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Effects of Estradiol and Tamoxifen on Feeding, Fattiness, and Some Endocrine Criteria in Hypothalamic Obese Hens

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JACCOBY, S., E. ARNON, N. SNAPIR AND B. ROBINZON. *Effects of estradiol and tamoxifen on feeding, fattiness, and some endocrine criteria in hypothalamic obese hens.* PHARMACOL BIOCHEM BEHAV **50**(1) 55-63, 1995.—In White Leghorn hens, basomedial hypothalamic (BMH) lesions result in two syndromes: a) obese, functionally castrated (OFC) hens, in which both the ventromedial hypothalamic nucleus (VMH) and the mammillary nuclei are damaged and plasma estrogen is very low; and b) obese laying (OL) hens, which have normal levels of plasma estrogen and are less obese than the former, and whose lesion is limited to the VMH. In the present study, the involvement of estrogen in regulation of fattiness and energy metabolism was assayed in OFC, OL, and control (CONT) hens. BMH lesions were made at 13 weeks of age. When the typical syndromes reached the static phase, 20 weeks later, CONT, OFC, and OL hens were divided into three subgroups and were injected for 10 weeks on each alternate day, with either 10 mg tamoxifen (TAM)/kg, 2 mg estradiol benzoate (E_2)/kg, or the vehicle, corn oil (0.5 ml). E_2 raised plasma total lipids and reduced plasma glucose, insulin, and hematocrit in all treated hens, and increased liver weight in OL and OFC, but not in CONT hens. In OFC hens only, E_2 reduced food intake (FI) and fattiness. In OL and CONT hens, E_2 increased plasma T_3, but raised the resting metabolic rate (RMR) only in CONT ones. In OFC hens, E_2 reduce plasma T_3 and T_4 without affecting RMR. E_2 reduced comb weight and egg production in CONT and more severely in OL hens. In the latter, E_2 diminished ovarian and oviduct weights, whereas in OFC hens it increased the size of the atrophied oviduct. TAM had no visible effect on OFC hens. However, in CONT and OL pullets, TAM decreased plasma total lipids, FI, liver, and ovarian and oviduct weights, abolished egg production, increased plasma glucose, insulin, T_3 and T_4, RMR, hematocrit, and comb weight, but had no effect on fattiness and body weight. It is well established that estrogen increases fattiness in cockerels and juvenile pullets. However, in adult hens in the present study, estrogen and its antagonist had no effect on fattiness in the laying ones, and even reduced body fat in the OFC hens. These results suggest that in hens, E_2 effects on fattiness alter with age. As E_2 increased plasma lipids in all hens, it may be assumed that in adult hens E_2 reduces fat deposition in depots to increase its availability for yolk production.

Estrogen Antiestrogen Thyroxin Triiodothyronine Insulin Resting metabolic rate Chicken Egg laying Functional castration

BASOMEDIAL hypothalamic lesions in 3-month-old White Leghorn (WL) hens produced two main sets of of hens with symptoms (18,19): a) obese, functionally castrated (OFC); and b) obese, laying (OL). After the hypothalamic lesion, the OFC hens developed transient hyperphagia, which was followed by hypophagia; they gained weight in both periods and became very obese. The OFC hens had high hematocrit values and atrophied ovary, oviduct, comb, and adenohypophysis. In these hens, plasma estrogen, androgen, and total lipids, as well as liver weight, were lower than in controls. The lesioned area included the ventromedial hypothalamic nucleus (VMH), mammillary nuclei (MN), arcuate nuclei (AN), and tuberal nucleus. The OL hens manifested transient hyperphagia that subsided into normophagia with the development of obesity. These hens were less obese than the OFC ones and showed normal reproductive traits. Their hypothalamic lesion was limited to the VMH.

In juvenile hens and cockerels before and after puberty, estrogen increases food intake (FI), liver lipogenesis, and fattiness (1,17,26,42). In juvenile hens estrogen delays the onset of

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egg production (9). Tamoxifen (TAM), a nonsteroidal antiestrogen, acts as a pure estrogen antagonist in chickens (24,46). Long-term administration of low doses of TAM advances the onset of egg laying in juvenile hens (20). However, at high doses, TAM delays the onset of laying and decreases liver lipogenesis and fattiness (20).

Obese, functionally castrated hens are fattier than OL and control hens, although their plasma estrogen is very low. These results are in conflict with the fattening role suggested for estrogen in chickens. There is no documentation regarding the role of estrogen and its antagonist in the regulation of feeding and fattiness in adult laying hens or in functionally castrated hens; we have studied this role of estrogen and report our findings here.

METHODS

Animals

Three-month-old WL hens were kept in individual cages and were fed (Commercial Breeder and Layers Mash, Matmor, Ashdod, Israel) and watered ad lib. They were subjected to 14 h light daily. Hypothalamic surgery was performed in 60 hens, and 24 were kept intact as controls (CONT).

