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Reduction of Mammary and Liver Lipogenesis and Alteration of Milk Composition during Lactation in Rats by Hypothyroidism

María Belén Hapon,[1,2] Silvia M. Varas,[2] María S. Giménez,[2] and Graciela A. Jahn[1]

Objective: The profound impairment in litter growth produced by untreated maternal hypothyroidism (HypoT) may be a consequence of maternal metabolic dysfunctions affecting lactation. In this work we studied the effects of HypoT on mammary and liver lipid metabolism and its consequences on milk quality. *Design*: We studied the effects of prolonged 6-propyl-2-thiouracil (PTU)–induced HypoT (0.01% PTU in drinking water starting 8 days before mating until sacrifice) on milk macronutrient composition, liver and mammary lipid metabolism and content and serum lipid, and glucose and insulin concentrations in rats on days 7, 15 (L15), and 20 (L20) of lactation. Mammary and hepatic mRNA abundances of lipogenic enzymes were measured using semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) on L15 and L20. *Main Outcome*: Milk lactose and triglycerides (TG) were reduced by HypoT, as well as mammary acetyl CoA carboxylase (ACC) activity on L15 and L20, and ACC and lipoprotein lipase (LPL) mRNA on L20. HypoT also decreased hepatic ACC activity on both days, ACC mRNA on L15 and liver [^{3}H]H_2O incorporation to TGs and TG content on L20. HypoT diminished insulinemia, increased serum total lipids, and decreased serum TGs on some or all the days of lactation studied. *Conclusion*: HypoT produces a drastic decrease in milk TGs; the main cause for this seems to be the decreases in liver TG synthesis and in circulating TGs, which, along with reduced mammary uptake of fatty acids caused by decreased LPL expression and possibly diminished mammary lipogenesis, result in an impaired mammary output of TGs to the milk. Thus, the impaired growth of the litters of HypoT mothers can be largely attributed to the low milk quality along with the impaired milk ejection.

Introduction

In rats, hypothyroidism (HypoT) has been associated with delayed delivery, subnormal number of fetuses, increased pup mortality, decreased pup growth, and altered circulating hormones (1,2). While most of these effects can be related to the hypothyroid state of the infants, we found impairments in milk ejection that may contribute to the growth retardation of the litters (2).

Thyroid hormones have significant effects on lipid metabolism, stimulating fatty acid and cholesterol synthesis (3,4), increasing mobilization of plasma cholesterol and triglycerides (TGs) (5,6), and stimulating fatty acid and cholesterol degradation (7,8). Disturbances in thyroid function are commonly associated with alterations in plasma lipid levels. Experimental HypoT induced by 6-propyl-2-thiouracil (PTU) treatment is characterized by the accumulation of plasma low-density lipoprotein (LDL) cholesterol and decreased very low-density lipoprotein (VLDL) and plasma TGs (9), generally reflecting reduced binding activity of hepatic LDL receptor, which can be normalized after substitution therapy with thyroid hormone (5,10).

Recently, we have observed in late pregnant HypoT rats the decreased lipogenic enzyme activity and expression that may affect milk quality during the subsequent lactation. The decrease in lipid synthesis, the decrease in lipogenic enzyme activity and expression, and the increase in the proportion of adipose tissue observed in mammary glands of HypoT pregnant rats may reflect increased lipid accumulation resulting from the diminished lipid metabolism characteristic of the hypothyroid state (11). We also found a decrease in hepatic lipid synthesis in virgin and pregnant HypoT rats, evidenced by the diminished expression and activity of fatty acid synthetase (FAS) and acetyl CoA carboxylase (ACC), and incorporation of 3H[H_2O] to TGs, which result in reduced hepatic TG synthesis and may be the cause of the diminished circulating TGs (11).

[1]Laboratorio de Reproducción y Lactancia, IMBECU, CRICYT-CONICET, Mendoza, Argentina.
[2]Laboratorio de Bioquímica Molecular, Departamento de Bioquímica y Ciencias Biológicas, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina.

When compared with non-pregnant values, plasma thyroid hormones are decreased during lactation in many species including humans and rats (2,12,13). A clinical state of HypoT during pregnancy and lactation may limit the capacity of the maternal organism to sustain itself and the newborn adequately, and of the mammary tissue to produce milk in sufficient quality and quantity, thus compromising nutrition of the newborn (2).

