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EFFECT OF LONG-TERM THERAPY WITH AN ORAL CONTRACEPTIVE ON SOME ASPECTS OF HEPATIC LIPID METABOLISM *IN VITRO*

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Abstract—Livers were obtained from untreated controls as well as from female rats which were treated with Enovid® (7·5 mg/kg of food) for either 4 days or 1 year. The livers were placed in an isolated perfusion system and the rate of release of cholesterol and triglyceride was measured. Long-term treatment with Enovid® resulted in a 38–72 per cent reduction below control level in the rate of release of hepatic triglyceride and cholesterol, respectively, while short-term treatment was without a significant effect. No significant effect of either short- or long-term treatment was observed on hepatic weight or on concentrations of either cholesterol or triglyceride in the liver at the end of the perfusion. Concentrations of triglyceride and cholesterol in the serum of the liver donors were also measured. The only significant change observed was a 30 per cent reduction in cholesterol concentration of the group treated with Enovid® for 4 days. Thus, treatment with Enovid® for 1 year was accompanied by a reduced rate of release of cholesterol and triglyceride by the liver, while serum triglyceride and cholesterol concentrations were unchanged. Studies of other aspects of the metabolism of these two substances will be necessary to explain the results.

Some women using oral contraceptive preparations containing progestational and estrogenic components have elevated serum triglyceride and depressed serum cholesterol concentrations [1–4]. The mechanisms relating ingestion of oral contraceptive agents to alteration of serum lipid levels are poorly understood. However, it is established that the liver is the major organ involved in the regulation of serum triglyceride and cholesterol concentrations. Hence, it seemed worthwhile to study the effect of treatment with the oral contraceptive, Enovid,* for both short and long periods of time on hepatic secretion of triglyceride and cholesterol *in vitro*. A preliminary report of some of the data has been presented [5].

METHODS

Female rats of the Blue Spruce Farms strain weighing initially from 180 to 230 g were used as liver donors in experiments involving both acute and chronic administration of Enovid. The animals were kept in a room at 26 ± 1° which was illuminated from 0800 to 1800 hr. The rats were given tap water and finely powdered Purina rat chow *ad lib*. The treated rats received Enovid† which was ground up and mixed with food at a concentration of 7·5 mg/kg of food. Controls received the same food without the drug in the same spill-resistant containers as used by the treated group [6]. Treated rats ingested Enovid in the food for either 4 days or 1 year. The period of 4 days was chosen because it was the shortest time for which changes in lipid metabolism have been noted [7]. The longer period of treatment was chosen to ascertain any compensatory adjustment in lipid metabolism induced by long-term therapy. Calculations of the average daily food intake indicated that the short-term treated group ingested 315 μg Enovid/kg of body weight/day and the long-term treated group ingested 375 μg Enovid/kg of body weight/day. Although this approximates three times the daily dose for humans, the effective dose for the rat might be similar to that of the human since mestranol, the estrogenic component of Enovid, is metabolized at much faster rates in rats than in humans [8].

The animals which served as liver donors were anesthetized with pentobarbital (35 mg/kg of body weight); the livers were removed [9] and placed in a perfusion apparatus [10]. The livers were perfused via the hepatic portal vein; perfusion pressure was 20 cm H_2O and the temperature in the perfusion apparatus was maintained at 37°.

The livers were allowed to equilibrate for 20 min during perfusion with a medium consisting of a mixture of 40 ml defibrinated rat blood, obtained from normal female rats, and 30 ml Krebs–Henseleit bicarbonate buffer, pH 7·4. Ninety mg palmitic acid (> 99 per cent purity, Schwarz–Mann Laboratories, Orangeburg, N.Y.) was complexed with 4·0 g bovine serum albumin (Pentex, Kankakee, Ill.). The albumin was prepared according to the method of Chen [11]. The complex was adjusted to 25 ml with 0·9% NaCl, and in this manner 105 μmoles palmitic acid was infused at a rate of 7·38 ml/hr. Aliquots of the perfusate were removed at hourly intervals, and at the end of a 3-hr perfusion period, a sample of liver was taken

* Enovid-5® (G. D. Searle & Co., Chicago, Ill.).

† Each Enovid tablet contained 5 mg norethynodrel and 0·075 mg mestranol.