Surgery and Injection Regimens

The hens were anesthetized by IV injection of 0.4–0.5 ml of 6% sodium pentobarbital solution (Nembutal, Abbot-Ceva, France) inserted into a specially designed stereotaxic instrument described elsewhere (25,41,43). Electrodes were aimed to the BMH area using the technique and coordinates described in the X-ray atlas of the chicken diencephalon (43). After verification of the electrode site (43), 3 mA anodal direct current was passed through the electrode for 20 s. This was repeated for the contralateral side, so that a bilateral lesion was produced. After recovery from anesthesia, the hens were returned to their home cage. A total of 18 hens showed the OFC syndrome and 24 showed OL. In the rest of the lesioned hens no change in any of the measured parameters was observed (18); these were excluded from the study.

At 33 weeks of age, when the static phase was well established in all OFC and OL hens, these and the CONT hens were divided into three groups (eight hens/group in the OL and CONT, six hens/group in the OFC). They were injected IM on alternate days with either 10 mg TAM/kg (20), 2 mg estradiol benzoate (E_2)/kg, or the vehicle, corn oil (0.5 ml). TAM and E_2 were obtained from Sigma Chemicals Co. (St Louis, MO). Injections regimen continued for 10 weeks, and then the experiment was terminated.

Procedure

Individual egg laying and FI were recorded daily. The resting metabolic rate (RMR) was determined at 42–43 weeks of age according to the methods describe elsewhere (30).

Heparinized blood samples were obtained from the jugular vein at 43 weeks of age, and hematocrit was measured. The samples were centrifuged, and plasma was stored at $-20°C$ pending determination of metabolites and hormone levels. Plasma total lipids were determined according to the methods of Zollner and Kirsch (49), and plasma glucose by the hexokinase method.

At the end of the experiment (43 weeks of age), the birds were killed by cervical dislocation and autopsy was performed. The brains were immediately removed and fixed in 10% neutral buffered formalin. The abdominal adipose tissue (AAT), liver, comb, ovary, and oviduct were immediately removed, cleaned from adhering tissues, and weighed.

Serial frozen frontal brain sections, each 25 μm in thickness, were prepared and stained with thionine (6). The sections were examined for localization of the lesion site.

Radioimmunoassays

Plasma insulin was determined using a Sorin Biomedica kit (anti-guinea pig-labeled porcine) (St Quentin Yvelines, France). Chicken insulin (Litron Laboratory, Rochester, NY) was used as a standard, according to the method described elsewhere (40). Plasma T_3 and T_4 were determined using a radioimmunoassay kit supplied by Diagnostic Products Corporation (Los Angeles, CA).

Statistical evaluation of the data was made using analysis of variance and Duncan's Multiple Range test (35).

RESULTS

After hypothalamic surgery all OFC hens developed marked hyperphagia, which subsided and turned into hypophagia after 9–10 weeks. The OL hens developed a moderate hyperphagia that turned into normophagia at about that time (Fig. 1a).

Administration of TAM reduced FI in the CONT and OL hens, but not in the OFC ones (Fig. 1b–d). Supplementation of E_2, however, reduced FI in the OFC, but not in OL and CONT hens (Fig. 1b–d).

The onset of egg laying was delayed in the OL hens, whereas OFC ones did not lay at all (Fig. 2a). TAM ceased, whereas E_2 reduced egg laying in CONT and OL hens (Fig. 2b and c). These effects were more drastic in the OL than in the CONT pullets.

At 43 weeks of age, the body weight (BW) and AAT of the OL and OFC hens were greater than those of the CONT ones (Table 1). The RMR was similar in the OL and OFC hens and lower than in the CONT ones (Table 1). Liver weight was significantly higher in the OL than in the OFC hens. The weights of the ovary, oviduct, and comb of the OL were similar to those of the CONT hens. These organs, however, were involuted in the OFC hens (Table 1). In the latter, hematocrit values were significantly higher and plasma total lipids levels were lower than in the OL and CONT hens (Table 1). Plasma glucose and insulin were similar in these groups, whereas plasma T_3 and T_4 were elevated only in the OFC hens (Table 1).

Figure 3 shows the location of the largest and the smallest brain lesions of the OL and OFC hens. In OFC hens, the lesion included the VMH, MN, and AN. In the OL hens the bilateral lesion was limited to the VMH and ventral section of the MN.

In the CONT groups, TAM (CONT-TAM) or E_2 (CONT-E_2) administration had no effect on BW and AAT weight. Both treatments, however, increased the RMR (Table 2). In the CONT-TAM hens, plasma total lipids, liver, ovary and oviduct weights were reduced, while plasma insulin, glucose, T_3, T_4, and hematocrit values were increased (Table 2). In the CONT-E_2 hens, the reproductive organs were not affected by the treatment. Plasma total lipids and T_3 levels were increased, whereas plasma insulin and glucose and hematocrit values were reduced in this group (Table 2). Comb weight in the

