Final Report

Comparing the Release Profile of Amino Acids from Vegan and Animal Type I Collagen Supplements in an *In Vitro* Model of the Digestive Tract.

Prepared for
VeCollal

By Aelius Biotech

August 2024

Aims & Background

Type I collagen supplements are typically found as hydrolysed collagen peptides from animal sources, such as porcine, bovine or marine sources. These animal collagens are not representative of human Type I collagen and are not suitable for vegetarians or vegans. As a solution, VeCollal have developed innovative, vegan friendly, amino acid supplements that contain an animo acid profile representative of human type I collagen.

This project aims to use Aelius Biotech's expertise in gastrointestinal physiology and analytical capabilities to understand the release and absorption profiles of free amino acids. Analysis will include all essential and non-essential amino acids including hydroxyproline. This stage of the project is focused on assessing the release profile of amino acids from two VeCollal products and two animal collagen products in the Aelius Biotech Model Gut System, which accurately simulates the environment of the digestive tract.

Samples

Sample doses are standardised to a VeCollal dose of 5 grams, which contains 4.505 g of amino acid. Amino acid/protein in the other products is matched to this 4.505 g of amino acid.

1. VeCollal Collagen Amino Acid Formulation (5 g dose; 4.505 g of amino acid) (n=3)
2. VeCollal II Amino Acid Formulation (7.958 g dose; 4.505 g of amino acid) (n=3)
3. Bovine Collagen Peptides (5.01 g dose; 4.505 g of amino acid) (n=3)
4. Marine Collagen Peptides (4.92 g dose; 4.505 g of amino acid) (n=3)

Conditions

1. Background (No collagen product)
2. Test (Collagen product added to model gut)

Methods

An *in vitro* simulation of gastrointestinal digestion cumulative of the mouth, stomach and small intestine was carried out as per the published methods of (Houghton et al., 2014).

Procedure

1. Add 10 mL saliva to 10 mL water [Volume 20 mL]
2. Add substrate if required.
    a. VeCollal Collagen Amino Acid Formulation (5 g dose) (n=3)
    b. VeCollal II Amino Acid Formulation (7.598 g dose) (n=3)
    c. Bovine Collagen Peptides (5.01 g dose) (n=3)
    d. Marine Collagen Peptides (4.92 g dose) (n=3)
3. Add 50 mL Gastric Juice
    a. At T0 also initiate the pumping of gastric secretions into the vessel at 0.5 mL/min (4.2 rpm) for 1 hour
4. At T60 add 25 ml Bile [65ml]
5. Pump in SI secretions at 0.5 mL/min for 2 hours

Sample at the following time course:

0, 15, 30, 60A (pre bile), 60B (post bile), 75, 90, 120, 150, 180 by adding 500 µL of sample to 500 µL of 10% trichloroacetic acid. Spin down samples at 13,000 xg for 10 min. Aliquot samples into new tubes and freeze until further analysis.

HPLC analysis to be carried out on an Agilent 1260/1290 system using the Advancebio amino acid analysis (AAA) kit (Agilent Technologies, CA, USA).

Results

Summary

To avoid confusion between the VeCollal supplements, they will be referred to as Type I and Type II.

In most cases the amount of free amino acid detected in the system was much greater for the VeCollal vegan collagen supplements than in the animal supplement. This is to be expected as most gut exopeptidases (the enzymes responsible for the liberation of amino acids from the N or C termini of proteins and peptides) are present in the brush border of the small intestinal epithelium – these enzymes would not be present in the digestive milieux, and so they are not modelled in the model gut system. Therefore, while the animal collagen proteins will be digested by gastric and pancreatic proteases such as pepsin, trypsin, chymotrypsin etc., only pancreatic carboxypeptidase will liberate amino acids from peptides and proteins outside of the brush border. Carboxypeptidase digestion will explain the small increase in amino acid content in most cases when using the animal collagen.

The data for the VeCollal collagen supplements also showed that amino acids were immediately detectable in most cases. This contrasts with the animal collagen where, if amino acids were detected, their appearance was not until the small intestinal phase – this will be due to the lack of exopeptidase in the gastric phase. We expect that this means far more amino acids are available for absorption for the VeCollal products in comparison to the animal products over the same time frame.

