

Analysis of Glutamine Synthetase (GS) Expression in Mice during Lactation

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Abstract

Although breast milk is considered the ideal source of nutrition for newborns, it has been shown that the amino acids in the milk, including glutamine, are not at optimal concentrations and could be improved. It has been found that synthesis of glutamine within the mammary gland only accounts for 50-60% of the glutamine that is found in milk. Finding the source of the other 40-50% of glutamine would allow us to determine how best to meet the nutritional needs of the newborn and promote maternal health during lactation.

In this study, we used western blotting to detect changes in glutamine synthetase (GS) abundance in order to determine the tissues within the body from which the additional glutamine originates, as GS is the only enzyme capable of synthesizing glutamine. We have found a statistically significant decrease in glutamine synthetase abundance in liver tissue during early and peak lactation (PD6 and PD16) as compared to the virgin control samples. There were no significant changes in glutamine synthetase levels found in muscle tissue. Glutamine synthetase abundance in adipose tissue was shown to increase significantly at peak lactation and involution. Therefore, the 40-50% of glutamine in the milk not synthesized in the mammary gland possibly comes from non-mammary adipose tissue.

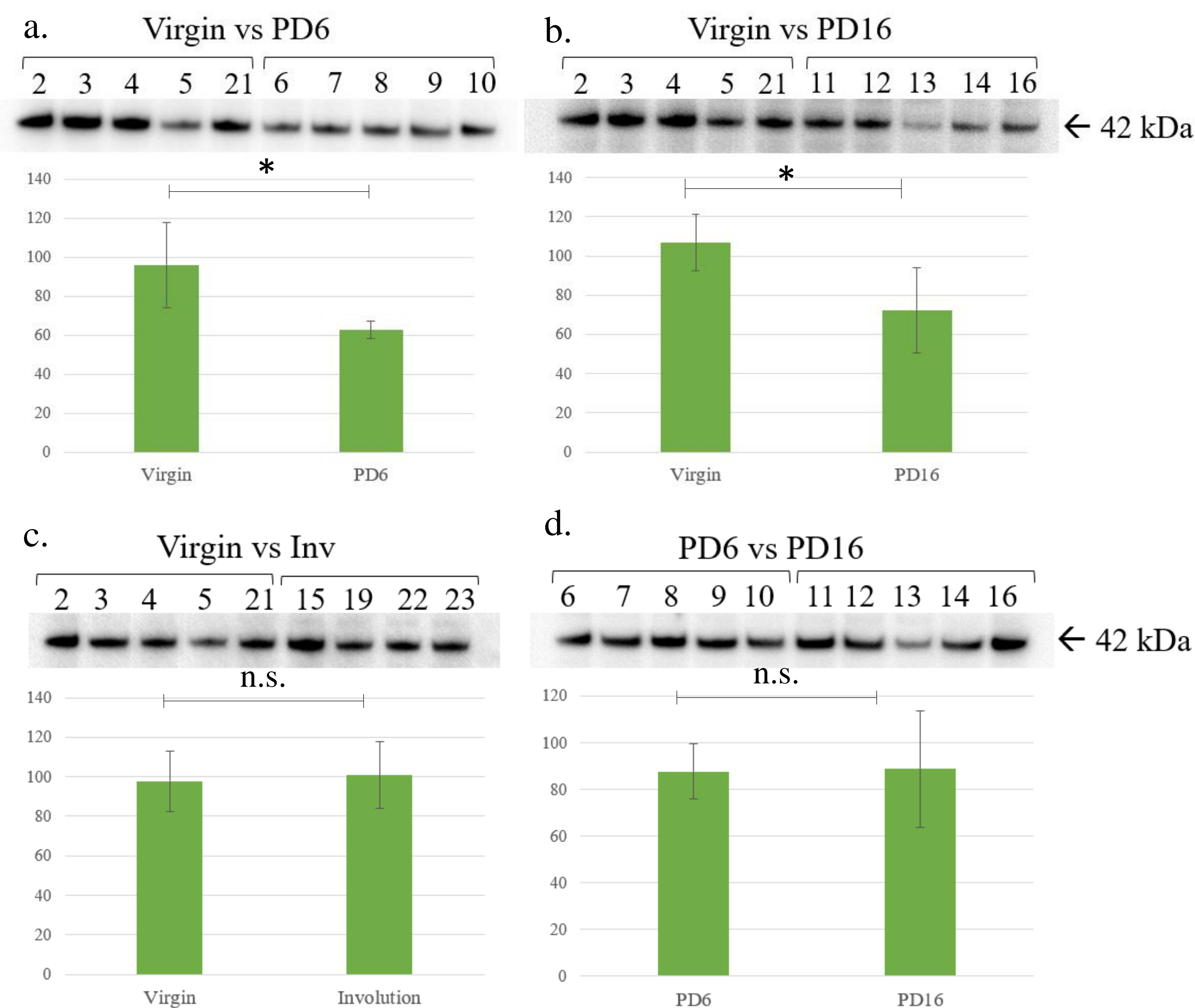


Figure 1. Analysis of the Abundance of GS in Liver Tissue. 5µg of protein was loaded in each lane.