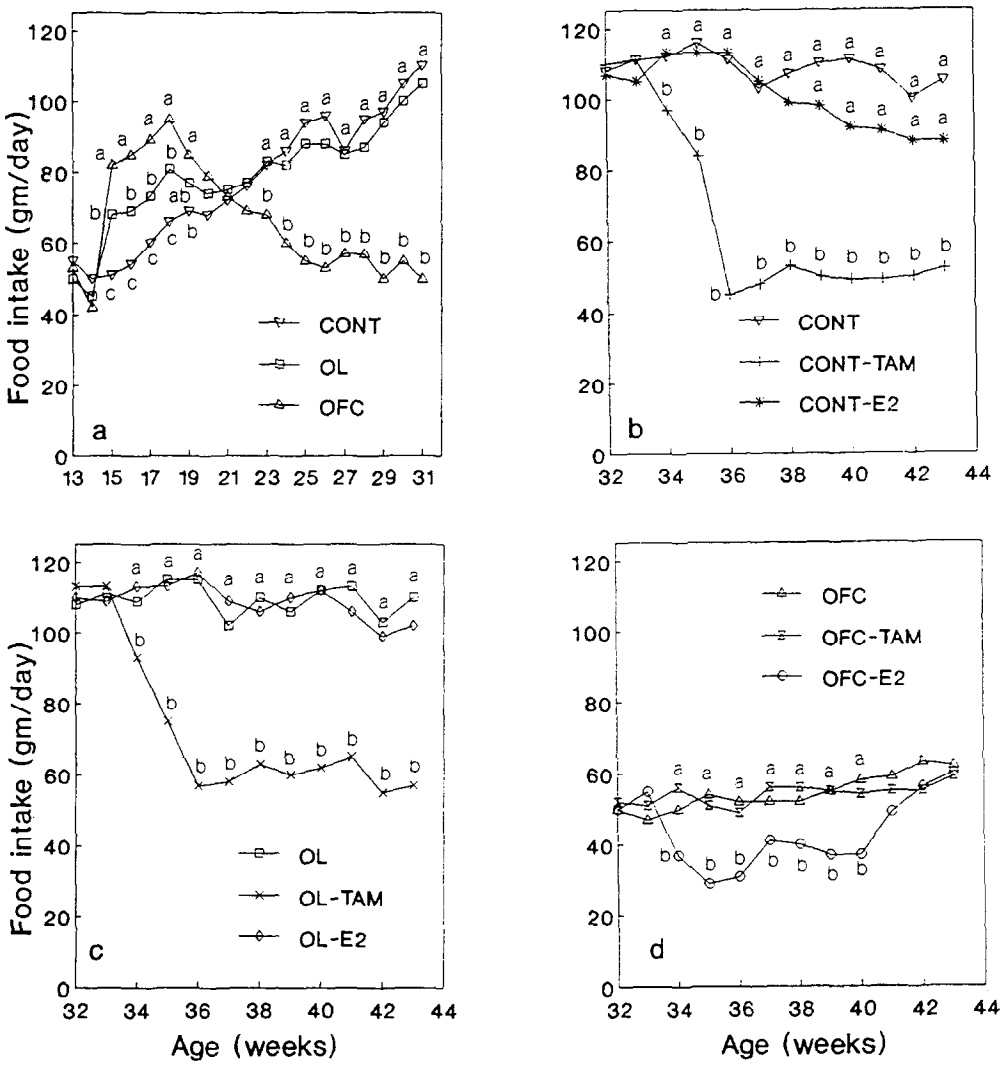

FIG. 1. Average daily food intake of obese functionally castrated (OFC; n = 18), obese laying (OL; n = 24), and control (CONT; n = 24) hens between 13 and 31 weeks of age. a) Control hens were injected with corn oil (CONT), 10 mg tamoxifen (TAM)/kg (CONT-TAM) or 2 mg estradiol benzoate (EB)/kg (CONT-E_2). b) n = 8/group; obese laying hens were injected with corn oil (OL), 10 mg TAM/kg (OL-TAM), or 2 mg EB/kg (OL-E_2). c) n = 8/group; obese functional castrated hens were injected with corn oil (OFC), 10 mg TAM/kg (OFC-TAM), or 2 mg EB/kg (OFC-E_2) on each alternate day from 33–43 weeks of age. d) n = 6/group). Values marked by different letters differed significantly; $p < 0.05$.

CONT-TAM hens was significantly higher than in the CONT-E_2 ones.

In the OL hens, neither TAM (OL-TAM) nor E_2 (OL-E_2) had any significant effect on BW and AAT weights (Table 3). TAM administration increased RMR, comb weight, hematocrit, plasma glucose, and insulin T_3 and T_4 levels, and reduced plasma total lipid levels and liver, ovary, and oviduct weights (Table 3). In the OL-E_2 hens, liver weight, plasma total lipids, and T_3 were increased, whereas reproductive organs weights, hematocrit, and plasma glucose were diminished.

In the OFC hens, TAM (OFC-TAM) administration affected none of the measured parameters (Table 4). Administration of E_2 to these hens (OFC-E_2) however, reduced BW, AAT weight, hematocrit, and plasma insulin, glucose, and T_3 and T_4 levels. Plasma total lipids and liver and oviduct weights were increased in the OFC-E_2 hens.