When observing the amino acid data for the VeCollal supplements, two scenarios were observed regarding the amount of each amino acid detected in the system.

1. The amount of amino acid detected in the system remained relatively consistent over time, indicating that the solubility of the amino acids does not improve over time while passing through the digestive tract.
2. The amount of amino acid detected in the system increased over time, indicating that amino acid solubility improves over time while passing through the digestive tract. The increase over time was mostly associated with the small intestinal phase rather than the gastric phase.

We also compared the amount of amino acid detected in the system to the amount we expected in the system based on the percentage of amino acid in each dose. The following observations were made:

1. In many cases the amount of amino acid detected in the system at the start of the experiment did not match with the expected amount, but steadily over time the amount increased to align with the amount expected.
2. In other cases, the amount of amino acid detected in the system did not align with the expected amount. Measured amounts in cases were either much lower, and did not rise over time to align with the expected amount; or much higher, and rose beyond what was expected in the system.

Total Amino Acid Data

This section will show the total amount of all amino acid detected in the system over time for the Type I and Type II VeCollal collagen supplements. This is presented as two graphs – one which shows how it compares to the total amino acid expected in the system, and a second showing a comparison of all collagens at the end of the model gut experiment. Also shown will be two separate graphs showing all the amount of amino acid detected in the system for all amino acids, for the Type I and Type II VeCollal products, respectively.

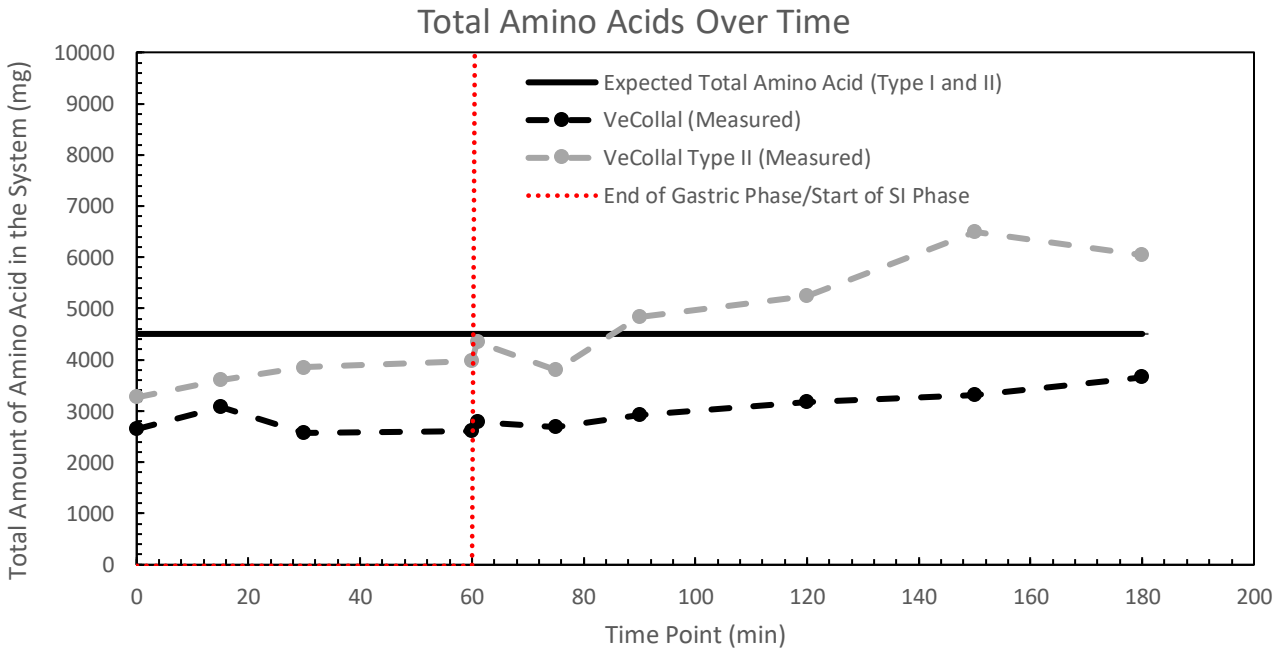

Figure 1. Total mg amount of amino acid detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I and Type II (Solid black line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); The mg amount expected for VeCollal Type I and VeCollal Type II was 4505 mg. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Point shows the sum of the means for each amino acid.

