Norethindrone acetate inhibition of splanchnic triglyceride secretion in conscious glucose-fed swine[1]

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Abstract The effects of conventional doses of two synthetic contraceptive steroids on the concentration and rate of secretion of plasma triglycerides from the splanchnic region were investigated. Studies were undertaken in miniature swine under steady state conditions produced by prolonged constant hypercaloric intravenous infusions of glucose. The steroids, alone or in combination, were administered with the high carbohydrate diet for at least 2 weeks prior to study of splanchnic metabolism and were also infused intravenously during the studies. Splanchnic triglyceride secretion was determined from measurements of plasma flow and transsplanchnic radiochemical gradients of plasma triglycerides. Compared with studies in the untreated animal, norethindrone acetate significantly reduced the arterial concentration (1.1 ± 0.1 vs. 0.7 ± 0.1 mM) and rate of splanchnic secretion of plasma triglyceride fatty acids (2.0 ± 0.4 vs. 0.8 ± 0.1 μmol/min·kg body wt$^{0.75}$) and decreased the percent of free fatty acids entering the splanchnic region that was converted to plasma triglycerides (22 ± 5 vs. $13 \pm 3\%$, $P < 0.05$). Ethynylestradiol, in the dose employed, had no significant effect on these variables; however, ethynylestradiol and norethindrone acetate together gave responses similar to norethindrone acetate alone. When the glucose was given intraduodenally vs. intravenously, values for splanchnic metabolism of triglycerides were unchanged. The hypolipemic effect of norethindrone acetate in glucose-fed swine was attributable to inhibition of hepatic triglyceride secretion.—**Wolfe, B. M., and D. M. Grace.** Norethindrone acetate inhibition of splanchnic triglyceride secretion in conscious glucose-fed swine. *J. Lipid Res.* 1979. **20:** 175–182.

Supplementary key words free fatty acids · glycogen · liver · metabolism · miniature swine · very low density lipoprotein

Myocardial infarction and cerebrovascular disease (2–7) have been associated with the use of oral contraceptive steroids. The hypertriglyceridemic effects of natural (8, 9) and synthetic (10, 11) sex hormones are of particular interest because hypertriglyceridemia may predispose to atherosclerosis (12, 13). Studies in postabsorptive women suggest that the rate of hepatic secretion of plasma TGFA is enhanced by estrogen (14), but is little affected by progestins (15). Avian hepatic TGFA secretion is increased by estrogen (16). However, the estrogen-induced elevation of plasma TGFA concentration in the rat may relate to either increased hepatic secretion (8) or impaired disposal of plasma VLDL TGFA (17). Norethindrone acetate has been reported to lower plasma TGFA in hypertriglyceridemic men and women (18); however, the lowering of plasma TGFA levels during the postpartum period in healthy women receiving this progestin may represent only a spontaneous return to basal level (19).

Since the fed or absorptive state comprises a major part of the day, there is a need for more studies of hormonal effects under these conditions. Miniature swine have been used successfully for metabolic studies in the glucose-fed state (20). The present studies were designed to elucidate the mechanisms whereby two commonly used synthetic contraceptive steroids, norethindrone acetate and ethynylestradiol (10, 11) produce changes in the concentration and transport of plasma TGFA in an unsedated animal. The same physiological conditions have permitted the study of splanchnic and hepatic carbohydrate and amino acid metabolism, the subject of a separate report.[2]

METHODS

Animal and surgical preparation

Seven female miniature swine weighing 30–58 kg and 9–24 months of age were studied (**Table 1**). None

Abbreviations: EE, ethynylestradiol; FFA, free fatty acids; NA, norethindrone acetate; TGFA, triglyceride fatty acid(s); VLDL, very low density lipoprotein(s).

[1] A preliminary report of this work has been published in abstract form (1).

[2] Wolfe, B. M., D. M. Grace, and E. B. Marliss. Effects of norethindrone acetate and ethynylestradiol on splanchnic carbohydrate and amino acid metabolism in conscious glucose-fed swine. Manuscript in preparation.

TABLE 1. Hemodynamic and other data for experimental swine

Treatment Period	No. of Swine	No. of Experiments	Weight[a]	Metabolic Body Size	Splanchnic Plasma Flow[b]
			kg	$kg^{0.75}$	$ml/min \cdot kg^{0.75}$
NA[c]	3	5[d]	42.3 ± 8.0	16.5 ± 2.3	42 ± 9
NA + EE[e]	3	5[d]	36.2 ± 2.9	14.7 ± 0.9	40 ± 2
EE[f]	3	4	46.8 ± 8.3	17.8 ± 2.4	34 ± 2
C[g]	5	9[h]	43.7 ± 4.9	16.9 ± 1.4	41 ± 5
ID[i]	3	3	47.9 ± 10.1	18.1 ± 2.8	49 ± 10

