PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 394–396 (1978)

Mammary Arterial and Venous Concentrations of Serum Insulin in Lactating Dairy Cows[1] (40355)

N. F. G. BECK[2] AND H. A. TUCKER

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48824

Insulin is essential for lactation (1). Concentrations of insulin in plasma increase with lactogenesis in rats (2) and increase in sera of cows as lactation progresses (3, 4). Furthermore, blood collected from cows immediately after milking contained greater concentrations of insulin than blood collected 2–4 hr before or 1 hr after milking (3). Presumably for insulin to affect mammary tissue it must be removed from blood and bound to mammary cells. Indeed *in vitro* studies of mammary epithelial cells from lactating mice showed that ^{125}I-insulin binds to membrane receptors (5). The primary objective of the present study was to measure arteriovenous (A-V) differences in serum insulin across the mammary glands of cows around milking.

Materials and methods. Twelve Holstein cows, six lactating 5–12 weeks and six lactating 37–57 weeks were used. Cows were maintained in stanchions and fed a ration of 18 kg of corn silage, 4.5 kg alfalfa-grass hay and 1 kg of grain concentrate per 2.5 kg of milk produced. Water was provided *ad libitum*.

One cannula was implanted surgically into an external pudendal artery and another into a subcutaneous abdominal mammary vein as previously described (6). Experiments commenced 3–5 days after surgery when milk yields approximated pre-surgery quantities.

Cannulas were flushed approximately 2 hr before each experiment and blood was collected and discarded every 15 min to accustom cows to sampling. Arterial and venous samples of blood were collected simultaneously on three consecutive afternoons at 30, 25, 20, 15, 10, 8, 6, 4, 2 and 0 min before milking and at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, and 60 min after milking. At time 0 the mammary glands were washed for 20–30 sec and then milked for 3–5 min with a mechanical milking machine. Milking occurred at approximately 1500 hr each day. Blood was stored at ≃25° for 2 hr, at 5° for 24–36 hr and then centrifuged at 2500*g* for 15 min. Sera were stored frozen at −20° prior to assay for insulin. Radioimmunoassay for insulin was as described previously (3, 7). Standard bovine insulin (Lot No. 795372; 24.2 units/mg) was provided by Eli Lilly and Co. (Indianapolis, IN). Hormone concentrations were determined in duplicate in each serum sample and accepted when agreement between duplicates was within ±5%. Within each time of sampling insulin concentrations were averaged across the three experimental replicates (days) for each cow. These values were used in a split-plot analysis of variance (8).

Results. Insulin in arterial and venous sera of cows lactating 5–12 weeks averaged (±SE) overall throughout the experiment 2.7 ± 0.4 and 2.6 ± 0.4 ng/ml, respectively (Fig. 1). In cows lactating 37–57 weeks insulin averaged 3.6 ± 0.2 and 3.4 ± 0.1 ng/ml, respectively. Insulin was greater in arterial ($P \simeq 0.08$) and venous ($P \simeq 0.09$) sera of cows lactating 37–57 weeks as compared with insulin in cows lactating 5–12 weeks. Stimuli associated with milking did not affect concentrations of serum insulin in either early or late lactating cows.

For the 30 min before milking, mammary arterial concentrations of insulin were 0.13 ± 0.04 ng/ml greater ($P < 0.05$) than venous concentrations in cows 5–12 weeks postpartum. In cows lactating 37–57 weeks the A-V difference was 0.22 ± 0.08 ng/ml, but this difference was not significant ($P > 0.05$). During the 20 min beginning at milking arterial concentrations of insulin were greater ($P < 0.05$) than venous concentrations in

[1] Michigan Agricultural Experiment Station Journal Article No. 8599. This research was supported in part by USPHS Grant No. AM-15899.

[2] Present address: Physiological Laboratory, Cambridge, England.

0037-9727/78/1593-0394$01.00/0

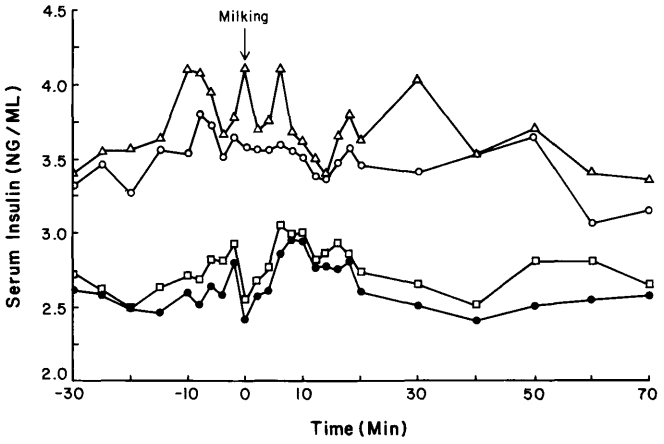

FIG. 1. Insulin concentrations in serum samples from early (5–12 weeks postpartum) lactating (arterial □——□; venous ●——●) and late (37 to 57 weeks postpartum) lactating (arterial △——△; venous ○——○) cows before, during and after milking which began at 0 min and lasted 3–5 min. Each point is the mean serum insulin concentration from three replicates in each of six cows. Pooled SE among early lactating cows were 0.4 and 0.4 ng/ml for arterial and venous samples, respectively. Among late lactating cows the pooled SE were 0.2 for arterial samples and 0.1 ng/ml for venous samples, respectively.

early (0.12 ± 0.03 ng/ml) and late (0.17 ± 0.04 ng/ml) lactating cows. Between 30 and 70 min postmilking, the A-V differences in concentrations of insulin in cows 5–12 weeks postpartum (0.20 ± 0.09 ng/ml) or 37–57 weeks postpartum (0.23 ± 0.14 ng/ml) were not significant ($P > 0.05$). Stage of lactation did not affect ($P > 0.05$) A-V differences in insulin.