TGs make up 97% of the lipid content of milk (14), and in rats they constitute 10% of the total milk volume (15). Fatty acids used for triacylglycerol synthesis in the mammary gland derive mainly from three different sources: de novo fatty acid synthesis, from lipoprotein lipase (LPL) action, and from the pool of non-esterified fatty acids that circulate bound to albumin (16). Administration of a moderate oral dose of triiodothyronine (T_3) to lactating rat and mice dams induces a higher growth rate in the pups, and this positive effect seems to be mainly due to augmented secretion of milk that in addition contains an elevated proportion of TGs (17,18). On the other hand, there is little information on the effects of thyroid hormone deficit during lactation; in particular, the impact of maternal HypoT on milk quality has not been explored. Any alterations in maternal metabolism that could lead to decreased milk quality could further complicate the panorama for the offspring.

The aim of the present investigation is to examine the effects of HypoT produced by PTU administration on maternal lipid metabolism and its impact on milk quality in rats. We measured the major milk components, lipid metabolism in liver and mammary gland, and serum lipid and hormonal parameters in order to determine the mechanisms that underlie the stunted growth and increased mortality of the pups. The focus of this investigation is the effect of HypoT on the maternal metabolic regulation of the major milk lipid, TGs.

Materials and Methods

Chemicals and radioisotopes

[^{3}H]-H_2O (3.70 GBq/g) and [^{14}C]-$NaHCO_3$ (1.8 GBq/mmol) were purchased from New England Nuclear Life Science Products (Boston, MA). Lipid standards were acquired from Sigma Chemical (St. Louis, MO). All the other chemicals were of reagent grade and were obtained from Merck Laboratory (Buenos Aires, Argentina) or from Sigma Chemical.

Animals and experimental design

Adult female Wistar rats bred in our laboratory, 3–4 months old and weighing 190–210 g at the onset of treatment, were used. The rats were kept in a light (lights on 06.00–20.00 hours)–controlled and temperature (22–24°C)–controlled room. Rat chow (Cargill, Cordoba, Argentina) and tap water or PTU solution were available *ad libitum*. HypoT was induced by administration of PTU at a concentration of 0.1 g/L in the drinking water. The treatment was started 8 days before the onset of pregnancy. The presence of spermatozoa in the vaginal smear the morning after caging with a fertile male in the night of pro-oestrus was indicative of pregnancy, and this day was counted as day 0 of pregnancy. PTU or control (Co) rats were killed on days 15 and 20 of lactation at 09.00–10.00 hours by decapitation.

On the day 1 of lactation, the number of pups in each litter was standardized to eight, and mothers and litters were weighed weekly. Animal maintenance and handling was performed according to the NIH guide for the Care and Use of Laboratory Animals (NIH publication N° 86-23, revised 1985 and 1991), the UK requirements for ethics of animal experimentation (Animals Scientific Procedures Act 1986), and the FRAME guidelines of 1999.

After decapitation, trunk blood was collected, and serum was separated by centrifugation and stored at − 20°C until used. The livers and inguinal mammary glands from the dams were removed, snap frozen in liquid nitrogen, and stored at − 70°C until they were analyzed. The values are means ± SEM for groups of eight rats.

Milk triglycerides, proteins, and lactose

Milk was obtained on days 2, 15, and 20 of lactation. Pups were separated from their mothers at 10.00 hours; 4 hours later, the dams were lightly anesthetized with ether, administered an intraperitoneal injection of 50 mU of oxytocin, and milk was extracted by gentle repeated pressing of the nipples. About 0.5–1 mL of milk was obtained from each rat and kept frozen in microvials until determinations. Concentration of TGs was determined by colorimetry (see "Serum lipid, protein, glucose, and insulin determinations"), protein concentration by the method of Lowry *et al*. (19), and lactose concentration was assessed by the method of Kuhn and Lowenstein (20).

Serum lipid, protein, glucose, and insulin determinations

Serum glucose, total lipids, TGs, phospholipids (PLs), total cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations were measured by colorimetric or enzymatic methods (kits from Boehringer, Buenos Aires, Argentina), using fresh serum from control or hypothyroid rats that had been fasted for 14 hours and bled on days 7, 15, and 20 of lactation as described previously (11).

Hormone determinations

Insulin was measured in the serum samples from the fasted rats used for lipid and glucose determinations, by heterologous radioimmunoassay using a commercial solid phase human insulin radioimmunoassay (RIA) kit (DPC Coat-a-Count Insulin, Diagnostic Products, Los Angeles, CA) and rat insulin (Linco rat insulin standard, Linco Research, St. Charles, MO) as standard. Results are expressed as ng/mL rat insulin. Assay sensitivity was less than 0.6 ng/mL, and intraassay variation was less than 5%. All samples were measured in the same assay.

