Increased milking frequency (IMF) during early lactation increases milk yield not only during the IMF period, but also during continuing lactation after a return to twice daily milking \((2\times)\). The increase in milk yield is locally regulated within the mammary gland; however the mechanism for the increase in milk yield is unknown. The objective of this study was to demonstrate a difference in milk and component yield, both during the IMF period and throughout remaining lactation, and examine potential local regulation mechanisms that increase production due to early lactation IMF. Eight multiparous dairy cows were assigned to unilateral frequent milking \([2\times \text{left udder half and } 4\times\text{-daily milking (}\times\text{right udder half)}\] for the first 21 d of lactation. Both udder halves were milked \(2\times\) for the remainder of lactation. Milk weights from each udder half were recorded at 7, 14, 21, 60, 120, and 180 DIM. Mammary biopsies were obtained from each rear quarter at 21 DIM, and tissue was used to measure protein and mRNA expression. During the IMF period, between 0 and 21 DIM, \(4\times\text{ milking increased milk yield } 5.5 \pm 0.5 \text{ kg/d more than } 2\times (P < 0.05).\) Between 60 and 180 DIM, the \(4\times\text{ half} \) tended to produce 4.4 \(\pm 0.9 \text{ kg/d more than the } 2\times \text{ half} (P < 0.1).\) Overall, between d 0 and d 180, IMF increased milk yield by 4.6 \(\pm 0.5 \text{ kg/d more than } 2\times (P < 0.05).\) The \(4\times\) gland had reduced phosphorylated and activated Akt \((\text{phospho:total Akt})\) compared with the \(2\times\) gland \((P < 0.05).\) There was no significant difference between the mRNA expression of IGF-1 or IGFBP-5 between \(4\times\) and \(2\times\) milking. There was no significant difference between total and phosphorylated STAT5, however the \(4\times\) gland had increased activation of STAT5 \((\text{phospho:total STAT5})\) compared with the \(2\times\) gland \((P < 0.06).\) There was no significant difference between the expression of total, phospho, or activated STAT3. We conclude that early lactation IMF significantly increased milk yield throughout lactation, and this increase in milk yield may be regulated by changes in cell activity resulting from activation of STAT5. 

**Key Words:** milking frequency, lactation, STAT5