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Failure of High Dietary Fat to Influence Serum Prolactin Levels during the Estrous Cycle in Female Sprague–Dawley Rats[1] (41760)

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Abstract. The influence of a 20% high-fat and a 4.5% control fat diet on circulating prolactin levels was determined during the estrous cycle of intact female rats, and during a progesterone-induced surge of prolactin in ovariectomized, estrogen-primed rats. An indwelling right atrial cannula was implanted into each rat to facilitate repeated blood sampling in conscious, undisturbed animals. No differences in serum prolactin levels were observed at any time during the estrous cycle or in the progesterone-induced surge of prolactin in rats fed either the high-fat or control fat diet. There also were no differences in the estrous cycles of rats on high- or low-fat diets. It is concluded that high dietary fat promotes mammary tumor development by a mechanism that does not involve alterations in circulating prolactin levels or of estrous cycles.

Consumption of high-fat (HF) diets has been shown repeatedly to enhance the development and growth of spontaneous, transplantable, and carcinogen-induced murine mammary tumors (1–4). Animals fed high levels of dietary fat generally show a greater number of tumors per rat, a higher frequency of rats with tumors, and a shorter latency period for tumor development than animals fed lower levels of dietary fat. Epidemiological studies show a strong positive correlation between per capita consumption of dietary fat and breast cancer incidence among women (1, 5).

Endocrine-related mechanisms have been proposed to explain the stimulatory influence of HF diets on mammary tumorigenesis (6, 7). Chan *et al.* (8) claimed that consumption of HF diets increased serum prolactin levels in rats on the afternoons of proestrus and estrus of the estrous cycle, and suggested that this accounts for the stimulation of mammary tumorigenesis by high dietary fat. Subsequent reports also have described increases in serum prolactin levels in female rats fed HF diets (9–11). However, other investigators have reported that high dietary fat does not influence serum prolactin levels in cycling female rats (12–15). These reports were based on single determinations of serum prolactin levels during different stages of the estrous cycle, and utilized stressful blood sampling procedures that could have influenced circulating levels of prolactin. Therefore, the effects of HF diets on circulating prolactin levels during the estrous cycles are still controversial.

By using surgically implanted, indwelling right atrial cannulas, blood can be serially collected from conscious, moving rats without disturbing them. This allows for more accurate assays of serum prolactin levels that are not influenced by drug-induced anesthesia or physiological stress. The purpose of this study was to observe in detail the effects of high dietary fat consumption on serum prolactin levels during the entire estrous cycle of female rats and on the proestrous-simulated surge of prolactin induced by injecting progesterone into ovariectomized, estrogen-primed rats (16, 17).

Materials and Methods. *Animals and dietary treatment.* Virgin female Sprague–Daw-

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ley rats, 55 days of age (Harlan Research Animals, Indianapolis, Ind.), and showing regular 4-day estrous cycles were housed in metal suspension cages in a temperature- (24.0 ± 0.5°C) and light-controlled (14 hr light:10 hr dark) room. They were fed *ad libitum* a semisynthetic high-fat (HF) diet (20% fat) or a control fat (CF) diet (4.5% fat) for 4 weeks (Table I). Tap water was provided *ad libitum*. Diets were prepared one to two times weekly and were stored at 4°C until fed. All rats were provided with fresh food every 2 days or more often when needed. Estrous cycle patterns were monitored in each rat by daily collection of vaginal smears.