Thyroid-stimulating hormone (TSH) was measured by double antibody radioimmunoassay using materials generously provided by Dr. Parlow and the National Hormone and Pituitary Program (Harbor-UCLA Medical Center, Torrance, CA). The hormone was radio-iodinated using the Chloramine T method and purified by passage through Sephadex G75. The results were expressed in terms of the rat TSH RP-3 standard preparation. Assay sensitivity was 0.5 μg/L serum, and the inter- and intraassay coefficients of variation were less than 10% for all hormones.

T_3 and tetraiodothyronine (T_4) in sera were measured by RIA using commercial kits for total hormones (DSL-3100 and DSL-3200 double-antibody RIAs, respectively; all from Diagnostic Systems Laboratories, Webster, TX).

Tissue preparation and enzymatic assays

Liver or mammary gland portions (1 g for 4 mL of buffer) were homogenized in an Ultra Turrax T25 homogenizer in 0.5 M potassium phosphate buffer (pH 7), 10 mM EDTA, and 10 mM D,L-dithiotreitol (DTT). The homogenates were centrifuged at 100.000 *g* for 1 hour to yield the cytosolic fraction in a Beckman model L8-80M ultracentrifuge with a Ti-80 rotor.

Cytosolic FAS activities were determined by modified versions of the methods of Alberts *et al*. (21) and as described in Hapon *et al*. (11). Total ACC activity was measured according to Burnol *et al*. (22). ACC activity was expressed as units/mg of protein, where 1 unit equals 1 pmol of [^{14}C]-bicarbonate incorporated into malonyl-Co A per minute at 37°C. The protein concentration was determined by the method of Lowry *et al*. (19), using fraction V bovine serum albumin as standard.

Lipid determinations

The lipids from hepatic or mammary tissue were extracted with chloroform/methanol (2:1) according to Folch *et al*. (23). The lipid fractions were separated by thin-layer chromatography with an *n*-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) solvent system. The scraped bands were eluted and aliquots were used for determination of the mass of the different lipids. Free and esterified cholesterol were determined according to the method of Zack *et al*. (24) after saponification (25). TGs were quantified by the method of Sardesai and Manning (26), and PLs were determined according to Bartlett (27). A recovery from thin-layer chromatography averaging 90% of cholesterol mass was obtained.

Incorporation of [^{3}H]H_2O from H_2O into lipids

Groups of *ad libitum* fed rats on day 20 of lactation were injected *i.p.* with 37 mBq (1 mCi)/rat [^{3}H]-H_2O in 1 mL saline and killed 2 hours later by decapitation. One gram of liver and mammary gland was extracted with 20 mL of chloroform/methanol (2:1) according to the method of Folch *et al*. (23). The lipid fractions were separated by thin-layer chromatography (see "Lipid determinations"); the bands were scraped and vacuum dried, and the radioactivity incorporated into each fraction was counted in a β counter (Wallac-Pharmacia 1209, Bromma, Sweden). The results are expressed as ng of ^{3}H incorporated/(h g) of tissue.

RNA isolation and RT-PCR analysis

Total RNA from mammary glands and livers was prepared using the guanidinium isothiocyanate-acid phenol method (28) as modified by Puissant and Houdebine (29). Ten micrograms of total RNA was reverse transcribed at 42°C using random hexamer primers and Moloney murine leukemia virus RT (Invitrogen/Life Technologies, Buenos Aires, Argentina) in a 20 μL reaction mixture. FAS, ACC, glycerol-3-phosphate acyltransferase (GPAT), acylCoA oxidase (ACO), carnitine palmitoyltransferase (CPT), and LPL mRNA concentrations were estimated by semiquantitative RT-PCR using the previously described rat-specific primers and reaction conditions (11). The PCR products were analyzed on 1.5% agarose gels containing 0.5 mg/mL ethidium bromide and photographed with a Polaroid camera. Band intensities of RT-PCR products were quantified using NIH Image software. Relative levels of mRNA were expressed as the ratio of signal intensity for the target genes relative to that for L 19 cDNA.