Table 1. Effect of short- and long-term administration of Enovid on food intake, body weight and liver weight of female rats

	Daily food intake* (g/100 g body wt)	P†	Body wt (g)	P	Liver wt wet (g)	P
Short-term‡						
Control (5)	6·2 ± 0·4		207 ± 5		6·22 ± 0·31	
		<0·05		<0·05		NS
Enovid (5) (7·5 mg/kg food)	4·4 ± 0·6		191 ± 2		5·84 ± 0·13	
Long-term						
Control (6)	4·7 ± 0·2		373 ± 25		9·77 ± 0·94	
		NS		<0·05		NS
Envoid (6) (7·5 mg/kg food)	5·1 ± 0·1		282 ± 20		9·37 ± 0·95	

* Values for food intake (g/100 g of body wt), body and liver weights are means ± S. E.

† P values (probability) indicate the significance between comparable groups as derived from a two-tailed table of Student's values for *t*. Non-significant differences are indicated by NS.

‡ The number of observations is shown in parentheses.

and both perfusate and liver sample were treated as described previously [12]. Glucose [12] was determined in an additional aliquot of perfusate. Triglyceride [13], cholesterol [14] and free fatty acid [15] concentrations were determined colorimetrically in samples of liver donor blood serum, cell-free perfusate and liver. The adrenal glands were removed from the donor animal after surgical removal of the liver. The glands were freed of non-glandular tissue, weighed rapidly, and ground in an all-glass tissue grinder with chloroform–methanol (2:1, v/v). The total lipid extract was treated as previously described for samples of liver [12], and total cholesterol [14] was determined. The statistical significance of the differences between data from control and experimental groups was evaluated by means of the *t*-test and the 95 per cent confidence limit [16].

* I. Weinstein and M. Veldhuis, unpublished observations.

RESULTS

Table 1 shows the effects of acute and chronic administration of Enovid on body weight, food intake (g/100 g of body weight) and liver weight of the four groups of liver donor animals. Body weights were reduced significantly ($P < 0.05$) by both acute and chronic treatments. A reduction in body weight by Enovid is a consistent finding in many laboratories [7]*. The effect of Enovid on body weight was observed even when the groups were pair fed [7, 17]. The effect of the drug on body weight is not a result of reduced food intake in the chronically treated group, since no differences were found in the amount of food consumed between the treated or control groups of animals. However, acute administration of Enovid significantly lowered food intake, which may, in part, account for the observed reduction in body weight. No significant effect of either acute or chronic treatment on liver weight was observed.

Table 2. Effect of short- and long-term administration of Enovid on bile production and perfusate flow rate in livers obtained from female rats

	Bile production* (ml/g liver)	P†	Perfusate flow rate‡ (ml/min/g liver)	P
Short-term§				
Control (5)	0·153 ± 0·015		2·9 ± 0·4	
		<0·05		NS
Enovid (5) (7·5 mg/kg food)	0·076 ± 0·022		2·9 ± 0·3	
Long-term				
Control (6)	0·049 ± 0·009		1·4 ± 0·1	
		NS		<0·02
Enovid (6) (7·5 mg/kg food)	0·064 ± 0·008		2·3 ± 0·3	

* Bile production is the amount produced during 3 hr of perfusion.

† P values (probability) indicate the significance between comparable groups as derived from a two-tailed table of Student's values for *t*. Non-significant differences are indicated by NS.

‡ Perfusate flow rate is the average flow during 3 hr of perfusion.

§ The number of observations is shown in parentheses; values are means ± S. E.

Table 3. Effect of short- and long-term administration of Enovid on rate of release of glucose, cholesterol and triglyceride into the perfusate of livers of female rats*

	Glucose (mg/g liver)	P†	Cholesterol (μmoles/g liver)	P	Triglyceride (μmoles/g liver)	P
Short-term‡						
Control (5)	13·56 ± 0·87	<0·05	1·30 ± 0·35	NS	2·31 ± 0·21	NS
Enovid (5) (7·5 mg/kg food)	9·45 ± 1·69		2·05 ± 0·46		1·99 ± 0·31	
Long-term						
Control (6)	9·91 ± 2·70	<0·05	2·51 ± 0·49	<0·05	2·88 ± 0·41	<0·05
Enovid (6) (7·5 mg/kg food)	3·49 ± 0·67		0·69 ± 0·38		1·79 ± 0·23	

* The values represent net production or release after 3 hr of perfusion.

† P values (probability) indicate the significant difference between comparable groups as derived from a two-tailed table of Student's values for *t*. Non-significant differences are indicated by NS.

‡ The number of observations is shown in parentheses; values are means ± S.E.