DISCUSSION

The location of the lesion sites and their consequential OL and OFC syndromes were similar to those we observed earlier (18). Estrogen supplementation reduces FI and BW in ovariectomized (44) and hypothalamic obese rats (3). These effects are mediated by both, a direct action in the VMH to suppress FI, and local effects in the adipose tissue to reduce lipoprotein lipase and enhance hormone sensitive lipase activities (47).

In chickens, estrogen has a lipogenic role (1,17,26,42). Thus, its administration increases FI, liver lipogenesis, plasma

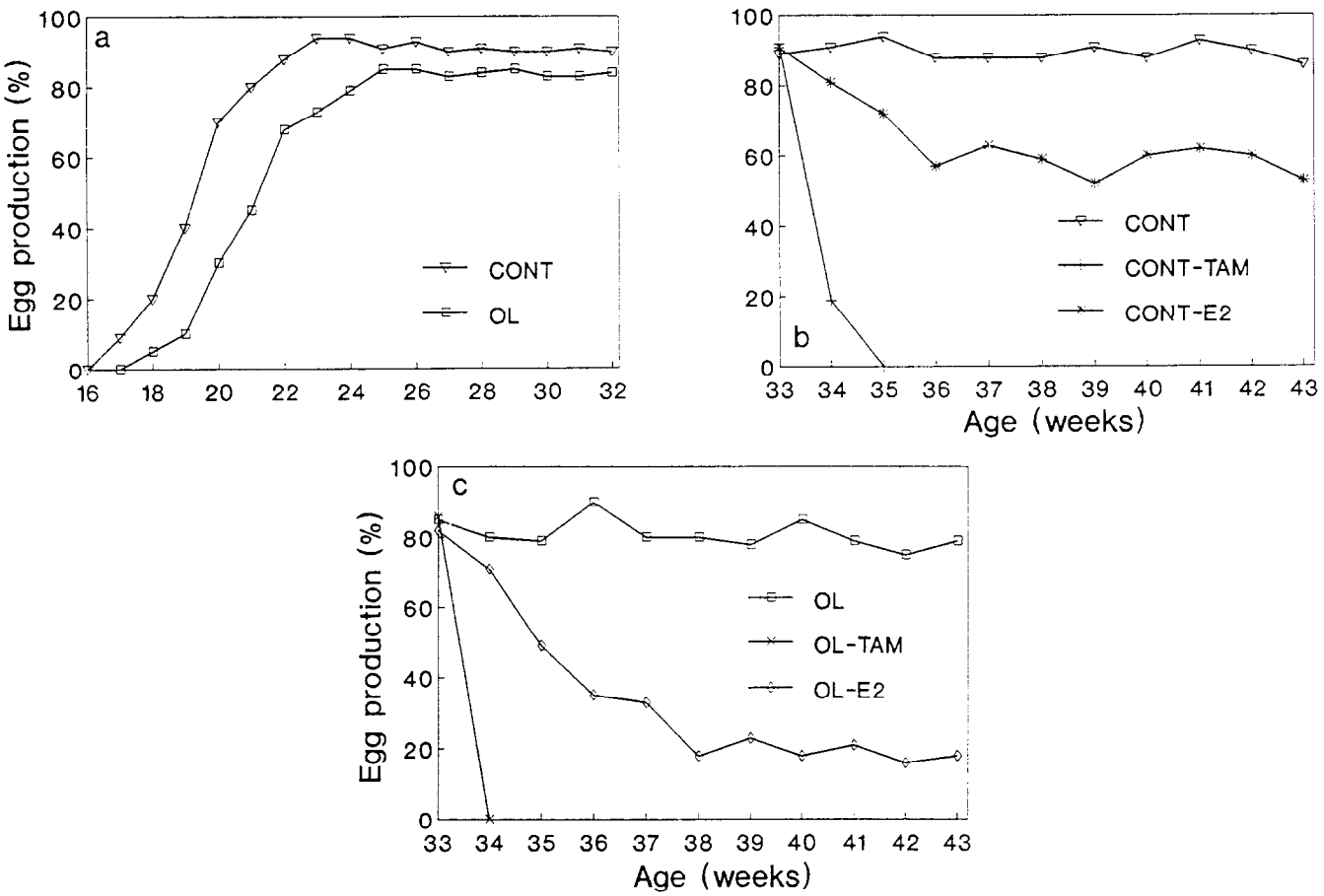

FIG. 2. Egg production by obese laying (OL; n = 24), control (CONT; n = 24) hens between 16 and 32 weeks of age. a) Control hens were injected with corn oil (CONT), 10 mg tamoxifen (TAM)/kg (CONT-TAM), or 2 mg estradiol benzoate (EB)/kg (CONT-E_2). b) n = 8/group; obese laying hens were injected with corn oil (OL), 10 mg TAM/kg (OL-TAM), or 2 mg EB/kg (OL-E_2) on each alternate day from 33-43 weeks of age. c) n = 8/group.