Statistics

Statistical analysis was performed using two-way ANOVA followed by the Bonferroni *post hoc* test to compare any two individual means or using Student's *t*-test when only two groups were compared (30). When variances were not homogeneous, we performed log transformation of the data. Differences between means were considered significant at the $p < 0.05$ level.

Results

Effects of PTU treatment on maternal T_3, T_4, and TSH serum concentrations during lactation

Table 1 shows that PTU treatment induced significant decreases in circulating T_3 and T_4 as well as a marked increase in serum TSH on all days of lactation studied, confirming the hypothyroid state of the rats.

Effects of PTU treatment on milk concentrations of lactose, triglycerides, and proteins and on pup growth

Since survival of newborn mammals depends upon an adequate milk supply produced by the mother, we evaluated whether the severe impairment in pup growth seen previously and in the present experiment (Fig. 1A) in litters from

Table 1. Effects of PTU Treatment on Serum Concentrations of T3, T4, and TSH in Lactating Rats

	Day 7 of lactation		*Day 15 of lactation*		*Day 20 of lactation*	
	Control	*HypoT*	*Control*	*HypoT*	*Control*	*HypoT*
T_3 (ng/dL)	45.1 ± 6.7	15.1 ± 2.5^a	44.5 ± 2.0	26.2 ± 5.9^a	54.9 ± 3.0	33.1 ± 5.0^a
T_4 (ng/mL)	24.3 ± 1.2	0.6 ± 0.4^a	18.0 ± 0.7	1.9 ± 0.7^a	19.4 ± 0.7	1.6 ± 0.3^a
TSH (ng/mL)	0.56 ± 0.03	9.59 ± 1.11^a	0.58 ± 0.07	14.51 ± 2.54^a	0.56 ± 0.08	11.86 ± 0.89^a

Values are means ± SEM for groups of eight rats.
[a] $p < 0.05$ compared with the respective control groups using two-way ANOVA and the Bonferroni *post hoc* test.
PTU, 6-propyl-2-thiouracil; T_3, triiodothyronine; T_4, tetraiodothyronine; TSH, thyroid-stimulating hormone; HypoT, hypothyroidism.

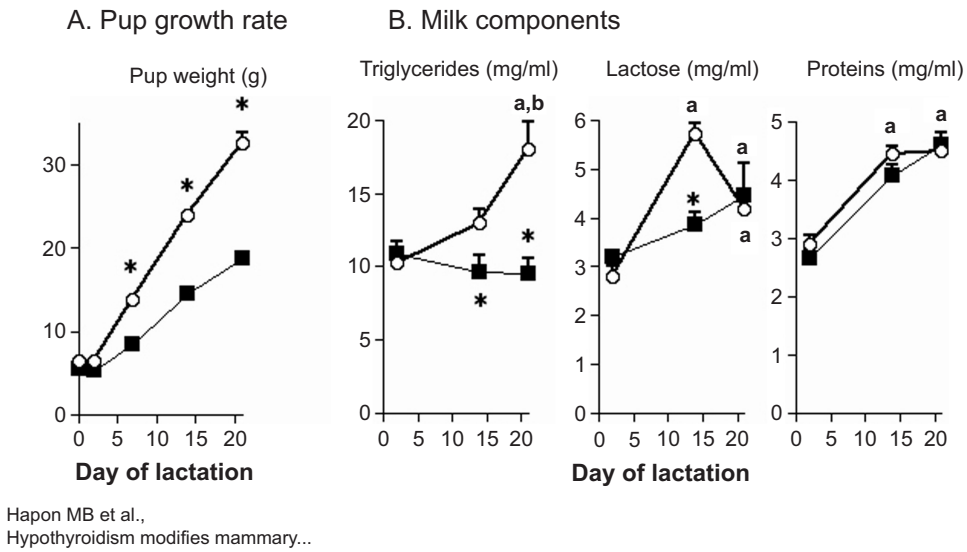

FIG. 1. Effects of 6-propyl-2-thiouracil treatment on litter growth rate, measured as pup weight increase (**A**) and milk concentrations of lactose, triglycerides, and proteins of rats during lactation (**B**). Values are means ± SEM for groups of eight rats. *$p < 0.05$ compared with the respective control groups using two-way ANOVA and the Bonferroni *post hoc* test. [a]$p < 0.05$ compared with the respective day 2 group using two-way ANOVA and the Bonferroni *post hoc* test. [b]$p < 0.05$ compared with the respective day 15 group using two-way ANOVA and the Bonferroni *post hoc* test.