Right atrial cannulation and blood sampling procedures. After 4 weeks on the different dietary treatments, 24 rats with normal estrous cycles (12 from each group) were each implanted with a right atrial cannula and maintained on their respective dietary regimen. Under ether anesthesia, a Silastic cannula (0.025-in. i.d.; 0.047-in. o.d.) was surgically inserted into the right external jugular vein, and threaded through the superior vena cava into the right atrium. The free end of the cannula was threaded subcutaneously to the back of the neck and exited 1–2 cm caudal from the base of the skull. After the cannula was secured in place, it was flushed with approximately 0.2 ml of sterile heparinized saline (100 IU/ml) and closed with a knot at the free end of the cannula. Cannulated rats were housed in individual wire cages. To collect a blood sample, the free end of the cannula was cut immediately proximal to the closure knot and a Silastic extension tube was attached to the cannula which extended outside the cage. Cannulated rats with extension tubes were able to move freely in their cages and were free to consume food and water. After each blood collection the cannula and extension tube were flushed with 0.85% sterile NaCl solution and appropriate volumes of saline were injected through the cannula to replace the blood volume lost in the blood sample.

TABLE I. COMPOSITION OF CONTROL FAT AND HIGH-FAT DIETS (PERCENTAGE OF TOTAL DIET)

	Control fat (4.5%)[a]	High fat (20.0)[a]
Casein[b]	25	25
Sucrose[c]	59.5	42.0
Corn oil[c]	4.5	20.0
Cellulose[b]	7	8
Salt mixture[b]	4	5

[a] Twenty one grams of Total Vitamin Supplement was added to each kg of diet mixture.

[b] Obtained from U.S. Biochemical Corp., Cleveland, Ohio.

[c] Purchased from local sources.

Beginning on the day after cannulation and continuing for the next 4 days, serial blood samples (0.7 ml each) were taken at 4-hr intervals during each stage of the estrous cycle. Additional blood samples (0.4 ml each) were taken at hourly intervals between 1600 and 1900 hr during the afternoon of proestrus. Estrous cycle patterns were monitored during the blood sampling period by taking daily vaginal smears immediately after the 0900-hr bleeding.

In a separate experiment, preovulatory-like surges of prolactin were induced as follows: 20 female Sprague–Dawley rats, 200–225 g in body weight, were bilaterally ovariectomized. Two weeks after ovariectomy rats were placed on either the HF or CF diet (10 rats per group). Six weeks after ovariectomy, or 4 weeks after initiation of dietary treatment, all rats were implanted with indwelling right atrial cannulas and injected (sc) with 20 μg of estradiol benzoate (EB). Seventy-two hours later, all rats were injected sc with 5 mg progesterone. Blood was collected serially (0.4 ml/sample) via the indwelling cannula immediately prior to (1100 hr) and following progesterone administration at 1400, 1600, 1700, 1800, 1900, and 2200 hr. In both experiments, serum was separated from each blood sample by centrifugation and stored at −20°C until radioimmunoassayed for prolactin according to the method of Niswender *et al.* (18).

Differences in serum prolactin levels between the two dietary fat treatment groups, at each time of blood sampling during the estrous cycle or hormone-induced prolactin release, were evaluated statistically by Student's t test, with $P < 0.05$ as the level of significance.

Results. The effects of high and low dietary fat on serum prolactin levels during the estrous cycle are shown in Fig. 1. Rats fed either the CF or the HF diet showed similar serum prolactin levels throughout the estrous cycle and