[a] Means ± SEM for swine in each group.
[b] Means ± SEM for all experiments in each group.
[c] NA received 100 μg norethindrone acetate/day·kg body $wt^{0.75}$ for at least 2 wk prior to each experiment. (See Experimental Procedures for details of dosage regimen).
[d] Two experiments in each of two swine, single experiment in the remainder.
[e] NA + EE received 100 μg norethindrone acetate/day·kg body $wt^{0.75}$ plus 5 μg ethynylestradiol/day·kg body $wt^{0.75}$ for at least 2 wk prior to each experiment.
[f] EE received 5 μg ethynylestradiol/day·kg body $wt^{0.75}$ for at least 2 wk prior to each experiment.
[g] Controls (untreated) received intravenous glucose (108 μmol/min·kg body $wt^{0.75}$).
[h] Three experiments in one swine, two in each of two other swine, and single experiments in the remainder.
[i] ID (untreated) received glucose intraduodenally instead of intravenously.

of the swine had been bred. Swine were maintained on approximately 36 g daily/kg body $wt^{0.75}$ of commercial pig starter consisting of a minimum of 17% crude protein and 3% crude fat and a maximum of 6% crude fiber, the remainder being carbohydrate. This diet met National Research Council minimum standards and allowed a weight gain of 1–2 kg per month.

Swine were prepared for surgery and indwelling carotid arterial and jugular, common portal and hepatic venous catheters were installed and maintained as previously described (20). Indwelling silicone rubber duodenal catheters were implanted 2 cm below the pylorus in the three swine that also received intraduodenal infusions of glucose.

Materials

[1-^{14}C]Palmitic acid (5.4 mCi/mmol) and [9,10-^{3}H]-palmitic acid (320 mCi/mol), certified over 98% pure, were obtained from New England Nuclear Corp., Boston, MA. Miniature swine were obtained from the Hormel Institute, Austin, MN, and later from the same strain maintained by Sinclair Research Farms, Columbia, MO. Pig starter was obtained from Master Feeds, Toronto, Ont. Porcine serum albumin, ethynylestradiol, and norethindrone acetate were obtained from Sigma Chemical Co., St. Louis, MO.

Experimental procedures

None of the animals was used for experiments until at least 1 wk after the catheters had been implanted and after consumption of three full feeds daily for at least 3 days (150% of the maintenance diet). However, the regular feed was withdrawn 8 hr before the constant infusion of glucose was started. The order of experiments was random. Each animal was used as its own control for studies with NA and/or EE; however, no contraceptive steroids were given for at least 4 wk before a control experiment. Prior to each study of splanchnic metabolism during administration of contraceptive steroids, the swine usually received a 2–3 wk course of either 100 μg NA, 5 μg EE, or 100 μg NA plus 5 μg EE/day·kg body $wt^{0.75}$. However, one swine received NA for 4 wk, another received EE for 10 wk, and a third received NA + EE for 5 wk (without any discernable effect related to more prolonged treatment). These doses of steroids were chosen because they were similar to the conventional doses used by women for contraceptive purposes prior to the introduction of low-dose estrogen preparations. The steroids were mixed with the morning feed. All studies involving contraceptive steroids and their controls were done during intravenous glucose administration. In addition, three swine were studied during similar prolonged (9 hr) constant intraduodenal infusions of glucose (in the absence of hormonal therapy). Two of these swine were studied under control conditions during intravenous glucose infusion. The control for the third swine given glucose intraduodenally was another female swine, of similar age and size, that received glucose intravenously. Experiments were performed on unsedated animals as previously described (20), except that the glucose (108 μmol/min·kg body $wt^{0.75}$), when given intravenously, the indocyanine green (1 mg/ml), and either the [1-^{14}C]palmitate or [9,10-^{3}H]palmitate (ca. 2 μCi/ml) bound to porcine

serum albumin (21) (molar ratio of FFA:albumin = 0.4) were infused into a jugular instead of a femoral vein. The glucose solution (1.23 ml/min) and the albumin-bound palmitate and indocyanine green (0.23 ml/min) were infused under sterile conditions. During experiments in which NA and/or EE were given, 9/24 of the daily dose was bound to 0.75 g of porcine serum albumin dissolved in 0.15 M sodium chloride and also infused intravenously at a constant rate (0.23 ml/min) for 9 hr. NA and EE were given intravenously on the day of the study because the animals did not receive their usual oral feeding on that day. Controls received albumin only.

The hydrophobic contraceptive steroids were bound to porcine albumin as follows. The dose of the steroid(s) required for a 9-hr study (for an average animal, 0.08 ml of a solution of NA dissolved in absolute ethanol to a concentration of 10 mg/ml and/or 0.04 ml of a solution of EE dissolved in absolute ethanol to a concentration of 1 mg/ml) was added to 20 ml of 0.15 M sodium chloride; the sodium chloride solution was heated to 50°C, mixed with 3.75 ml of 20% porcine albumin, and then purified by passage through a 0.8 μm diameter filter. Hepatic venous, portal venous (when available), and arterial blood samples were obtained simultaneously at approximately 20–30 min intervals during the last 3 hr of the glucose infusion. Samples of arterial blood were obtained for plasma cholesterol determinations 8 hr after the onset of the 9-hr glucose infusion.