Key Points

- For VeCollal Type I, the expected amount of amino acid based on the dose of 5g was 4505 mg. The total amino acid observed in the system at the start of the experiment was 2656.4 mg, which is 58.9% of the expected total. This then rose over the course of the experiment to 3656.86 mg, which is 81.2% of the total expected amount.
- For VeCollal Type II, the expected amount of amino acid based on the dose of 7.958 g was 4505 mg. The total amino acid observed in the system at the start of the experiment was 3297.3 mg, which is 73.1% of the expected total. This then rose over the course of the experiment to 6043.2 mg, which is

134% of the total expected amount. Much more proline and glutamine was observed than expected, resulting in the higher total amino acids than expected.

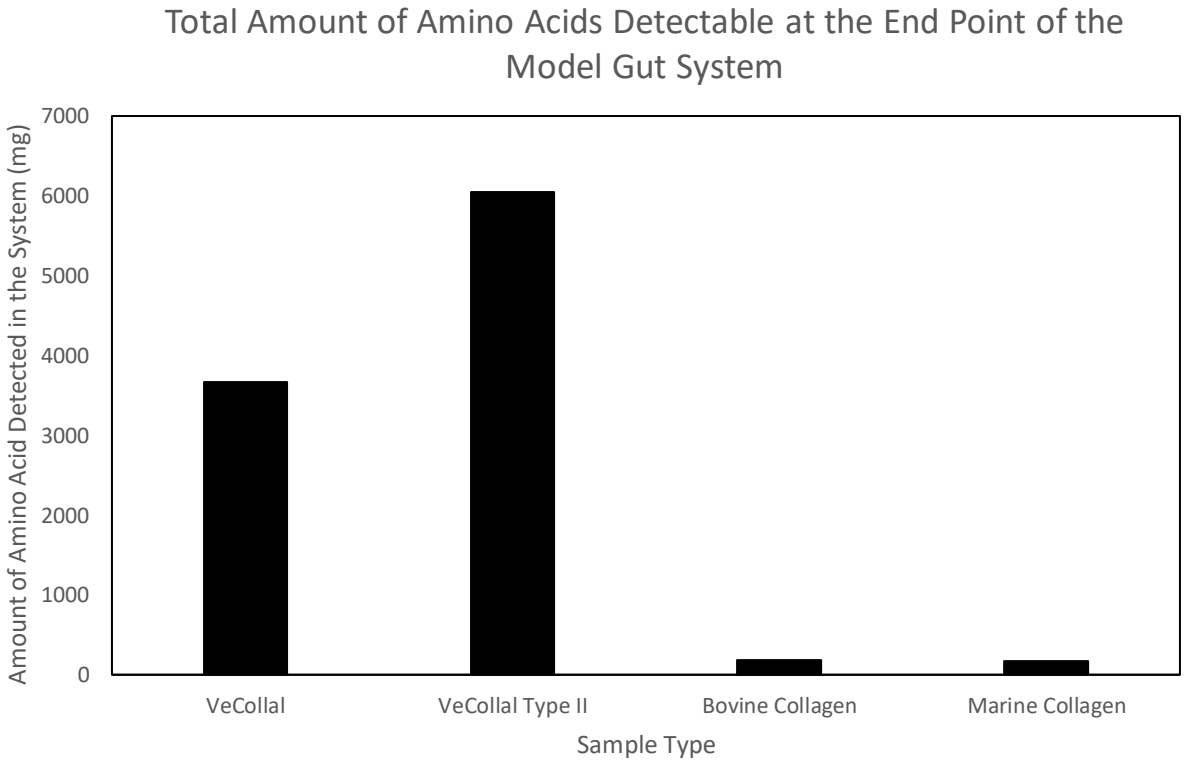

Figure 2. Total mg amount of amino acid detected in the Aelius Biotech Model Gut System after 180 minutes after dosing with collagen supplements. The x-axis shows sample type. Samples include VeCollal, VeCollal Type II, Bovine Collagen and Marine Collagen. The y-axis shows the total mg amount of amino acid in the system. VeCollal Type II yielded the most amino acids (6050.3 mg), followed by VeCollal (3667.4 mg). The bovine and marine collagen supplements yielded far less amino acids than the VeCollal supplements, with 188.4 mg and167.0 mg respectively.