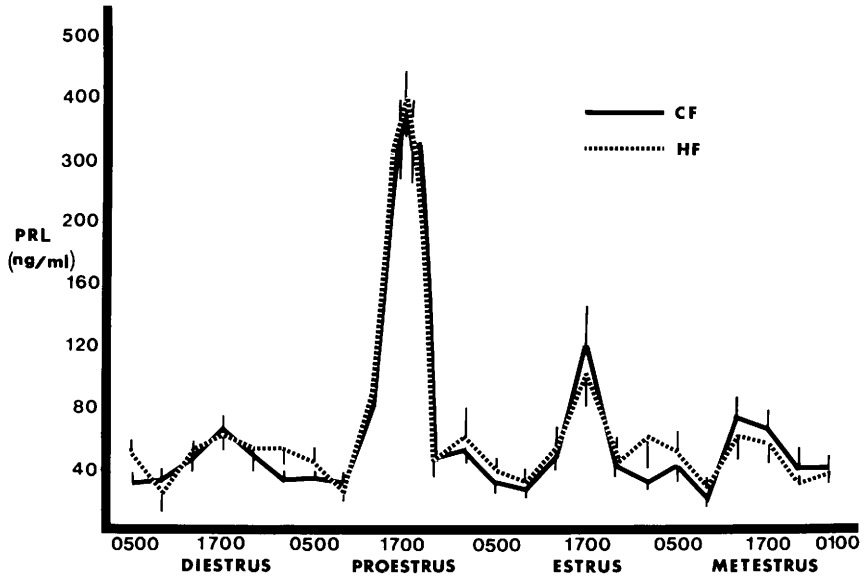

FIG. 1. Effect of high-fat diet on serum prolactin levels during the estrous cycle of female Sprague–Dawley rats. CF represents 4.5% control fat diet. HF represents 20.0% high-fat diet. Vertical bars represent SEM. $N = 10$ for each point.

a typical surge of prolactin occurred on the afternoon of proestrus. Basal serum prolactin levels for both the CF and HF rats ranged from 20 to 60 ng/ml throughout the cycle. Prolactin levels during the afternoon of proestrus were elevated to approximately 400 ng/ml in both treatment groups. Additionally, a smaller surge of prolactin was observed in both treatment groups on the afternoon of estrus. No significant differences in serum prolactin were detected at any time during any stage of the estrous cycle between rats fed the CF or the HF diet.

Estrous cycle patterns were not altered by consumption of the HF diet during the 4-week treatment period. A total of 20 of 28 rats fed the CF diet and 19 of 29 rats fed the HF diet showed consistent 4- to 5-day estrous cycles, as indicated by daily vaginal smears. Additionally, in both dietary treatment groups, 10 of 12 rats implanted with indwelling cannulas maintained their normal estrous cycles during the 4-day blood sampling period.

The effects of high dietary fat on serum prolactin levels in progesterone-treated, ovariectomized EB-primed rats are summarized in Fig. 2. Treatment of ovariectomized rats with EB followed by progesterone showed a surge of prolactin in both the CF and HF treatment groups. Prior to progesterone injection, basal serum prolactin levels in both the CF- and HF-fed rats were approximately 30 ng/ml. Five hours after injection of progesterone, serum prolactin levels peaked at nearly 400 ng/ml in both the CF and HF treatment groups. Serum prolactin levels subsequently declined to nearly 100 ng/ml at 2200 hr, 11 hr after progesterone treatment. At no time were serum prolactin levels significantly different between rats fed either the HF or the CF diet.

Discussion. The stimulatory influence of high levels of dietary fat on murine mammary tumorigenesis has been well established (1–4). However, no mechanism has been proposed which can totally account for these effects. One of the most extensively investigated and widely accepted views for stimulation by dietary lipids of mammary tumorigenesis involved a presumed role for the endocrine system, particularly anterior pituitary prolactin secretion (6–9). Prolactin and estrogen have been shown to be the two primary hormones involved in murine mammary tumor development and growth (19, 20). The hypothesis proposed by Chan *et al.* that HF diets stimulate mammary tumorigenesis by increasing anterior pituitary prolactin secretion was partially based on the observations that drug-induced