Analyses and calculations

Analyses of blood glucose and of the content and radioactivity of FFA in plasma and of TGFA in plasma and plasma VLDL were performed as previously described (20). Since 14 hr had elapsed between the last regular feeding and the start of the sampling of blood for plasma TGFA, it was assumed that chylomicrons were not present. Acetoacetate and β-hydroxybutyrate were determined using enzymatic microfluorimetric techniques (22). Cholesterol was determined as previously described (23). Serum insulin was measured by a coated-charcoal radioimmunoassay (24) employing porcine insulin as standard, porcine monoiodinated [^{125}I]-labeled insulin as tracer, and an antiserum. Mean values for plasma insulin and blood glucose were based on three determinations during the last hour of the glucose infusion. Splanchnic (hepatic) plasma flow was determined by the method previously described (20). The general equations used for calculations have been described (20, 25). It was assumed, on the basis of our previous studies (20), that 50% of the blood entering the liver was derived from the hepatic artery and 50% from the portal vein. Average values for FFA and TGFA were derived from four to eight complete simultaneous sets of arterial, portal venous, and hepatic venous blood samples. Measurements of splanchnic plasma (or blood) flow were based on determination of indocyanine green in serum on three or four sets of arterial and hepatic venous blood samples. Splanchnic and hepatic secretion of TGFA, in particular, were both measured radiochemically. Mean values for each experiment were based on four to eight determinations of transsplanchnic or transhepatic radiochemical concentration gradients for labeled FFA and labeled TGFA. The following formula was used for the calculation: Secretion of TGFA (or VLDL TGFA) from liver (or splanchnic region) = [hepatic (splanchnic) secretion of TGFA (or VLDL TGFA) (cpm/ml plasma)/ hepatic (splanchnic) uptake of labeled FFA (cpm/ml plasma)] ×

$$\left[\left(SA_{FFA\ \frac{a+h}{2}}\right)_{\bar{x}} \Big/ (SA_{VLDL\ TGFA})_{\bar{x}}\right]$$

× hepatic (splanchnic) uptake of FFA (μmol/min), where $(SA_{VLDL\ TGFA})_{\bar{x}}$ is the mean specific activity of TGFA of arterial VLDL and

$$\left(SA_{FFA\ \frac{a+h}{2}}\right)_{\bar{x}}$$

is the mean value for specific activity of FFA in arterial and hepatic venous blood plasma during the terminal period when values for this ratio reached a plateau (20).

Differences between groups of experiments were evaluated according to Snedecor and Cochran for both paired and unpaired samples (26). Kg body wt$^{0.75}$ was used as a unit of body size (20, 27) to facilitate comparisons among species differing widely in size. The terminology used in this report was generally as recommended (28) and recently summarized (29); however, splanchnic secretion of TGFA was used instead of "splanchnic net inflow transport of TGFA".

RESULTS

Hemodynamic data

Values for splanchnic plasma flow were similar during treatment and control periods (Table 1). Likewise, there was no significant difference in the values for flow between studies in which glucose was given intraduodenally ($n = 3$) vs. control studies ($n = 3$) in which it was given intravenously (49 ± 10 vs. 40 ± 3/ min·kg body wt$^{0.75}$, respectively, $P > 0.5$). Flow did not change systematically during the period of blood

TABLE 2. Arterial concentrations of metabolites[a]

Comparison of Treatment Periods	Period	No. of Experiments	Blood Glucose	Plasma		
				FFA	TGFA	VLDL TGFA
				μmol/ml		
NA vs. C	NA	5	4.5 ± 0.3	0.115 ± 0.012	0.73 ± 0.12[b]	0.53 ± 0.12[b]
	C	5	4.9 ± 0.5	0.115 ± 0.014	1.11 ± 0.09	0.95 ± 0.14
NA + EE vs. C	NA + EE	5	4.3 ± 0.2	0.086 ± 0.015	0.61 ± 0.10[b]	0.47 ± 0.11[b]
	C	5	5.0 ± 0.5	0.095 ± 0.020	1.27 ± 0.33	0.88 ± 0.20
EE vs. C	EE	4	5.2 ± 0.5	0.102 ± 0.012	0.97 ± 0.14	0.73 ± 0.17
	C	4	4.3 ± 0.3	0.115 ± 0.016	0.98 ± 0.19	0.91 ± 0.20
ID vs. IV	ID	3	4.1 ± 0.4	0.083 ± 0.019	0.79 ± 0.23	0.54 ± 0.14
	IV	3	4.6 ± 0.3	0.099 ± 0.031	0.90 ± 0.19	0.77 ± 0.27

[a] Means ± SEM. Mean values for individual experiments were derived from four to eight samples.
[b] Significantly different from control period, $P < 0.05$.

sampling from 6 to 9 hr after starting the hypercaloric infusions of glucose.