HypoT mothers (2) is related to the quality of milk components. Figure 1B shows that the milk TGs and proteins increased in controls throughout lactation. HypoT blocked the increase in TGs on days 15 and 20 without affecting milk protein concentrations. In contrast, milk lactose peaked on day 15 in control rats and decreased on day 20 to values that were still significantly higher compared with day 2. HypoT rats had similar milk lactose values to controls on days 2 and 20, but the peak of day 15 was abolished.

Effect of hypothyroidism on mammary lipid metabolism in lactating rats

In order to investigate the possible causes of the decrease in milk TGs, we measured some parameters of mammary lipid metabolism, such as lipid synthetic activities and mRNA abundances of lipogenic enzymes on days 15 and 20 of lactation.

HypoT significantly diminished mammary ACC activity on both days of lactation (Table 2), but did not modify mammary FAS activity (not shown) and $[^3H]H_2O$ incorporation to any lipid fraction (Table 2). In addition, HypoT had no effect on mammary proteins, total lipids, TGs, PLs, or free and esterified cholesterol concentrations.

The mRNA abundances of ACC, FAS, GPAT, enzymes that participate in TG synthesis, and LPL, which participates in the uptake and hydrolysis of circulating TGs, were measured using semiquantitative RT-PCR from mammary RNA of rats on days 15 and 20 of lactation (Fig. 2). HypoT significantly reduced ACC and LPL abundances on day 20 of lactation, demonstrating that PTU treatment impairs mammary lipogenic enzyme expression at the end of lactation.

Since mammary lipid degradation processes often are inversely correlated with secretory activity (31), we also mea-

Table 2. Effects of PTU Treatment on Mammary and Liver ACC Activity and on $[^3H]H_2O$ Incorporation to TGs on Days 15 and 20 of Lactation

	ACC (U/mg total protein)			
	Day 15 of lactation		*Day 20 of lactation*	
	Control	*HypoT*	*Control*	*HypoT*
Mammary glands	30.38 ± 5.04	11.33 ± 4.78[a]	42.21 ± 5.34	15.37 ± 4.32[a]
Liver	9.64 ± 0.38	4.03 ± 0.38[a]	10.20 ± 0.94	7.38 ± 0.73[a]
	$[^3H]H_2O$ incorporation to TGs (ng ^3H/[h g] tissue)			
Mammary glands	ND	ND	63.30 ± 6.63	73.57 ± 16.12
Liver	ND	ND	11.95 ± 2.69	5.05 ± 1.00[a]

Values are means ± SEM for groups of six to seven rats.
[a]$p < 0.05$ compared with the respective control groups using ANOVA and the Bonferroni *post hoc* test.
PTU, 6-propyl-2-thiouracil; ACC, acetyl CoA carboxylase; TGs, triglycerides; HypoT, hypothyroidism; ND, not determined.