To investigate mammary uptake of insulin, mammary blood flow (MBF) was calculated using the equation of Kronfeld *et al.* (9) in which MBF = 1.0 + 0.42 ×, where × is daily milk yield. Daily milk yields averaged 22.9 kg in early lactating cows and 13 kg in late lactators. Thus, MBF were estimated to be 10.6 and 6.5 liters/min. A-V differences averaged 0.15 and 0.21 ng/ml for cows in early and late lactation, respectively. Theoretical mammary uptakes of insulin (calculated by multiplying MBF by A-V differences) were 1.6 and 1.4 μg/min in early and late lactating cows, respectively.

Discussion. Serum insulin concentrations in both early and late lactating cows remained reasonably constant in the 25 samples of serum collected between 30 min prior to milking through 70 min after milking. This is in contrast with the previous report of Koprowski and Tucker (3) who observed greater concentrations of insulin in sera collected within 5 min of milking compared with sera collected 2–4 hr before or 1 hr after milking. The cause of the discrepancy is unknown, but may be associated with differences in feeding schedule relative to milking. In any event the present study strongly suggests that milking does not cause an acute increase in concentrations of insulin in cows.

Insulin A-V differences across the mammary gland were positive and remarkably similar regardless of time relative to milking or stage of lactation (and milk yield). Maintenance of positive A-V differences in serum insulin during the interval from 30 min before through 70 min after milking suggests the possibility of continuous mammary uptake of the hormone from arterial blood. We speculate this uptake of insulin is probably essential for regulation of uptake of metabolites and maintenance of lactation (1).

The greater concentrations of serum insulin observed in late lactating cows producing 43% less milk per day compared with early lactating cows agrees with previous reports (3, 4). In dairy cows, serum insulin concentrations are negatively correlated with milk yield (3), and greater concentrations of serum insulin in beef cattle, compared with dairy cattle, may be associated with their lower rate of milk production (10). Also, administration of insulin suppresses milk yields in cattle unless exogenous glucose is supplied simultaneously (11). Since numbers of mammary

secretory cells decrease with advancing lactation or decreasing milk yields (12) while total uptake of insulin remained essentially constant, the uptake of insulin per mammary cell theoretically increases with advancing lactation. If and how the theoretically greater uptakes of insulin per mammary cell are associated with suppression of milk synthesis remains to be determined. On the other hand, serum insulin increases as feed intake increases relative to maintenance requirements (13). In our study early and late lactating cows were fed the same rations. Most likely the late lactating cows were fed in excess of requirements for milk yield. Thus, the increased serum insulin during late lactation may be related to diet and only coincidently related to milk production.

Summary. Insulin averaged 2.6 ng/ml in mammary arterial and venous sera collected from 30 min before to 70 min after milking of cows lactating 5–12 weeks. During the same period in cows lactating 37–57 weeks insulin increased to 3.5 ng/ml. Milking did not affect insulin concentrations during early or late lactation. Arteriovenous (A-V) differences averaged 0.17, 0.14 and 0.22 ng/ml for 30 min before, 0–20 min after and 30–70 min after milking. Stage of lactation (and yield of milk) did not affect A-V differences. Mammary uptakes of insulin averaged 1.6 and 1.4 μg/min in early and late lactating cows, respectively. Maintenance of uptakes of insulin may be associated with uptake of metabolites essential for maintenance of lactation.

The authors gratefully acknowledge the assistance of Drs. W. D. Oxender, D. J. Krahwinkle and T. W. Riebold for developing surgical techniques, and Dr. Roger Neitzel for statistical and computer programming help.

1. Tucker, H. A., *in* "Lactation: A Comprehensive Treatise" (B. L. Larson and V. R. Smith, eds.) Vol. 1, p. 277. Academic Press, New York (1974).
2. Sutter-Dub, M. Th., Leclercq, R., Sutter, B. Ch. J., and Jacquot, R., Horm. Metabol. Res. **6,** 297 (1974).
3. Koprowski, J. A., and Tucker, H. A., Endocrinology **93,** 645 (1973).
4. Smith, R. D., Hansel, W., and Coppock, C. E., J. Dairy Sci. **59,** 248 (1976).
5. O'Keefe, E., and Cuatrecasas, P., Biochim. Biophys. Acta **343,** 64 (1974).
6. Beck, N. F. G., Tucker, H. A., and Oxender, W. D., Endocrinology, accepted for publication.
7. Grigsby, J. S., Oxender, W. D., Hafs, H. D., Britt, D. G., and Merkel, R. A., Proc. Soc. Exp. Biol. Med. **147,** 830 (1974).
8. Gill, J. L., and Hafs, H. D., J. Anim. Sci. **33,** 331 (1971).
9. Kronfeld, D. S., Raggi, F., and Ramberg, C. F., Amer. J. Physiol. **215** (1968).
10. Hart, I. C., Bines, J. A., Balch, C. C., and Cowie, A. T., Life Sci. **16,** 1285 (1975).
11. Kronfeld, D. J., Mayer, G. P., Robertson, J. M., and Raggi, F., J. Dairy Sci. **46,** 559 (1963).
12. Tucker, H. A., J. Dairy Sci. **52,** 721 (1969).
13. Trenkle, A., J. Anim. Sci. **31,** 389 (1970).

Received July 10, 1978. P.S.E.B.M. 1978, Vol. 159.