Indices of isolated perfused rat liver function are bile production and perfusate flow rate. Harkavy and Javitt [18] reported that acute administration of ethinyl estradiol reduced the rate of bile flow in the intact rat. Table 2 shows that bile production was reduced approximately 50 per cent in the rats treated with Enovid for 4 days. In rats treated with Enovid for 1 year, no effect was attributable to the oral contraceptive. However, in the control animals of this group, bile production was reduced 70–75 per cent when compared to bile production of livers obtained from younger animals. For unexplained reasons, perfusate flow rate through the livers of rats treated with Enovid for 1 year was significantly faster ($P < 0·02$) than that through livers of the control group.

Table 3 shows the effects of Enovid on the release of glucose, cholesterol and triglyceride into the perfusate of livers obtained from animals fed the oral contraceptive for either 4 days or 1 year. Glucose production was reduced by both the short- and long-term drug treatment. In the chronic treatment group, this was not the result of reduced food intake since the control and experimental groups consumed the same amount of food. However, in the short-term treated group reduced food intake may have affected the production of glucose. In the group treated with Enovid for 1 year, release of cholesterol and triglyceride into the perfusate was reduced; this effect was not observed in the short-term treated group.

Enovid has no effect on the concentration of either hepatic triglyceride or cholesterol regardless of the treatment regimen (Table 4). Although triglyceride and cholesterol release from livers treated with Enovid for 1 year was inhibited, no accumulation of hepatic triglyceride or cholesterol was evident.

Hypertriglyceridemia was reported in humans taking oral contraceptives [1–4] and, recently, in rats treated for 14 days with ethinyl estradiol [19]. It was of particular interest to correlate the changes in the concentrations of triglyceride and cholesterol in the serum of donor animals induced by Enovid with the changes in release of these lipids from perfused livers of these same animals. Under the conditions of our experiments, Enovid did not produce an elevation of

Table 4. Effect of short- and long-term administration of Enovid on concentration of hepatic triglyceride and cholesterol in the perfused liver of female rats*

	Triglyceride (μmoles/g liver)	Cholesterol (μmoles/g liver)
Short-term		
Control (5)	15·29 ± 1·39	7·80 ± 0·31
Enovid (5) (7·5 mg/kg food)	14·24 ± 1·69	7·08 ± 0·48
Long-term		
Control (6)	15·04 ± 1·43	7·24 ± 0·36
Enovid (6) (7·5 mg/kg food)	16·20 ± 1·41	7·86 ± 0·63

* The number of observations is shown in parentheses; values are means ± S.E. and determined in the liver after 3 hr of perfusion. No significant differences were observed.

Table 5. Effect of short- and long-term administration of Enovid on serum triglyceride and cholesterol concentrations*

	Triglyceride (μmoles/ml)	P†	Cholesterol (μmoles/ml)	P
Short-term‡				
Control (5)	0·17 ± 0·02	NS	0·67 ± 0·04	<0·02
Enovid (5) (7·5 mg/kg food)	0·19 ± 0·02		0·47 ± 0·07	
Long-term				
Control (6)	0·26 ± 0·07	NS	1·38 ± 0·22	NS
Enovid (6) (7·5 mg/kg food)	0·22 ± 0·02		1·13 ± 0·19	

* Determinations were made on serum samples obtained just prior to cannulation of the liver.

† P values (probability) indicate the significant differences between comparable groups as derived from a two-tailed table of Student's values for *t*. Non-significant differences are indicated by NS.

‡ The number of observations is shown in parentheses; values are means ± S. E.

triglyceride concentration in the serum of donor animals (Table 5). In confirmation of results reported by others [7], we observed a decrease in serum cholesterol in rats fed Enovid in their food for 4 days. In the serum of animals held 1 year, treatment with Enovid had no effect, but total cholesterol concentrations of both treated and control groups were increased approximately 100 per cent above that of their younger counterparts.

Oral contraceptive therapy has been reported to affect adrenal cortical secretions [20–28]. Two criteria used for ascertaining adrenal function have been adrenal weight and cholesterol content. The effects of short- and long-term administration of Enovid upon these parameters are shown in Table 6. Enovid had no effect on the weights of adrenal glands obtained from animals treated for 4 days or 1 year. The total sterol content of the adrenal glands taken from animals treated with Enovid for 1 year was decreased significantly ($P < 0·05$) but not in the animals which were fed for 4 days with food containing Enovid.