TABLE 1

BODY AND ORGANS WEIGHTS, RESTING METABOLIC RATE (RMR), HEMATOCRIT AND PLASMA LEVELS OF METABOLITES AND HORMONES OF 43-WEEK-OLD CONTROL (CONT; n = 8), OBESE LAYING (OL; n = 8) AND OBESE FUNCTIONALLY CASTRATED (OFC; n = 6) HENS, INJECTED WITH CORN OIL ON EACH ALTERNATE DAY FROM 33-43 WEEKS OF AGE

	CONT	OL	OFC
Body weight (g)	1673 ± 73^{b}*	2060 ± 91^{a}	2066 ± 99^{a}
RMR (ml O_2/h per kg)	1133 ± 17^{a}	814 ± 38^{b}	804 ± 65^{b}
Abdominal adipose tissue (g)	51.1 ± 7.2^{b}	114.1 ± 10.9^{a}	134.1 ± 12.8^{a}
Liver (gm)	34.1 ± 1.5^{ab}	45.2 ± 4.8^{a}	28.9 ± 2.3^{b}
Ovary (g)	51.2 ± 3.6^{a}	45.1 ± 2.9^{a}	0.66 ± 0.03^{b}
Oviduct (g)	56.2 ± 3.2^{a}	63.8 ± 3.8^{a}	0.72 ± 0.11^{b}
Comb (g)	8.8 ± 1.3^{a}	6.4 ± 0.8^{a}	0.69 ± 0.08^{b}
Hematocrit (%)	30.5 ± 0.4^{b}	28.9 ± 0.5^{b}	35.6 ± 0.9^{a}
Plasma total lipids (mg/ml)	27.5 ± 2.0^{a}	33.8 ± 2.2^{a}	8.8 ± 1.1^{b}
Plasma glucose (mg/dl)	205.6 ± 4.2	203.7 ± 5.6	210.0 ± 6.7
Plasma insulin (pg/ml)	840 ± 90	790 ± 80	970 ± 50
Plasma T_3 (pg/ml)	810 ± 60^{b}	660 ± 60^{b}	1690 ± 90^{a}
Plasma T_4 (ng/ml)	11.5 ± 1.0^{b}	12.7 ± 0.5^{b}	18.6 ± 0.6^{a}

Data are represented as means ± SEM.
*Values marked by different letters differed significantly ($p < 0.05$).

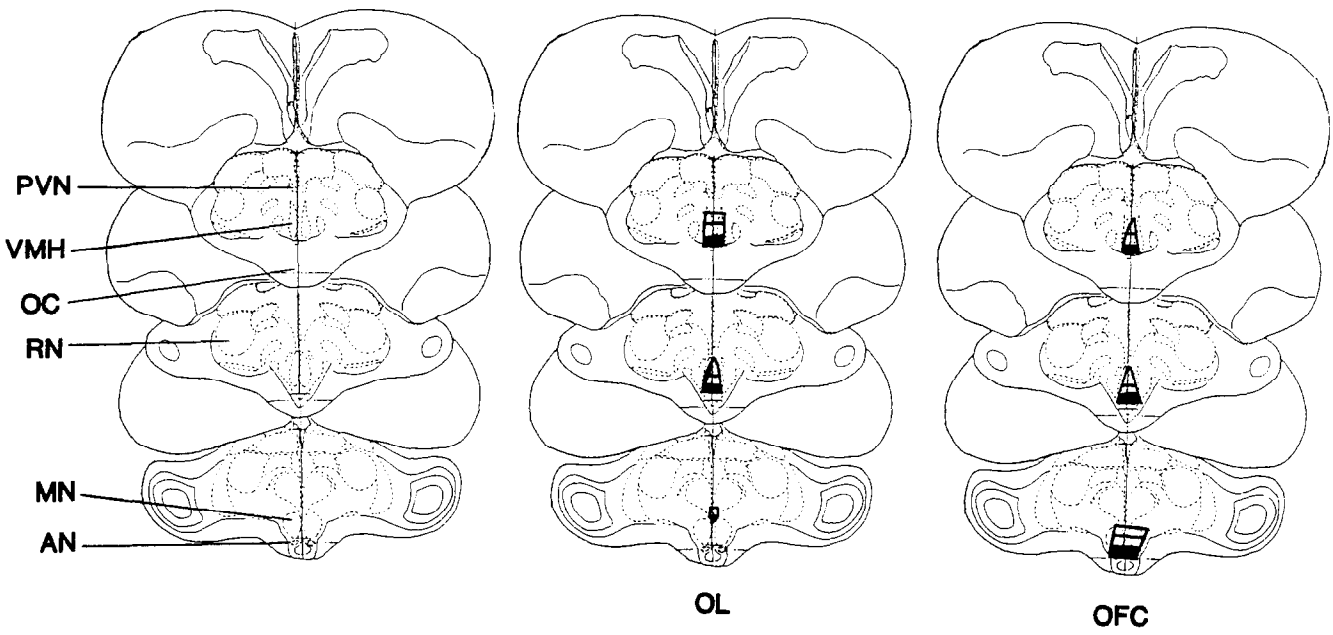

FIG. 3. Schematic drawing of the chicken brain (43) demonstrating the location of the hypothalamic lesions in obese functionally castrated (OFC) and obese laying (OL) hens. The blackened area covers the site of the smallest lesion. Heavy barred area = the largest lesion.