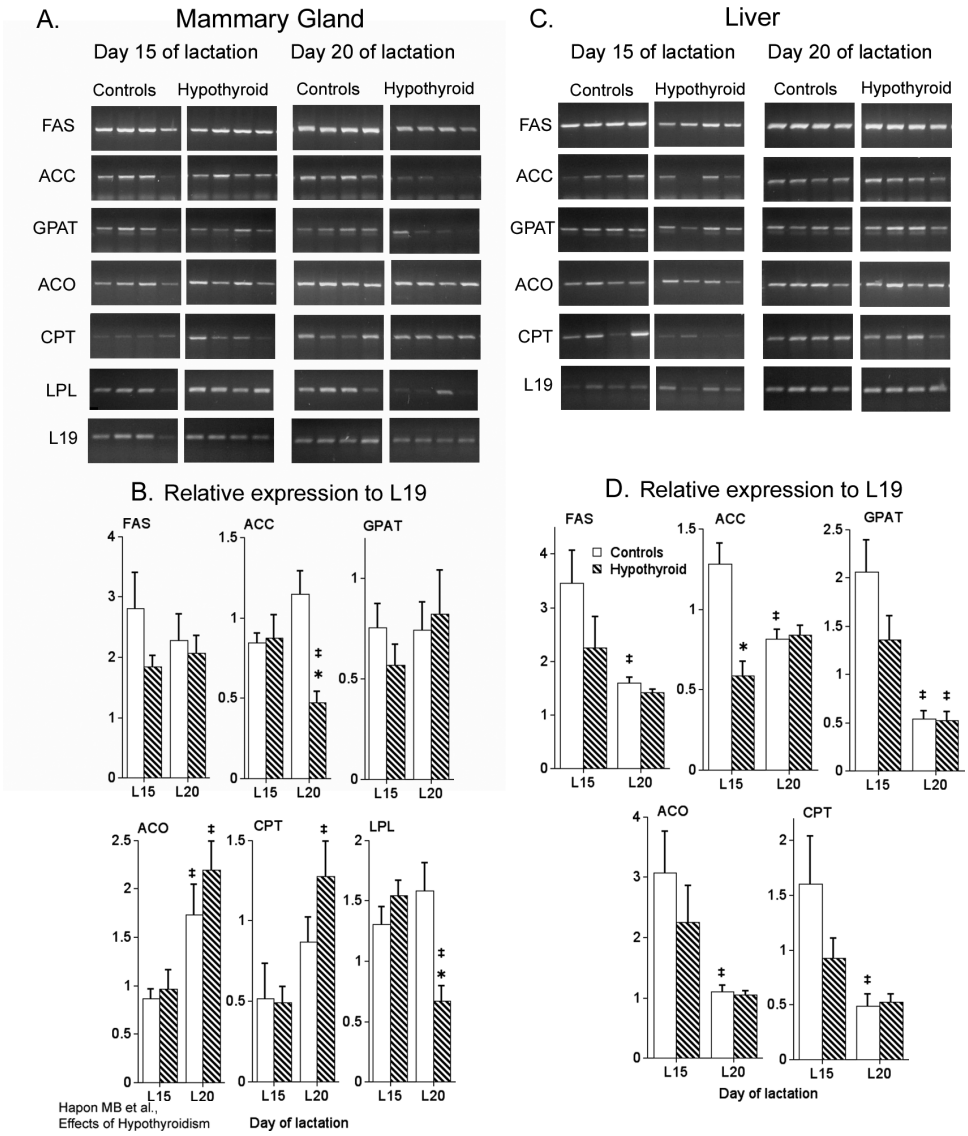

FIG. 2. Expression of mammary (**A, B**) and liver (**C, D**) enzymes related to lipid metabolism in control and HypoT rats on days 15 and 20 of lactation. Measurement by RT-PCR of expression of L19, FAS, ACC, GPAT, ACO, CPT, and LPL. (**A, C**) Ethidium bromide fluorescence photographs of the gel electrophoresis of the amplification products. (**B**) Relative expression of FAS, ACC, ACO, GPAT, CPT, and LPL relative to L19. The gel photographs were quantified using NIH Image and expressed as arbitrary units. Results are expressed as the mean ± SEM. $^{*}p < 0.05$ compared with the respective control groups using two-way ANOVA and the Bonferroni *post hoc* test. $^{\ddagger}p < 0.05$ compared with the respective L15 group using two-way ANOVA and the Bonferroni *post hoc* test.

sured the expression of ACO and CPT, enzymes related to fatty acid β oxidation. In controls, mammary ACO and CPT mRNAs increased between days 15 and 20 of lactation, but only the increase in ACO was significant. HypoT had no significant effect compared with controls (Fig. 2).

Effect of hypothyroidism on liver lipid metabolism in lactating rats

As an index of maternal lipid metabolism, we determined some hepatic parameters of lipid metabolism. Liver ACC activity was significantly diminished on days 15 and 20 of lactation (Table 2), FAS activity was not modified by HypoT (not shown), and $[^3H]H_2O$ incorporation to TGs was significantly diminished (Table 2), while there were no differences in $[^3H]H_2O$ incorporation to cholesterol or PL (not shown). HypoT also increased liver TG content on day 20 of lactation (controls: 11.34 ± 0.3, HypoT: 13.14 ± 0.6 mg/g tissue; $p < 0.05$ using Student's *t*-test), without modifying total lipid, cholesterol, PL, or total protein content (not shown). The mRNA abundance of ACC, FAS, GPAT, ACO, and CPT were measured using semiquantitative RT-PCR of liver RNA prepared from rats on days 15 and 20 of lactation. All mRNA abundances decreased significantly on both groups of rats on day 20 of lactation compared with day 15 (Fig. 2). HypoT decreased the mRNAs of all the enzymes on day 15, but only ACC achieved statistical significance (Fig. 2), while there was no effect on day 20.