References

1. Trottier, NL et al. (1997). *J. Anim. Sci.* 75: 1266-1278
2. Haynes, TW et al. (2009). *Amino Acids* 37: 131-142
3. Curthoys, NP, Watford, M. (1995) *Annu. Rev. Nutr.* 15:133-59.

Background

- Glutamine is the most abundant free alpha amino acid and plays an important role in the development of the neonatal intestine and immune system
- Previous studies have indicated that concentration of glutamine in the milk is not at optimal concentrations
- Synthesis of glutamine within the mammary gland only accounts for 50-60% of the glutamine that is found in milk
- Glutamine synthetase is the only enzyme able to synthesize glutamine
- In the non-lactating state, the liver, skeletal muscle, adipose tissue, and lung are sites of net glutamine synthesis

Methods

- Western blots were done to compare GS presence in virgin control vs post-delivery (PD) day 6 (early lactation), PD day 16 (peak lactation), and PD day 25 (involution)
- N=4-5 for each group
- 1:5000 dilution of anti-GS primary antibody and anti-mouse secondary antibody was used to detect GS presence in western blotting
- Bovine serum albumin (BSA) was used to block the membrane
- ImageJ was used to quantify the western blots

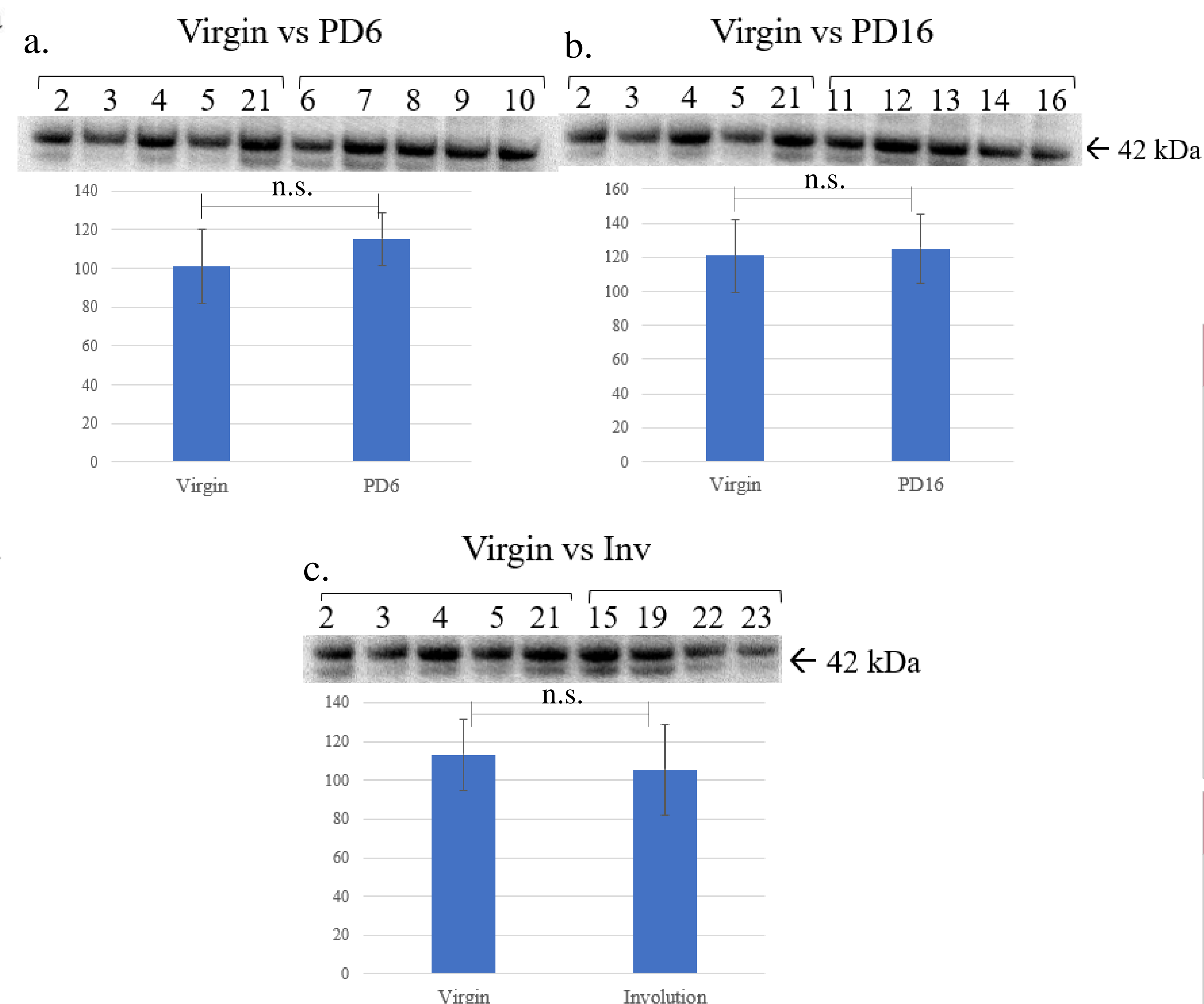


Figure 2. Analysis of the Abundance of GS in Muscle Tissue. 10µg of protein was loaded in each lane