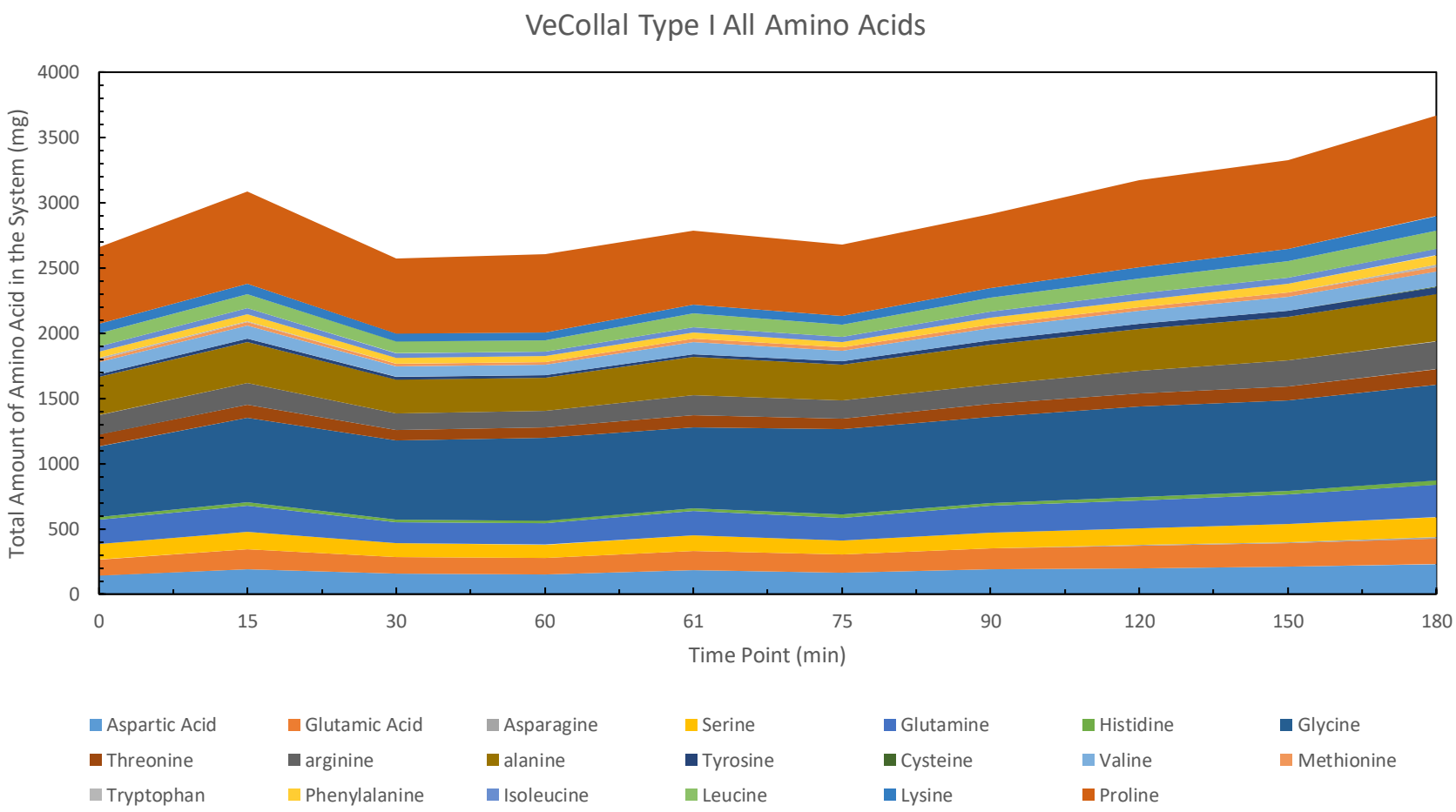

Figure 3. Stacked area chart showing the total mg amount of each individual amino acid analysed in this study, for the Type I VeCollal collagen supplement, detected in the Aelius Biotech Model Gut System over a time course of 180 minutes. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as per the legend. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value.