Serum parameters related to lipid metabolism

Since circulating insulin, glucose, and lipids play a role in the regulation of lipid tissue content, metabolism, and secretion, we measured the concentrations of insulin, TGs, cholesterol fractions, PLs, and glucose in the serum of lactating control and HypoT rats fasted on the previous night on days 7, 15, and 20 of lactation (Table 3). Circulating glucose concentrations in controls peaked on day 15, while in the HypoT rats they were significantly higher than controls on day 7 and fell progressively to values significantly lower than controls on day 20. Circulating insulin was always diminished in the HypoT rats, but the difference was significant only on day 15 (Table 3).

Total lipids and TGs were maximal on day 7 in controls and fell significantly on days 15 and 20, respectively. HypoT rats had increased total lipids on days 7 and 20 of lactation, while TGs were decreased on days 7 and 15 (Table 3). As previously found in virgin and pregnant rats (11), HypoT also increased total and [LDL + VLDL] cholesterol on the 3 days of lactation studied, but did not modify HDL cholesterol or PLs (Table 3).

Discussion

The main effect of PTU-induced HypoT during lactation found in the present experiments was the drastic diminution in milk quality, demonstrated by the decrease in TGs observed in mid and late lactation and in milk lactose on mid lactation, which may contribute significantly, along with the impaired milk ejection and the hypothyroid state of the pups (2), to the severe growth deficit previously observed in the litters born to HypoT mothers (2).

Lipid metabolism is very sensitive to alterations in the thyroid state, and since mammary lipid synthesis and utilization are essential for the normal function of the gland and for milk synthesis, the effects of HypoT on lipids during lactation may have important consequences for the well-being of the mother and newborn. Clinical HypoT during lactation may limit the capacity of the maternal organism to sustain itself and the newborn adequately, thus compromising its nutrition (2).

Fatty acid synthesis is under complex hormonal and nutritional control. Thyroid hormones regulate lipogenesis by altering levels of ACC and FAS mRNAs (32). We have previously found that in virgin and lactating rats, hyperthyroidism increased hepatic FAS and ACC activities (33,34), while PTU-induced HypoT decreased lipid synthesis and lipogenic enzyme activities and mRNAs in liver and mammary tissues from pregnant rats (11). In the present study we found a diminution in hepatic lipid synthesis evidenced by the decreases in incorporation of $[^3H]H_2O$ to TGs and ACC expression and activity in lactating HypoT rats, consistent with the described inhibitory effects of HypoT in male rats (35,36). Lactation is a state of decreased circulating VLDL. Smith *et al*. (37) suggest that TG-rich particles are rapidly cleared from circulation during lactation through the action of mammary gland LPL to supply lipids for milk production. In the HypoT mothers, the reduced hepatic TG synthesis associated with their decrease in serum could indicate less secretion of TG-rich particles into circulation during mid to late lactation. In agreement with our results, Davidson *et al*. described that in rats, HypoT suppressed hepatic TG assembly and secretion (38). Thus, the decreased liver TG synthesis in the lactating HypoT rats may be responsible for the decrease in circulating TGs, compromising the fulfillment of lactational TG requirements.

The lipid synthetic capacity of the mammary gland also seems to be compromised, as evidenced by the decreased ACC activity on days 15 and 20 and ACC mRNA on the latter day. In addition, the diminished mammary LPL mRNA on day 20 may indicate that the mammary tissue is incapable of incorporating sufficient exogenous TGs, which, along with the depressed TG synthetic capacity, may be directly responsible for the difference in TG milk content between controls and HypoT rats, which became more marked as lactation proceeded.

Thyroid hormones have profound effects on liver fatty acid oxidation, stimulating it in the hyperthyroid state (39,40), while hypothyroid states decrease it (40). In accordance with this, we found that HypoT tended to decrease liver ACO and CPT mRNA levels at mid lactation. Since CPT is regulated by malonyl CoA availability, the diminished

Table 3. Effects of PTU Treatment on Serum Concentrations of Glucose, Insulin, Proteins, and Different Lipids in Lactating Rats