DISCUSSION

The objectives of this study were to compare the effects of acute vs chronic administration of Enovid on release of triglyceride and cholesterol from the

Table 6. Effect of short- and long-term administration of Enovid on adrenal weight and total cholesterol content*

	Wet wt (mg/adrenal)	P†	Cholesterol (mg/g adrenal)	P
Short-term‡				
Control (5)	35·1 ± 3·1	NS	19·5 ± 2·2	NS
Enovid (5) (7·5 mg/kg food)	35·2 ± 1·2		25·6 ± 3·4	
Long-term				
Control (6)	26·2 ± 1·9	NS	20·9 ± 3·3	<0·05
Enovid (6) (7·5 mg/kg food)	24·1 ± 1·1		9·7 ± 1·9	

* Determinations were made on adrenal glands removed from animals just after extirpation of the liver.

† P values (probability) indicate the significant differences between comparable groups as derived from a two-tailed table of Student's values for *t*. Non-significant differences are indicated by NS.

‡ The number of observations is shown in parentheses; values are means ± S. E.

livers of rats and to correlate these observations with the concentrations of triglyceride and cholesterol in the serum of the donor animal prior to removal of its liver. The contrasting effects of short- and long-term treatment were manifested by the differences in the rate of release of cholesterol and triglyceride from the perfused livers (Table 3). Long-term treatment with Enovid significantly reduced the rate of release of both cholesterol and triglyceride. The rate of glucose release was significantly reduced by both long- and short-term treatment with Enovid (Table 3). The hepatic concentrations of both triglyceride and cholesterol measured at the end of the perfusion study were unaffected by either long- or short-term treatment (Table 4). Long-term treatment failed to alter serum triglyceride and cholesterol concentrations in this study but a reduction in serum cholesterol concentration was observed after short-term treatment with Enovid (Table 5).

These results suggest that maintenance of a normal serum concentration of triglyceride and cholesterol in chronically treated rats concurrent with a reduction in the rate of hepatic release of these substances can only be accounted for by reduced peripheral metabolism. Conversely, the reduced serum cholesterol concentration in rats treated with Enovid for 4 days occurred without change in the rate of hepatic release of this substance. An appropriate explanation for this may be an increased rate of peripheral metabolism of cholesterol accompanying short-term treatment. Additional studies will be required to explain these contrasting effects of short- and long-term treatment with Enovid.

The failure to demonstrate a change in serum triglyceride concentration in rats treated either acutely or chronically with Enovid contrasts with the many reports of elevated serum triglyceride concentration in women ingesting Enovid or certain other oral contraceptive drugs [1–4]. Heimberg *et al.* [9] reported a decreased rate of triglyceride release in the perfusate of livers obtained from fasted animals. A possibility exists that the treated rats used in both the acute and chronic studies were in a fasted state relative to controls. Both treated groups weighed significantly less than controls and the treated rats in the acute study ate significantly less food (Table 1). Since chronic treatment did not affect food intake (Table 1) and digestibility of foodstuffs is not affected by either mestranol or norethynodrel [29], it is difficult to account for the reduced rate of triglyceride secretion in chronically treated rats on the basis of these parameters. A possibility exists that blocks in the synthesis or utilization of fat and protein may occur in treated animals and account for their reduced body weight. Increased energy expenditure by way of increased spontaneous locomotor activity cannot account for the weight reduction, since administration of Enovid is accompanied by a reduction in locomotor activity in both rats [17] and humans [30].

Cortisol levels in the serum of women are elevated during therapy with oral contraceptives [21, 27, 28]. It has been suggested that an elevation of blood concentration of glucocorticoid hormones may account for the hypertriglyceridemia observed in women taking oral contraceptives [27]. It is not clear at present how the glucocorticoids function. Thus, in contrast to the aforementioned studies, in women ingesting Enovid the rate of secretion of cortisol was significantly reduced [23], as it is in women and rats treated with estrogenic agents [24–26]. In these studies, no measurements of serum triglyceride were made. Drill [31] reported that rats treated with Enovid for 1 year had a slight decrease in adrenal weight but no change in adrenal to body weight ratio. We observed no significant change in adrenal weight with Enovid therapy (Table 6). However, the adrenal glands of rats treated with Enovid for 1 year had a reduced cholesterol concentration relative to controls. The depletion of cholesterol under these conditions could suggest a reduced rate of synthesis or an increased rate of secretion of glucocorticoid. It is recognized, however, that the measurement of cholesterol is a poor predictor of adrenal cortical secretion rate in rats treated for protracted periods.

The inhibition of triglyceride release by Enovid in the long-term treated group may be manifested through a direct action of the drug on the liver. Since the oral contraceptive is composed of mestranol and norethynodrel, the results obtained may be attributed to metabolic effects of the individual components. Experiments are currently in progress which will examine the effect of the individual components on hepatic triglyceride transport.

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