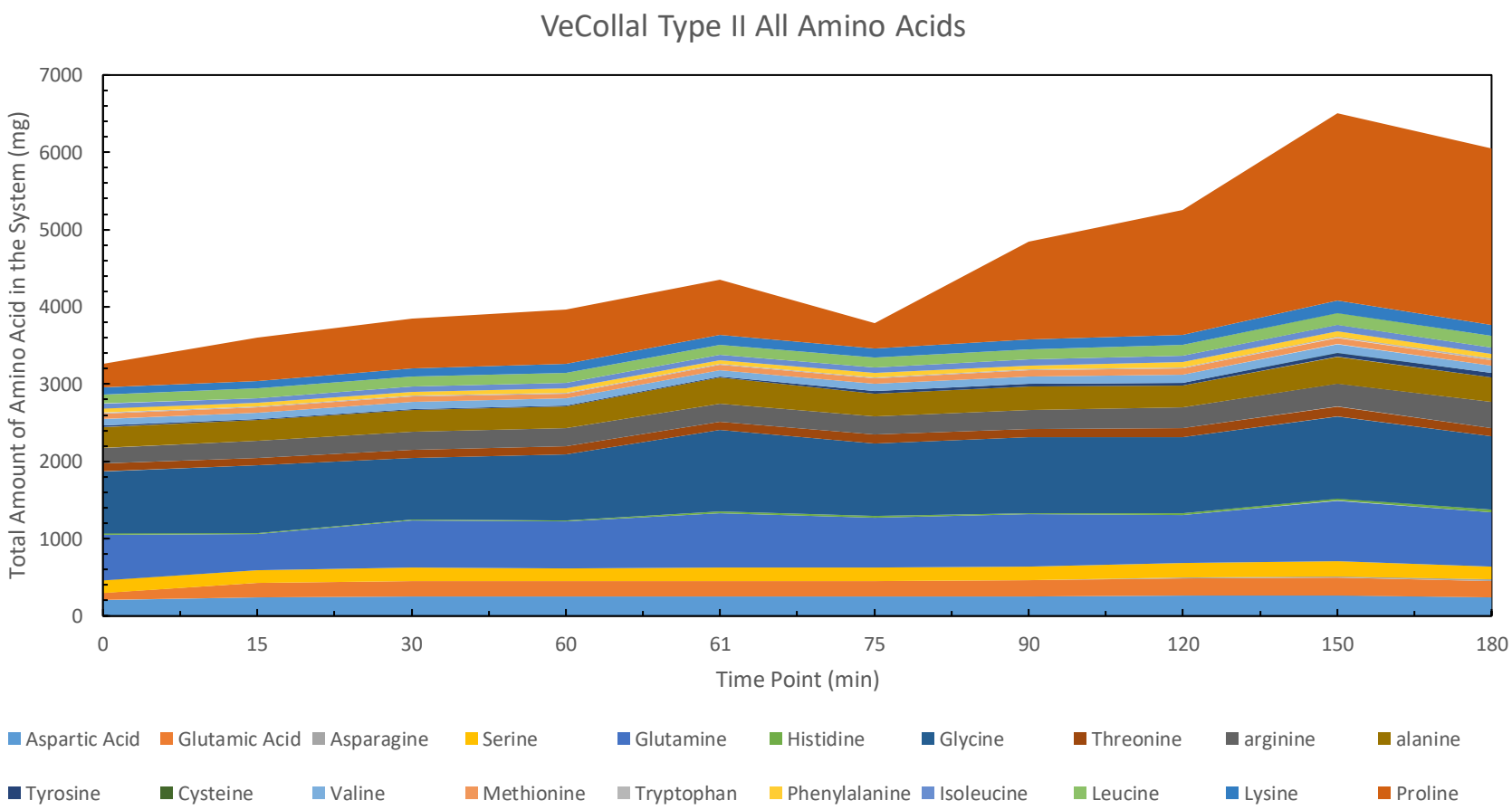

Figure 4. Stacked area chart showing the total mg amount of each individual amino acid analysed in this study, for the Type II VeCollal collagen supplement, detected in the Aelius Biotech Model Gut System over a time course of 180 minutes. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as per the legend. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value.