lipids, and fattiness in cockerels (42) and juvenile hens (Jacoby et al., unpublished). In the present study, E_2 increased plasma lipids in all experimental hens. However, it had no effect on FI and fattiness in the laying (CONT and OL) hens, and even reduced FI and AAT weight in the OFC ones. Several mechanisms might cause the leaning effect of E_2 in OFC hens. Plasma of laying hens, but not of juvenile hens and cockerels, contains an estrogen-induced lipoprotein lipase inhibitor (11,12,16,21). Furthermore, estrogen administration depresses lipoprotein lipase activity in the adipose tissue of young chicks (14). In chickens, most of the lipoprotein lipase activator is presented in the HDL fraction, whereas the VLDL contained only a minute amount of it (13). In laying hens and estrogen-treated chicks, plasma HDL is lower and VLDL is higher than in juvenile hens (22). Thus, estrogen may shift plasma lipoprotein profile to reduce their hydrolysis by lipoprotein lipase, and by this, to diminish lipid flow to adipose tissue and increase their level in plasma and availability for

TABLE 2

BODY AND ORGANS WEIGHTS, RESTING METABOLIC RATE, HEMATOCRIT, AND PLASMA LEVELS OF METABOLITES AND HORMONES OF 43-WEEK-OLD HENS INJECTED WITH CORN OIL, 10 mg TAMOXIFEN/kg (CONT-TAM), OR 2 mg ESTRADIOL BENZOATE/kg (CONT-E_2) ON EACH ALTERNATE DAY FROM 33-43 WEEKS OF AGE

	CONT	CONT-TAM	CONT-E_2
Body weight (g)	1673 ± 73	1656 ± 52	1716 ± 72
RMR (ml O_2/h per kg)	1133 ± 17^{b}*	1224 ± 55^{a}	1275 ± 23^{a}
Abdominal adipose tissue (g)	51.1 ± 7.2	58.4 ± 8.1	52 ± 6.6
Liver (g)	34.1 ± 1.5^{a}	25.9 ± 1.3^{b}	37.2 ± 3.3^{a}
Ovary (g)	51.2 ± 3.6^{a}	18.9 ± 1.9^{b}	56.3 ± 6.8^{a}
Oviduct (g)	56.2 ± 3.2^{a}	27.1 ± 2.6^{b}	57.0 ± 3.9^{a}
Comb (g)	8.8 ± 1.3^{ab}	12.4 ± 1.3^{a}	5.9 ± 0.4^{b}
Hematocrit (%)	30.5 ± 0.4^{b}	39.2 ± 0.9^{a}	26.8 ± 0.7^{c}
Plasma total lipids (mg/ml)	27.5 ± 2.0^{b}	7.4 ± 0.5^{c}	118.5 ± 11.6^{a}
Plasma glucose (mg/dl)	205.6 ± 4.2^{b}	224.0 ± 5.3^{a}	186.6 ± 6.2^{c}
Plasma insulin (pg/ml)	840 ± 90^{b}	1150 ± 80^{a}	460 ± 60^{c}
Plasma T_3 (pg/ml)	810 ± 60^{b}	1260 ± 50^{a}	1190 ± 90^{a}
Plasma T_4 (ng/ml)	11.5 ± 1.0^{b}	17.4 ± 1.5^{a}	11.0 ± 0.5^{b}

n = 8/group. Data are represented as means ± SEM. Abbreviations as in Table 1.
*Values marked by different letters differed significantly ($p < 0.05$).

TABLE 3

BODY AND ORGANS WEIGHTS, RESTING METABOLIC RATE, HEMATOCRIT, AND PLASMA LEVELS OF METABOLITES AND HORMONES OF 43-WEEK-OLD OBESE LAYING HENS INJECTED WITH CORN OIL, 10 mg TAMOXIFEN/kg, OR 2 mg ESTRADIOL BENZOATE/kg (OL-E_2) ON EACH ALTERNATE DAY FROM 33-43 WEEKS OF AGE

	OL	OL-TAM	OL-E_2
Body weight (g)	2060 ± 91	1942 ± 83	2100 ± 32
RMR (ml O_2/h per kg)	814 ± 38[b]*	1060 ± 42[a]	794 ± 24[b]
Abdominal adipose tissue (g)	114.1 ± 10.9	115.2 ± 11.9	127.0 ± 8.7
Liver (g)	45.2 ± 4.8[b]	28.0 ± 1.6[c]	57.2 ± 4.0[a]
Ovary (g)	45.1 ± 2.9[a]	14.7 ± 2.7[b]	35.0 ± 11.8[a]
Oviduct (g)	63.8 ± 3.8[a]	25.6 ± 3.7[c]	45.6 ± 6.0[b]
Comb (g)	6.4 ± 0.8[b]	12.5 ± 1.3[a]	3.8 ± 0.6[c]
Hematocrit (%)	28.9 ± 0.5[b]	37.1 ± 0.6[a]	22.3 ± 0.9[c]
Plasma total lipids (mg/ml)	33.8 ± 2.2[b]	6.7 ± 0.2[c]	134.8 ± 9.3[a]
Plasma glucose (mg/dl)	203.7 ± 5.6[b]	227.0 ± 5.3[a]	183.1 ± 6.2[c]
Plasma insulin (pg/ml)	790 ± 80[b]	1330 ± 70[a]	570 ± 70[c]
Plasma T_3 (pg/ml)	660 ± 60[c]	1540 ± 70[a]	1230 ± 30[b]
Plasma T_4 (ng/ml)	12.7 ± 0.5[b]	15.9 ± 1.1[a]	12.3 ± 1.2[b]