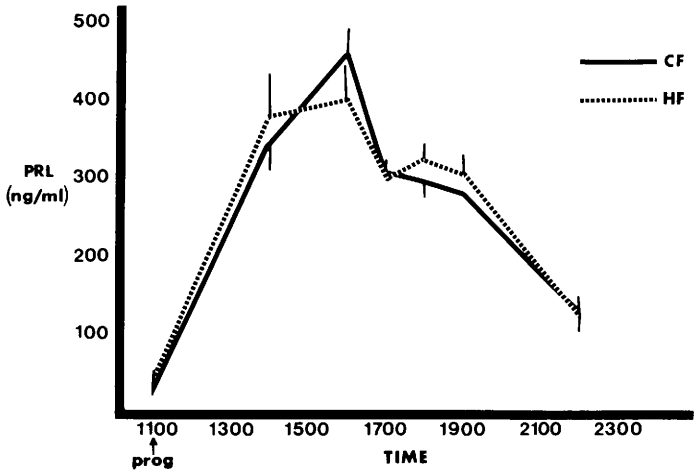

FIG. 2. Effect of high-fat diet on the surge of prolactin induced by progesterone in ovariectomized EB-primed female rats. CF represents 4.5% control fat diet. HF represents 20.0% high-fat diet. Vertical bars represent SEM. PROG: indicates progesterone injection (5 mg/rat). $N = 10$ for each point.

decreases of serum prolactin levels could block the stimulatory effect of HF diets on mammary tumorigenesis (21). Furthermore, these investigators reported that HF diets elevated serum prolactin levels at specific periods (on proestrous afternoon and on estrous day) during the estrous cycle (8, 9). These observations led to the conclusion that the stimulatory effects of HF diets on mammary tumorigenesis were mediated indirectly through the hypothalamic–hypophysial system, resulting in the elevation of serum prolactin levels (6, 7).

The data reported here clearly demonstrate that the consumption of a 20% HF diet does not significantly influence serum prolactin levels at any time during the estrous cycle. Similar HF-diet treatment has been shown by our laboratory (22) and others (1–4) to enhance carcinogen-induced mammary tumorigenesis in rats. Discrepancies between the previous reports and the data presented here may be due to use of single-blood sample assays for assessing the influence of HF diets on circulating levels of prolactin. The collection of single blood samples under drug-induced anesthesia could have influenced serum prolactin values, as such methods are stressful (23). In the present study, serial blood samples were taken from conscious, unstressed rats, and therefore are believed to represent more accurate circulating prolactin levels. Discrepancies in the literature also may be due to the fact that some investigators compared serum prolactin levels in animals fed a 0.5% low-fat (LF) diet with animals fed a 20.0% HF diet. A 0.5% LF diet may be marginally deficient in essential fatty acids. Therefore, differences in serum prolactin levels between rats fed the 0.5% LF and the 20.0% HF diet may actually reflect suppression of prolactin levels in the LF group. In the study reported here, serum prolactin levels were compared in rats fed a 4.5% CF diet with rats fed a 20.0% HF diet. Other differences in the results reported here and those by Chan *et al.* (8, 9) and Ip *et al.* (11) may be due to the dietary regimens used. Ip *et al.* (11) began their dietary treatment at weaning and continued treatment for 24–26 weeks. Chan *et al.* (8, 9) began dietary treatment at 50 days of age and continued for 8 to 20 weeks (8) or 24 weeks (9). However, our laboratory (22) and others (24) have shown that a HF diet can enhance mammary tumor development and growth after only 3 to 4 weeks (22) or 4 to 6 weeks of treatment (24).

The influence of HF diets on serum prolactin levels at night had not been examined previously. Since rats are most active and consume most of their daily ration at night, any acute or short-term effects of HF consumption on serum prolactin levels might be overlooked if blood were collected only during the day. In the present study, nightly blood samples were taken and no differences in serum pro-

lactin levels were observed between rats fed either the CF or HF diets.

The present results also show that long-term ovariectomized EB-primed rats fed either the CF or the HF diet demonstrate similar progesterone-induced surges of prolactin. These results support our observations that HF diets do not increase the preovulatory surge of prolactin on the afternoon of proestrus, and are in agreement with previous results from this laboratory showing that when estrogen and prolactin were maintained at similar levels in rats fed CF or HF diets, mammary tumorigenesis was still enhanced in rats fed the HF diet (22). Regularity of estrous cycles also was not altered by the HF diet in this and previous studies, suggesting that estrogen secretion was unaffected. It is concluded, therefore, that the stimulating effects of HF diets on mammary tumorigenesis are not mediated by altering prolactin or estrogen secretion.

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