Arterial concentrations

Mean concentrations of blood glucose and plasma FFA during the period 6–9 hr after starting the glucose infusions were in the expected range for glucose-fed swine (20). Mean concentrations of blood glucose in fed swine during treatment with NA or NA + EE were slightly, but not significantly, lower than control (4.5 ± 0.3 vs. 4.9 ± 0.5 and 4.3 ± 0.2 vs. 5.0 ± 0.5 mM, respectively, $P > 0.5$, **Table 2**). By contrast, mean arterial plasma insulin levels were significantly lower than controls during treatment with either NA (14 ± 2 vs. 41 ± 12 μU/ml, $P < 0.05$) or NA + EE (13 ± 2 vs. 28 ± 6 μU/ml, $P < 0.05$).[3]

Concentrations of plasma TGFA and VLDL TGFA during treatment with NA or NA + EE were significantly lower than control. Although the mean arterial plasma levels of TGFA and VLDL TGFA were slightly lower during intraduodenal vs. intravenous glucose, the difference was not significant ($P > 0.2$). The mean arterial concentration of acetoacetate during NA + EE was similar to control (49 ± 5 vs. 73 ± 29 mM) as was that of β-hydroxybutyrate (16 ± 1 vs. 21 ± 4 mM, respectively, $n = 3$ per group).

Metabolism of FFA

Values for rates of net inflow transport, splanchnic extraction fraction (radiochemical), and splanchnic uptake of FFA were similar during treatment and control periods (**Table 3**). There was also no difference in FFA metabolism between studies in which glucose was given intraduodenally vs. intravenously. Total splanchnic production of ketone bodies was small and could account for at most 22 ± 5% of FFA uptake during administration of NA + EE vs. 12 ± 3% in controls ($n = 3$ per group). Splanchnic production of acetoacetate during treatment with NA (0.8 ± 0.1 μmol/min·kg body $wt^{0.75}$) was similar to NA + EE (1.0 ± 0.1 μmol/min·kg body $wt^{0.75}$); however, NA + EE gave significantly higher values than controls (0.4 ± 0.1 μmol/min·kg body $wt^{0.75}$, $P < 0.05$). Splanchnic production of β-hydroxybutyrate during NA + EE was similar to control (0.3 ± 0.1 vs. 0.2 ± 0.1 μmol/min·kg body $wt^{0.75}$, respectively).

Secretion of plasma TGFA

In control experiments, an average of about one-fifth of the FFA removed by the splanchnic region was secreted as TGFA into hepatic venous plasma (**Table 4**). Treatment with either NA or NA + EE significantly reduced this fraction. By contrast to the experiments in which NA was given, EE alone had no significant effect on splanchnic conversion of FFA to plasma TGFA ($P > 0.6$).

As in previous studies in glucose-fed miniature swine (20), values for the ratio of specific activity of arterial VLDL TGFA to estimated specific activity of FFA entering liver reached a plateau within 180 min of starting the infusion of radioactive palmitate. Only a small fraction of plasma VLDL TGFA was derived from FFA; this was unaffected by NA and/or EE or by the route of glucose administration. Most of the VLDL TGFA (73–89%) were derived from precursors other than circulating FFA (released from adipose tissue stores and other sources).

Total splanchnic secretion of plasma TGFA fell significantly in response to treatment with either NA or NA + EE ($P < 0.05$, Table 4); by contrast, EE alone had no significant effect ($0.3 > P > 0.4$). Two mechanisms can explain the decreased secretion of TGFA. During NA therapy there was a major reduction in splanchnic secretion of plasma TGFA derived from precursors other than FFA, as compared to matched

[3] Wolfe, B. M., and E. B. Marliss. Unpublished observations.

TABLE 3. Metabolism of free fatty acids[a]

Comparison of Treatment Periods	Period	No. of Experiments	Net Inflow Transport	Splanchnic		Splanchnic Uptake/ Net Inflow Transport
				Extraction Fraction[b]	Uptake	
			$\mu mol/min \cdot kg^{0.75}$		$\mu mol/min \cdot kg^{0.75}$	
NA vs. C	NA	5	8.0 ± 2.3	0.35 ± 0.05	2.26 ± 0.94	0.25 ± 0.04
	C	5	7.0 ± 1.0	0.33 ± 0.06	1.70 ± 0.29	0.25 ± 0.04
NA + EE vs. C	NA + EE	5	5.1 ± 0.7	0.34 ± 0.03	1.41 ± 0.17	0.28 ± 0.02
	C	5	5.3 ± 1.1	0.32 ± 0.05	1.56 ± 0.36	0.29 ± 0.02
EE vs. C	EE	4	5.6 ± 0.3	0.45 ± 0.02	2.03 ± 0.38	0.36 ± 0.05
	C	4	7.3 ± 0.8	0.38 ± 0.07	2.13 ± 0.49	0.28 ± 0.05
ID vs. IV	ID	3	5.2 ± 1.3	0.35 ± 0.03	1.52 ± 0.19	0.31 ± 0.04
	IV	3	5.2 ± 1.6	0.34 ± 0.07	1.59 ± 0.53	0.30 ± 0.03

[a] Means ± SEM. Mean values for individual experiments were derived from four to eight sets of samples of arterial and hepatic venous blood obtained over 3 hr.
[b] Based on extraction of radioactive palmitate.

control experiments (0.63 ± 0.14 vs. 1.68 ± 0.39 μmol/min·kg body $wt^{0.75}$, respectively, $P < 0.05$), and a smaller decrease in the secretion of TGFA that were derived from FFA (0.20 ± 0.06 vs. 0.32 ± 0.16 μmol/min ·kg body $wt^{0.75}$ in control experiments, $0.1 > P > 0.2$). The decrease in splanchnic secretion of TGFA during combined therapy with NA + EE was due to significant decreases in both the secretion of TGFA derived from FFA (0.10 ± 0.03 vs 0.34 ± 0.10 μmol/min ·kg body $wt^{0.75}$) and of TGFA derived from non-FFA precursors (0.58 ± 0.22 vs 1.51 ± 0.32 μmol/min·kg body $wt^{0.75}$, $P < 0.05$).

