the effect on serum FFA. A direct evidence of this is presented by our finding, that the incorporation of ^{14}C-palmitic acid into cholesterol and cholesterol ester fractions of serum and liver lipids was not changed significantly by pretreatments with either nicotinic acid or 3-methylpyrazole-5 carboxylic acid. This permits the conclusion that despite the pharmacologically induced reduction of serum FFA pool a sufficient amount of substrates is still available for cholesterol biosynthetic pathways.

As to the possible mechanism of the cholesterol lowering effect observed for nicotinic acid we must refer to previous studies which have shown that nicotinic acid may decrease synthesis of cholesterol[19] or increase its oxidative catabolism.[20]

In contrast to the effect on serum cholesterol the triglyceride lowering effect of lipolysis inhibitors seems to be a common feature of an agent with such a pharmacological profile. In fasting state it has been well established for both the pyridine and pyrazole type of lipolysis inhibitors.[11, 12, 15, 17] Our observations showing that two structurally different lipolysis inhibitors, namely nicotinic acid and 3-methylpyrazole-5 carboxylic acid can similarly reduce the incorporation of ^{14}C-palmitic acid into the triglyceride fractions of serum and liver lipids of intact animals point directly to the importance of

serum FFA pool as a source of substrate for triglyceride biosynthesis. Our results with nicotinic acid are in accordance with those of Carlson[21] and Solyom[22] reported previously.

While the fasting state is associated with a high level of serum FFA, in carbohydrate-induced lipemia the serum FFA level is low. Therefore it would seem rather surprising that all lipolysis inhibitors tested could reduce the serum triglyceride level significantly in this condition. Since the level of FFA remained either unchanged or was increased the assumption that in this condition the lipolysis inhibitors act via their effect on serum FFA level must be ruled out.

Concerning the possible mechanism of triglyceride lowering effect of lipolysis inhibitors in carbohydrate-induced lipemia the finding of Nikkila seems to be of importance.[23] This author observed an increase in adipose tissue lipoprotein lipase activity after nicotinic acid injection. The degree of induction of lipoprotein lipase in serum and tissues other than adipose tissue and its role in the "clearing" effect of lipolysis inhibitors deserves further experimentation.

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Research Instute for Pharmaceutical Chemistry, Budapest, Hungary

G. TAMÁSI*
J. BORSY
R. GYENGE

* Present address: Radioisotope Research Veterans Administration Center, Los Angeles, Calif. 90073, U.S.A. For reprints write to G. T.

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