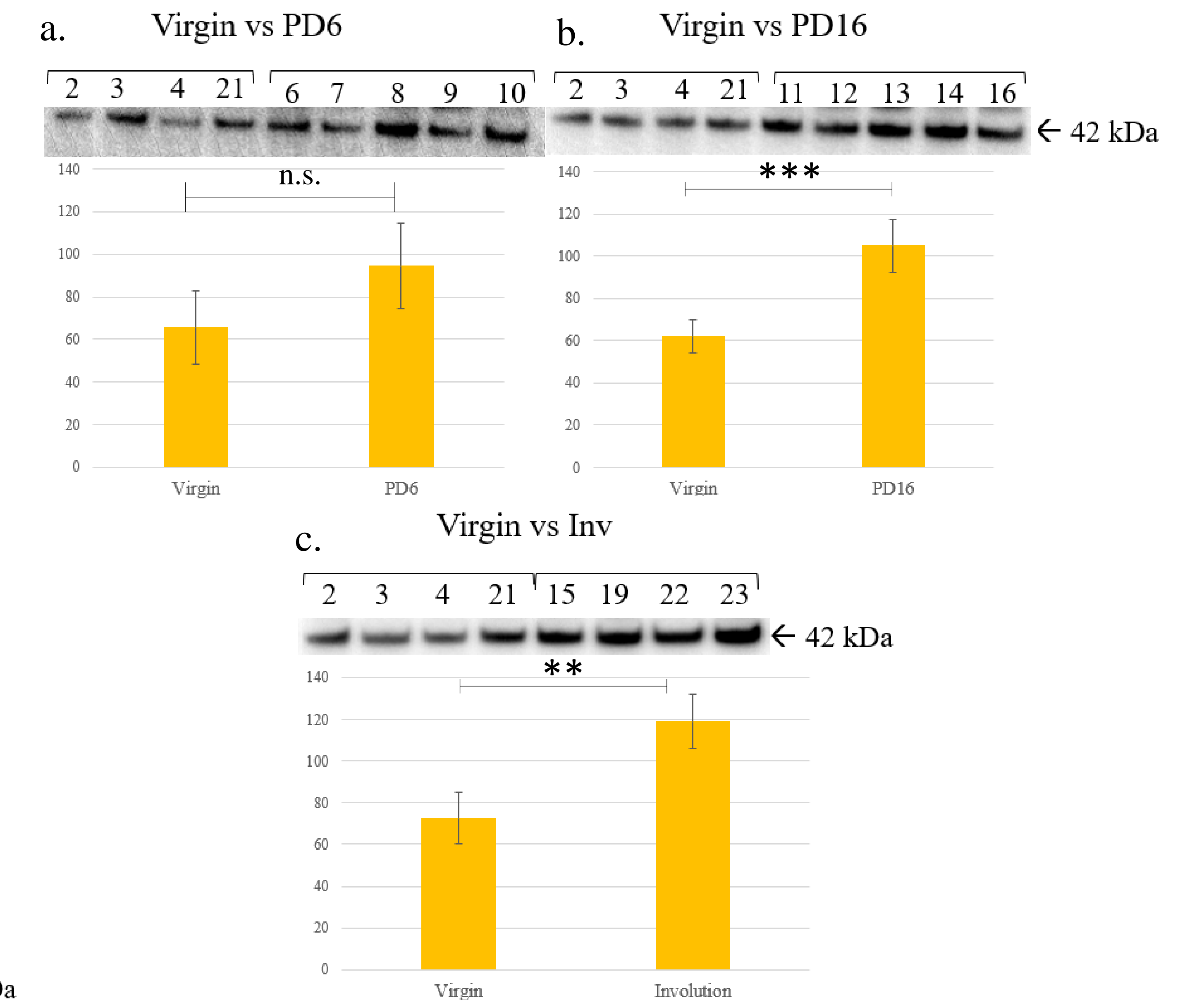


Figure 3. Analysis of the Abundance of GS in Retroperitoneal Adipose Tissue. 10µg of protein was loaded in each lane

Results

- Statistically significant decrease was seen between virgin control and PD6 ($p < 0.05$) and virgin and PD16 ($p < 0.05$) liver samples, indicating that GS abundance decreases in liver tissue during lactation (Fig 1a and b)
- No significant difference in GS presence between virgin and involution liver samples or between PD6 and PD16 liver samples (Fig 1c and d)
- No significant changes in GS levels were seen in muscle tissue (Fig 2)
- An increase was observed in the GS abundance at PD6 compared to virgin control in adipose tissue (not statistically significant) (Fig 3a)
- Statistically significant increase was seen between virgin control and PD16 ($p < 0.001$) as well as virgin control and involution ($p < 0.01$) adipose tissue samples (Fig 3b and c)

Conclusion

- Glutamine synthesized outside of mammary gland during lactation potentially comes from non-mammary adipose tissue
- Activity assays will need to be conducted to verify that the GS detected were enzymatically active
- In vivo studies measuring the flux of glutamine out of the cell in these tissues

Acknowledgement

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