n = 8/group. Data are represented as means ± SEM. Abbreviations as in Table 1 and 2.
*Values marked by different letters differed significantly ($p < 0.05$).

yolk formation (13). However, although estrogen shifts plasma lipid profiles in both adult hens and juvenile chicks, in cockerels and juvenile hens, estrogen has a fattening effect. Unlike in adult hens, in juvenile pullets and cockerels, estrogen causes a transient phase of hyperphagia. This hyperphagia increases levels of plasma portomicrons that have high affinity for lipoprotein lipase (13). It is possible that although estrogen shifts the plasma lipoprotein profile, the increase in plasma protomicrons is sufficient to promote lipoprotein lipase activity and lipid flow into the fat depots.

Ovarian activity and steroids may be presumed to release during the process of a hen's maturation, and to alter lipid metabolism and its response to estrogen in the adult life, different from those of cockerels and juvenile hens. However, in OFC hens, when ovarian development soon ceased after the destruction of the BMH, estrogen increased plasma lipids and reduced fattiness. This suggests that hens have an age-dependent, rather than ovarian-dependent, mechanism that alters the fattiness response to estrogen.

Only in OFC hens did E_2 administration reduce AAT

TABLE 4

BODY AND ORGANS WEIGHTS, RESTING METABOLIC RATE, HEMATOCRIT, AND PLASMA LEVELS OF METABOLITES AND HORMONES OF 43-WEEK-OLD OBESE FUNCTIONAL CASTRATED HENS INJECTED WITH CORN OIL (OFC), 10 mg TAMOXIFEN/kg, OR 2 mg ESTRADIOL BENZOATE/kg (OFC-E_2) ON EACH ALTERNATE DAY FROM 33-43 WEEKS OF AGE

	OFC	OFC-TAM	OFC-E_2
Body weight (g)	2066 ± 99	2060 ± 92	1816 ± 126
RMR (ml O_2/h per kg)	804 ± 65	828 ± 58	901 ± 34
Abdominal adipose tissue (g)	134.1 ± 12.8[a]	138.4 ± 12.6[a]	96.0 ± 6.1[b]
Liver (g)	28.9 ± 2.3[b]	31.9 ± 4.4[b]	57.3 ± 9.7[a]
Ovary (g)	0.66 ± 0.03	0.71 ± 0.05	0.72 ± 0.06
Oviduct (g)	0.72 ± 0.11[b]	0.72 ± 0.08[b]	26.55 ± 5.09[a]
Comb (g)	0.69 ± 0.08	0.72 ± 0.08	0.51 ± 0.11
Hematocrit (%)	35.6 ± 0.9[a]	36.0 ± 1.1[a]	20.5 ± 0.9[b]
Plasma total lipids (mg/ml)	8.8 ± 1.1[b]	7.2 ± 0.4[b]	197.2 ± 30.7[a]
Plasma glucose (mg/dl)	210.0 ± 6.7[a]	206.0 ± 6.1[a]	135.0 ± 6.7[b]
Plasma insulin (pg/ml)	970 ± 50[a]	990 ± 10[a]	320 ± 30[b]
Plasma T_3 (pg/ml)	1690 ± 90[a]	1690 ± 90[a]	1040 ± 120[b]
Plasma T_4 (ng/ml)	18.6 ± 0.6[a]	17.6 ± 0.7[a]	14.1 ± 1.4[b]

n = 8/group. Data are represented as means ± SEM. Abbreviations as in Tables 1 and 2.
*Values marked by different letters differed significantly ($p < 0.05$).

weight and FI. In these hens, in which plasma total lipid levels were the lowest, E_2 increased plasma lipids to the highest magnitude. It seems that OFC hens, in which ovarian activity and plasma E_2 are very low (18), are hypersensitive to estrogenic effects on lipid metabolism.

In intact cockerels, estrogen administration increases FI, fattiness, and plasma lipids (42). In castrated cockerels, similar estrogen supplementation results in a much higher increase in plasma lipids, but only in short-lasting hyperphagia, which is followed by hypophagia. Thus, in castrated cockerels estrogen causes a lesser degree of fattiness than in intact ones (42). It was assumed that the higher degree of hyperlipidemia in castrated cockerels suppresses their feeding response to estrogen (42). In accordance, only in the OFC hens, in which E_2 caused the highest degree of hyperlipidemia did it reduced FI. The reduction in FI observed in estrogen-treated old hens (45) could be evoked by a similar mechanism.