In ten experiments, portal venous blood was also sampled so that it was possible to calculate both hepatic and splanchnic secretion of plasma TGFA. Splanchnic secretion of TGFA under control conditions averaged 91 ± 5% of hepatic secretion ($n = 4$, $0.1 > P > 0.2$); during administration of the contraceptive steroids the mean value was 96 ± 5% ($n = 6$, two in each group, $P > 0.4$).

Arterial plasma cholesterol levels were measured after 8 hr of glucose infusion in control experiments as well as in those in which NA and/or EE was given. There were insignificant decreases in cholesterol levels below control values during administration of NA in three of four swine given EE (61 ± 7 vs. 67 ± 6 mg/dl,

TABLE 4. Splanchnic metabolism of plasma TGFA[a]

Comparison of Treatment Periods	Period	No. of Experiments	Secretion of Labeled TGFA/ Uptake of Labeled FFA	$SA_{VLDL\ TGFA}$[b]/ $\left(SA_{FFA\ \frac{a+h}{2}}\right)$[c]	Secretion of TGFA[d]
					$\mu mol/min \cdot kg^{0.75}$
NA vs. C	NA	5	0.13 ± 0.03[e]	0.265 ± 0.086	0.8 ± 0.1[e,f]
	C	5	0.22 ± 0.05	0.189 ± 0.041	2.0 ± 0.4
NA + EE vs. C	NA + EE	5	0.07 ± 0.02[e]	0.140 ± 0.035	0.7 ± 0.3[e,f]
	C	5	0.18 ± 0.03	0.140 ± 0.033	1.9 ± 0.4
EE vs. C	EE	4	0.19 ± 0.03	0.154 ± 0.024	1.9 ± 0.3
	C	4	0.28 ± 0.05	0.178 ± 0.041	2.6 ± 0.4
ID vs. IV	ID	3	0.17 ± 0.03	0.154 ± 0.047	1.7 ± 0.4
	IV	3	0.14 ± 0.04	0.112 ± 0.029	1.8 ± 0.7

[a] Means ± SEM derived from four to eight simultaneous sets of arterial and hepatic venous blood samples for each experiment.
[b] Specific activity of arterial VLDL TGFA relative to the mean specific activity of arterial and hepatic venous FFA during the time interval when the value for this ratio was constant (after 180 min infusion of the albumin–palmitate complex).
[c] Value based on average specific activity of FFA of arterial and hepatic venous blood plasma.
[d] Value for each experiment based on four to eight determinations of transsplanchnic radiochemical gradients of TGFA (see Methods).
[e] Significantly different from control period, $P < 0.05$.
[f] Significantly different from EE, $P < 0.05$.

$0.4 > P > 0.5$), and four of five swine given NA + EE (52 ± 6 vs. 64 ± 2 mg/dl, $0.1 < P < 0.2$). Cholesterol levels in glucose-fed animals during treatment with NA alone were similar to control values (65 ± 4 vs. 66 ± 3 mg/dl, respectively).

DISCUSSION

The present studies demonstrate that norethindrone acetate markedly lowers the concentration and rate of transport of plasma TGFA in glucose-fed swine. The studies were performed in a nutritional setting that would be expected to promote high rates of TGFA synthesis from carbohydrate and high rates of TGFA transport, with low rates of FFA mobilization. Under these conditions, the potential inhibitory effects of an agent on hepatic TGFA synthesis from carbohydrate precursors might be more readily demonstrable than in the fasting state when lipogenesis is low.

Metabolism of FFA

Values for net inflow transport and splanchnic uptake of FFA in the present study were based on the constant infusion of albumin-bound radioactive palmitate. For technical reasons, they may be slightly high even though they are 5-fold lower than values calculated for swine fed ad lib (30).[4]

Neither plasma concentration (Table 2) nor other measures of transport of FFA (Table 3) were significantly influenced by EE and/or NA, in accord with findings in women receiving a variety of oral contraceptive steroids (31) and in oöphorectomized women given estradiol benzoate or progesterone (32). By contrast to NA, progesterone has been reported to elevate insulin levels in both fed and fasted rats; however, FFA levels were unaffected (8). FFA transport was unaffected despite the lower serum insulin concentrations during treatment with NA or NA + EE. This may relate to the potent antilipolytic effect of even low concentrations of insulin (33). The failure of plasma glucose levels to change despite the lower insulin levels during NA treatment suggests that fewer insulin molecules achieved the same hormonal ends. Thus NA treatment may have resulted in an alteration in the insulin–receptor interaction which was opposite in direction to that occurring in insulin resistance (34). By contrast, estradiol benzoate has been reported to elevate plasma FFA levels in fasted rats (8, 35).