	Day 7 of lactation		*Day 15 of lactation*		*Day 20 of lactation*	
	Control	*HypoT*	*Control*	*HypoT*	*Control*	*HypoT*
Glucose (mg/mL)	0.75 ± 0.03	1.02 ± 0.08^{a}	0.91 ± 0.03	0.89 ± 0.05	0.78 ± 0.03	$0.67 \pm 0.02^{a,b}$
Insulin (ng/mL)	6.08 ± 1.2	4.17 ± 0.48	6.94 ± 0.98	4.25 ± 0.69^{a}	5.51 ± 0.58	4.14 ± 0.46
Total lipids (mg/mL)	3.37 ± 0.15	4.55 ± 0.24^{a}	3.12 ± 0.25	2.69 ± 0.25^{b}	$2.39 \pm 0.16^{b,c}$	$3.98 \pm 0.24^{a,c}$
Triglycerides (mg/mL)	0.83 ± 0.15	0.48 ± 0.05^{a}	0.45 ± 0.06^{b}	$0.22 \pm 0.06^{a,b}$	0.47 ± 0.05^{b}	0.47 ± 0.05^{c}
Phospholipids (mg/mL)	1.28 ± 0.03	1.26 ± 0.08	1.29 ± 0.09	1.28 ± 0.08	1.35 ± 0.05	1.23 ± 0.07
Total cholesterol (mg/mL)	0.59 ± 0.03	0.69 ± 0.01^{a}	0.58 ± 0.05	0.79 ± 0.05^{a}	0.55 ± 0.06	$0.97 \pm 0.06^{a,b,c}$
HDL cholesterol (mg/mL)	0.35 ± 0.02	0.30 ± 0.02	0.33 ± 0.05	0.36 ± 0.04	0.35 ± 0.04	0.39 ± 0.03
[LDL + VLDL] cholesterol (mg/mL)	0.25 ± 0.03	0.38 ± 0.02^{a}	0.28 ± 0.04	0.42 ± 0.05^{a}	0.28 ± 0.04	$0.56 \pm 0.04^{a,b,c}$

Values are means ± SEM for groups of eight rats.

[a] $p < 0.05$ compared with the respective control groups using two-way ANOVA and the Bonferroni *post hoc* test.

[b] $p < 0.05$ compared with the respective day 7 group using two-way ANOVA and the Bonferroni *post hoc* test.

[c] $p < 0.05$ compared with the respective day 15 group using two-way ANOVA and the Bonferroni *post hoc* test.

PTU, 6-propyl-2-thiouracil; HypoT, hypothyroidism; HDL, high-density lipoprotein; LDL, low-density lipoprotein;VLDL, very low-density lipoprotein.

ACC expression may result in decreased malonyl CoA that may compensate for the effects of HypoT, resulting in no significant change in expression, as observed *in vitro* by Muller *et al*. (41). HypoT had no significant effect on mammary fatty acid oxidation enzymes, but we observed an increase in these enzymes between days 15 and 20 of lactation, which was more marked in the HypoT rats, and which may be related to the initiation of involution triggered by the gradual instauration of weaning (31) plus a degree of milk accumulation caused by the impaired milk ejection reflex observed in HypoT mothers (2).

Thyroid hormones affect carbohydrate metabolism at various levels, ranging from modulation of insulin secretion and receptor levels to direct actions on glucose uptake and utilization (42,43). HypoT lowered insulin in male (42) and nonpregnant females (11) and did not modify the low levels found during late pregnancy (11), but decreased circulating insulin in the lactating rats. There is clear evidence that lactation increases mammary insulin receptors (44) and that liver and mammary lipogenesis is markedly stimulated by insulin in the lactating rat. Thus, the decreased circulating insulin could be a possible cause of the diminution of lipogenesis in these two tissues and indirectly of the lower levels of TG in milk.

In conclusion, HypoT had a significant effect on milk composition, evidenced mainly by a reduction in TGs. This condition seems to be a result, primarily, of impaired mammary lipid synthetic capacity accompanied with reduced availability of fatty acids from circulation caused by the decrease in circulating TGs and insulin as well as in the expression of LPL on late lactation. The decrease in circulating TGs, in turn, may be a direct consequence of the diminished liver lipogenic activity and lipid content, which is a well-known effect of the thyroid dysfunction. The diminished milk quality complicates further the previously described lactational deficit of the hypothyroid mothers (2).

As has been suggested by Matsuno *et al*. (45), the implication of our findings for humans may be limited, given that the proportion of total energy required for lactation in rats is much greater than that in humans. However, because mammary gland physiology is similar across species, biological concepts developed from the lactating rat model may be instructive for human lactation.

Acknowledgments

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Address reprint requests to:
Graciela A. Jahn
Laboratorio de Reproducción y Lactancia
IMBECU-CRICYT-CONICET
C.C. 855
5500 Mendoza
Argentina

E-mail: gjahn@lab.cricyt.edu.ar