Exogenous E_2 reduced plasma glucose in all experimental hens, probably as a result of an increase in glucose use for lipogenesis (23). This was accompanied by a reduction in plasma insulin in these hens.

Tamoxifen reduced plasma lipids and FI in CONT and OL hens, but not in OFC ones. However, it had no effect on fattiness in any of the hens. These effects of TAM probably merged from its antiestrogenic activity as it reduced plasma lipids and FI only in the CONT and OL hens, to the level normally presented in the OFC pullets, in which plasma estradiol is very low (18). In juvenile hens TAM reduces not only plasma lipid and FI, but also the amount of body fat (20). This further supports our hypothesis that estrogen serves as a fattening factor in juvenile, but not in mature hens.

In CONT and OL hens, E_2 reduced egg laying and TAM abolished it. The deleterious effect of E_2 and TAM on egg production was reported previously (45). In chickens, TAM stimulates the hypothalamo-hypophysial gonadotropic activity, whereas estrogen inhibits it (20,32). In accordance, TAM increased comb size and hematocrit values in the CONT and OL hens, probably as a result of an increase in testosterone production (20). On the other hand, TAM reduced plasma lipids and oviduct weight, probably by in situ inhibition of estrogen activity. Because in hens developing yolks are the major component of the ovarian mass, the reduction in synthesis of lipoproteins for incorporation in yolks diminished the ovarian weight and abolished egg production. In the OFC hens, in which the hypothalamic-gonadotropic system is damaged to the extend of functional castration, TAM could not activate this system, and thus had no effect on hematocrit value or comb size. As the estradiol level in plasma of these hens is very low (18,19), they had an involuted oviduct upon which TAM had no further retarding effect. In these hens, E_2 promoted some growth of the oviduct, but not to the normal size. This may point to the absence of other factors necessary for normal oviduct development (5).

The metabolic response to hypothalamic lesions and drugs administration did not always correlate with that of the T_3 and T_4. OL and OFC hens had lower RMR than that of CONT hens. However, OL and CONT hens had similar levels of plasma T_3 and T_4, significantly lower than that of the OFC ones. Furthermore, even though E_2 reduced plasma T_3 and T_4 in OFC hens, it had no effect on their RMR. A lack of metabolic response to increased levels of thyroid hormones was also found in Japanese quails at high ambient temperatures (4), and in malnourished rats (28,29). In the latter, it was attributed to reduced availability of serum thyroid hormones to tissues (28,29). TAM increased plasma levels of T_3 and T_4 and raised RMR in laying (CONT and OL), but not OFC hens. In several avian species, thyroid and gonadal activities are inversely related (15,48); administration of gonadal steroids reduces thyroid activity and serum T_4 (27,36) and castration increases plasma T_3 and T_4 (37). In chickens, gonadotropins can stimulate the thyroid (7,8). Castration and sex-steroid administration are known to increase and reduce gonadotropin secretion, respectively. As TAM increased gonadotropic activity in laying (CONT and OL), but not OFC hens, it enhanced the thyroid to secrete higher amounts of T_3 and T_4 in the former, but not the latter. In CONT and OL hens, E_2 increased plasma T_3 but did not change plasma T_4. Enhancement of T_4 to T_3 conversion by estrogen should be considered. In the CONT hens, increased plasma T_3 was accompanied by an elevation in RMR values. However, in the OL-E_2 hens the rise in plasma T_3 had no effect on RMR. In mammals, VMH stimulation accelerates norepinephrine turnover in peripheral tissues (34); norepinephrine is essential for the induction of uncoupling protein synthesis, and thus thermogenesis by T_3 (38,39). In pigeons, T_4 enhances, and thiouracil reduces, the thermogenic effect of norepinephrine (33). As in mammals, the hypothalamic damage in the OL-E_2 hens could reduce the sympathetic tone, and thus prevent the manifestation of the full capacity of the thermogenic effect of T_3. This could also be the case for the OFC hens, in which in the presence of the highest levels of thyroid hormones in plasma, RMR was very low. In OFC hens, the high levels of plasma T_3 and T_4 are certainly not the result of increased gonadotrpic activity, as this is very low. Furthermore, in BMH lesioned FC cockerels, the pituitary and thyroids do not respond to goiterogen (31). In birds, thyroxin-binding prealbumin is a major carrier of T_3 and T_4 in blood (2,10). Its synthesis and release from the liver is inversely related to gonadal activity (10). Thus, the increased levels of thyroid hormones in the plasma of OFC hens is probably not a reflection of higher thyroid activity, but of a higher binding capacity in plasma that lowers the clearance rate. In these hens, E_2 administration reduced thyroid hormones in plasma, probably by reducing the plasma-binding capacity. The increase in plasma T_3 and T_4 in CONT and OL hens by TAM could also involve a change in the plasma level of the carrier protein.

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