Metabolism of TGFA

The mean fraction of FFA removed by the splanchnic region that appeared in hepatic venous TGFA was significantly reduced by either NA or NA + EE (Table 4). Since only a minor portion of the secreted TGFA was derived from FFA (as indicated by the ratio

$$SA_{VLDL\ TGFA}/SA_{FFA\ \frac{a+h}{2}}$$

the quantitative effect of this change in the handling of FFA on overall total TGFA secretion was small. However, the inhibitory effect of NA or NA + EE on secretion of TGFA derived from non-FFA precursors was much larger (see Results).

The marked inhibition of splanchnic secretion of TGFA by NA or NA + EE in the present studies is in striking contrast to findings in other species that have been treated with various progestational and androgenic steroids. A large dose of progesterone (16,000 μg/day·kg body $wt^{0.75}$) was found not to influence entry of TGFA into blood plasma of the rat (8). Likewise, the triglyceride-lowering androgenic steroid oxandrolone has been found not to significantly influence entry of VLDL TGFA into human blood plasma (36). NA causes an increase in postheparin lipolytic activity concurrent with the lowering of plasma triglycerides in man (18); however, it is not known whether this increased postheparin plasma lipolytic activity reflects increased hepatic lipase (as in the case of oxandrolone) (37) or increased lipoprotein lipase activity. It is unnecessary to postulate an increase in lipoprotein lipase activity in glucose-fed swine during NA treatment because splanchnic secretion and disposal of plasma TGFA declined together.

The metabolic explanation for the NA-induced reduction of splanchnic secretion of TGFA derived from precursors other than FFA likely relates to inhibition of one or more steps in the synthesis of triglycerides from their precursors. Ultrastructural changes, such as depletion of glycogen granules, have been reported to occur in the livers of baboons treated

[4] In two studies under control conditions and in three during administration of EE, [9,10-^{3}H]palmitic acid complexed to porcine serum albumin with a lower molar ratio (FFA:albumin = 0.2) was infused simultaneously with [1-^{14}C]palmitic acid similarly complexed with the higher molar ratio (FFA:albumin = 0.4) that was generally used in these studies. The lower FFA:albumin ratio gave significantly lower values for total net inflow transport of FFA (mean 47 ± 3% of respective values using the higher ratio of FFA:albumin) and splanchnic uptake of FFA (mean 45 ± 3%, respectively); however, the mean value for the ratio of specific activity of arterial VLDL TGFA relative to the estimated specific activity of FFA entering liver was also proportionately lower (mean 45 ± 3%, $P < 0.01$). The lower values for the ratio:

$$SA_{VLDL\ TGFA}/S_{FFA\ \frac{a+h}{2}},$$

with the lower palmitate:albumin ratio compensated for the lower splanchnic uptake of FFA so that the respective values for splanchnic secretion of TGFA averaged 95 ± 14% of those obtained using the higher FFA:albumin ratio ($P > 0.4$).

with norethindrone (38). Indirect evidence suggests that liver glycogen may be a precursor of plasma TGFA in the glucose-fed state (20, 29). It is tempting to speculate that the lower serum insulin levels during NA or NA + EE may play a role in both the decreased hepatic secretion of plasma TGFA and the increased acetoacetate production. Failure of β-hydroxybutyrate production to increase during NA administration may reflect a shift in the redox potential of the mitochondria to a more oxidized state. Such a change is thought to occur in fasted human subjects during clofibrate treatment (39). Insulin deficiency depresses hepatic lipogenesis (40, 41) while enhancing ketone body production (22). However, it remains uncertain as to whether an excess of insulin beyond a certain point stimulates normal lipogenesis.

Although administration of the conventional dose of EE to glucose-fed miniature swine failed to increase plasma levels or hepatic secretion of TGFA and failed to increase either the incorporation of FFA into plasma TGFA or the fraction of plasma VLDL TGFA derived from precursors other than FFA (as an indication of an effect on de novo synthesis of fatty acids in liver), the present studies do not constitute a proof for the lack of effect of EE. Further studies involving perhaps different diets and dose levels of EE will be required to evaluate this matter. EE has been reported to increase the incorporation of oleate into hepatic and perfusate triglyceride in the livers of fasted rats (42), whereas diethylstilbesterol increases triglyceride production in chick livers (16). Stimulation of de novo synthesis of VLDL has been reported with 17-β estradiol using rooster livers (9) and with diethylstilbesterol using chick livers (16). Intramuscular injection of 17 μg of estradiol benzoate/day·kg body $wt^{0.75}$ has also been reported to enhance TGFA entry into the blood plasma in fed rats (8). Postmenopausal women studied in the postabsorptive state while receiving conjugated equine estrogens have also been found to have a marked increase in VLDL TGFA production rate (14), as have healthy young women given various combinations of oral contraceptive steroids (15). Since we did not study incorporation of precursors into hepatic lipids, we can not exclude the possibility that EE might have stimulated lipogenesis, but that VLDL secretory rates were limiting to TGFA secretion. It is conceivable that rates of VLDL TGFA secretion were already maximal under control conditions of hypercaloric glucose infusion and that any possible effect of EE on hepatic lipogenesis could therefore not be manifested in increased secretion.

The similarity of hepatic metabolism of FFA and TGFA during intraduodenal and intravenous glucose infusion suggests that the latter route of substrate administration yields physiologically valid information. No evidence was obtained in the swine to support the notion that the intestinal mucosa plays a major role in carbohydrate-induced hypertriglyceridemia (43). The present studies of lipid transport were performed using conventional doses of NA and/or EE (10, 11) under physiological conditions of glucose feeding. Similar studies are planned to explore the metabolic effects of these agents in the fed state in women. The lowering of plasma cholesterol levels in three of four glucose-fed swine during treatment with EE (although statistically not significant) is in accord with findings in fasted human subjects (32, 44).

We are grateful to A. Elworthy, T. Huang, Y. Kang, C. Naus, and B. Tan for expert technical assistance and to E. Wolfe who assisted with all aspects of this investigation. This work was supported by a grant from the Ontario Heart Foundation (3–16).

Received 7 September 1977 and in revised form 21 April 1978; accepted 2 August 1978.

REFERENCES

1. Wolfe, B., and M. Grace. 1976. Inhibition of triglyceride secretion by norethindrone acetate in conscious glucose-fed swine. *Circulation.* **54:** 211.
2. Radford, D. J., and M. F. Oliver. 1973. Oral contraceptives and myocardial infarction. *Br. Med. J.* **3:** 428–430.
3. Maleki, M., and R. L. Lange. 1973. Coronary thrombosis in young women on oral contraceptives: Report of two cases and review of the literature. *Am. Heart J.* **85:** 749–754.
4. Mann, J. I., M. P. Vessey, M. Thorogood, and Sir R. Doll. 1975. Myocardial infarction in young women with special reference to oral contraceptive practice. *Br. Med. J.* **2:** 241–245.
5. Collaborative Group for the Study of Stroke in Young Women. 1973. Oral contraception and increased risk of cerebral ischemia or thrombosis. *N. Engl. J. Med.* **288:** 871–878.
6. Collaborative Group for the Study of Stroke in Young Women. 1975. Oral contraceptives and stroke in young women. Associated risk factors. *J. Am. Med. Assoc.* **231:** 718–722.
7. de Gennes, J. L., G. Turpin, and J. Truffert. 1976. Vascular disease and oral contraceptives: Familial hyperlipoproteinaemia as a predisposing factor. *Diabete Metab.* **2:** 81–86.
8. Kim, H-J., and R. K. Kalkhoff. 1975. Sex steroid influence on triglyceride metabolism. *J. Clin. Invest.* **56:** 888–896.
9. Luskey, K. L., M. S. Brown, and J. L. Goldstein. 1974. Stimulation of the synthesis of very low density lipoproteins in rooster liver by estradiol. *J. Biol. Chem.* **249:** 5939–5947.
10. Stokes, T., and V. Wynn. 1971. Serum-lipids in women on oral contraceptives. *Lancet.* **2:** 677–680.
11. Kekki, M., and E. A. Nikkilä. 1971. Plasma triglyceride turnover during use of oral contraceptives. *Metabolism.* **20:** 878–889.

12. Goldstein, J. L., W. R. Hazzard, H. G. Schrott, E. L. Bierman, and A. G. Motulsky. 1973. *J. Clin. Invest.* **52:** 1533–1543.
13. Carlson, L. A., and L. E. Böttiger. 1972. Ischaemic heart-disease in relation to fasting values of plasma triglycerides and cholesterol. *Lancet.* **1:** 865–868.
14. Glueck, C. J., R. W. Fallat, and D. Scheel. 1975. Effects of estrogenic compounds on triglyceride kinetics. *Metabolism.* **24:** 537–545.
15. Fallat, R., and C. J. Glueck. 1974. Effects of anabolic and progestational agents upon triglycerides and triglyceride kinetics in normals and hyperlipemic patients. *Lipids.* **9:** 117–119.
16. Kudzma, D. J., F. St. Claire, L. DeLallo, and S. J. Friedberg. 1975. Mechanism of avian estrogen-induced hypertriglyceridemia: evidence for overproduction of triglyceride. *J. Lipid Res.* **16:** 123–133.
17. Hamosh, M., and P. Hamosh. 1975. The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. *J. Clin. Invest.* **55:** 1132–1135.
18. Glueck, C. J., R. I. Levy, and D. S. Fredrickson. 1971. Norethindrone acetate, postheparin lipolytic activity, and plasma triglycerides in familial types I, III, IV, and V hyperlipoproteinemia. *Ann. Int. Med.* **75:** 345–352.
19. Spellacy, W. M., W. C. Buhi, S. A. Birk, and S. A. McCreary. 1973. Metabolic studies in women taking norethindrone for 6 months' time (measurements of blood glucose, insulin, and triglyceride concentrations). *Fertil. Steril.* **24:** 419–425.
20. Wolfe, B. M., and L. W. Belbeck. 1975. Splanchnic and hepatic triglyceride secretion during hypercaloric intravenous glucose infusion in conscious swine. *J. Lipid Res.* **16:** 19–27.
21. Felts, J. M., and E. J. Masoro. 1959. Effects of cold acclimatization on hepatic carbohydrate and lipid metabolism. *Am. J. Physiol.* **197:** 34–36.
22. Basso, L. V., and R. J. Havel. 1970. Hepatic metabolism of free fatty acids in normal and diabetic dogs. *J. Clin. Invest.* **49:** 537–547.
23. Sperry, W. M., and M. Webb. 1950. A revision of the Schoenheimer–Sperry method for cholesterol determination. *J. Biol. Chem.* **187:** 97–106.
24. Herbert, V., K-S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay for insulin. *J. Clin. Endocrinol. Metab.* **25:** 1375–1384.
25. Havel, R. J., J. P. Kane, E. O. Balasse, N. Segal, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49:** 2017–2035.
26. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th ed. 114.
27. Kleiber, M. 1960. The Fire of Life. John Wiley and Sons, New York. 200.
28. Brownell, G. L., M. Berman, and J. S. Robertson. 1968. Nomenclature for tracer kinetics. *Int. J. Appl. Radiat. Isot.* **19:** 249–262.
29. Wolfe, B. M., and S. P. Ahuja. 1977. Effects of intravenously administered fructose and glucose on splanchnic secretion of plasma triglycerides in hypertriglyceridemic men. *Metabolism.* **26:** 963–977.
30. Freeman, C. P., D. E. Noakes, and E. F. Annison. 1970. The metabolism of glucose, acetate, palmitate, stearate and oleate in pigs. *Br. J. Nutr.* **24:** 705–716.
31. Wynn, V., and J. W. H. Doar. 1969. Longitudinal studies of the effects of oral contraceptive therapy on plasma glucose, non-esterified fatty acid, insulin and blood pyruvate levels during oral and intravenous glucose tolerance tests. *In* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids. H. A. Salhanick, D. M. Kipnis, and R. L. Vande Viele, editors. Plenum Press, New York, N. Y. 157–177.
32. Svanborg, A., and O. Vikrot. 1966. The effect of estradiol and progesterone on plasma lipids in oophorectomized women. *Acta Med. Scand.* **179:** 615–622.
33. Pozefsky, T., P. Felig, J. D. Tobin, J. S. Soeldner, and G. F. Chaill, Jr. 1969. Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. *J. Clin. Invest.* **48:** 2273–2282.
34. Kahn, C. R., J. S. Flier, R. S. Bar, J. A. Archer, P. Gorden, M. M. Martin, and J. Roth. 1976. The syndromes of insulin resistance and acanthosis nigricans. Insulin–receptor disorders in man. *N. Engl. J. Med.* **294:** 739–745.
35. Laron, Z., and A. Kowadlo-Silbergeld. 1965. Fat mobilising effect of oestrogens. *Acta Endocrinol.* **48:** 125–131.
36. Glueck, C. J., S. Ford, Jr., P. Steiner, and R. Fallat. 1973. Triglyceride removal efficiency and lipoprotein lipases: Effects of oxandrolone. *Metabolism.* **22:** 807–814.
37. Ehnholm, C., J. K. Huttunen, P. J. Kinnunen, T. A. Miettinen, and E. A. Nikkilä. 1975. Effect of oxandrolone treatment on the activity of lipoprotein lipase, hepatic lipase and phospholipase A_1 of human postheparin plasma. *N. Engl. J. Med.* **292:** 1314–1317.
38. Chai, L. S., J. G. Colson, R. Y. Kirdani, and A. A. Sandberg. 1974. Ultrastructural study of hepatic effects of a contraceptive steroid in baboon. *Contraception.* **10:** 79–93.
39. Wolfe, B. M., J. P. Kane, R. J. Havel, and H. P. Brewster. 1973. Mechanisms of the hypolipemic effect of clofibrate in postabsorptive man. *J. Clin. Invest.* **52:** 2146–2159.
40. Chernick, S. S., and I. L. Chaikoff. 1950. Insulin and hepatic utilization of glucose for lipogenesis. *J. Biol. Chem.* **186:** 535–542.
41. Boden, G., and B. Willms. 1966. Einfluβ von Insulin auf Kohlenhydrat- und Fettstoffwechsel der perfundierten Leber bei normalen und Alloxan-diabetischen Ratten. *Klin. Wochenschr.* **44:** 579–583.
42. Weinstein, I., C. Soler-Argilaga, and M. Heimberg. 1977. Effects of ethynyl estradiol on incorporation of [1-^{14}C]oleate into triglyceride and ketone bodies by the liver. *Biochem. Pharmacol.* **26:** 77–80.
43. DenBesten, L., R. H. Reyna, W. E. Connor, and L. D. Stegink. 1973. The different effects on the serum lipids and fecal steroids of high carbohydrate diets given orally or intravenously. *J. Clin. Invest.* **52:** 1384–1393.
44. Oliver, M. G., and G. S. Boyd. 1961. Influence of reduction of serum lipids on prognosis of coronary heart-disease. A five-year study using oestrogen. *Lancet.* **2:** 499–505.