Data for Individual Amino Acids

The total amount of each individual amino acid detected in the model gut system will be presented here. Each graph will show data for a single amino acid over the model gut time course for both VeCollal products and both animal collagen products. Also shown on the graph will be the expected amount of amino acid in the system based on the percentage of each animo acid in the sample dose.

Total milligram amounts in the system was calculated by determining the mg/mL concentration of each amino acid in the system at each time point and multiplying this by the volume (mL) in the system at those respective time points. Volumes at each time point, also accounting for samples taken, are shown in the table below.

Time (min)	0	15	30	60	61	75	90	120	150	180
Volume (mL)	60	72	79	93.5	119	125	132	145.5	160	174.5

The amount of amino acid naturally present in the model gut system was also determined by running the model gut system without sample. This was subtracted from the amount of amino acid determined for the samples to give the final value.

Aspartic Acid

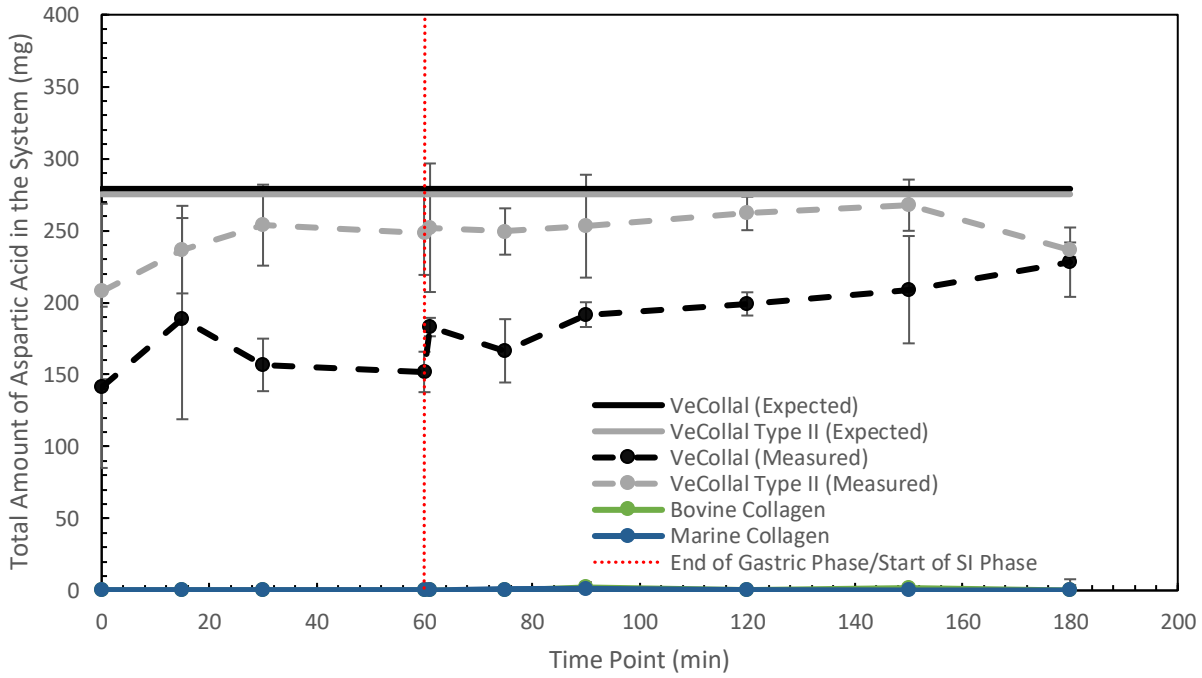

Figure 5. Total mg amount of aspartic acid detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 279 mg and 275.25 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little aspartic acid was observed for the animal collagen supplements (<2 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 141.1 mg, which is 50.5% of the expected amount. This rose over the duration of the experiment by 87 mg to 228.1 mg, which is 82% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 207.7 mg, which is 75.4% of the expected amount. This rose slightly over the duration of the experiment by 29 mg to 237.7 mg, which is 92.4% of the expected amount.

Glutamic Acid

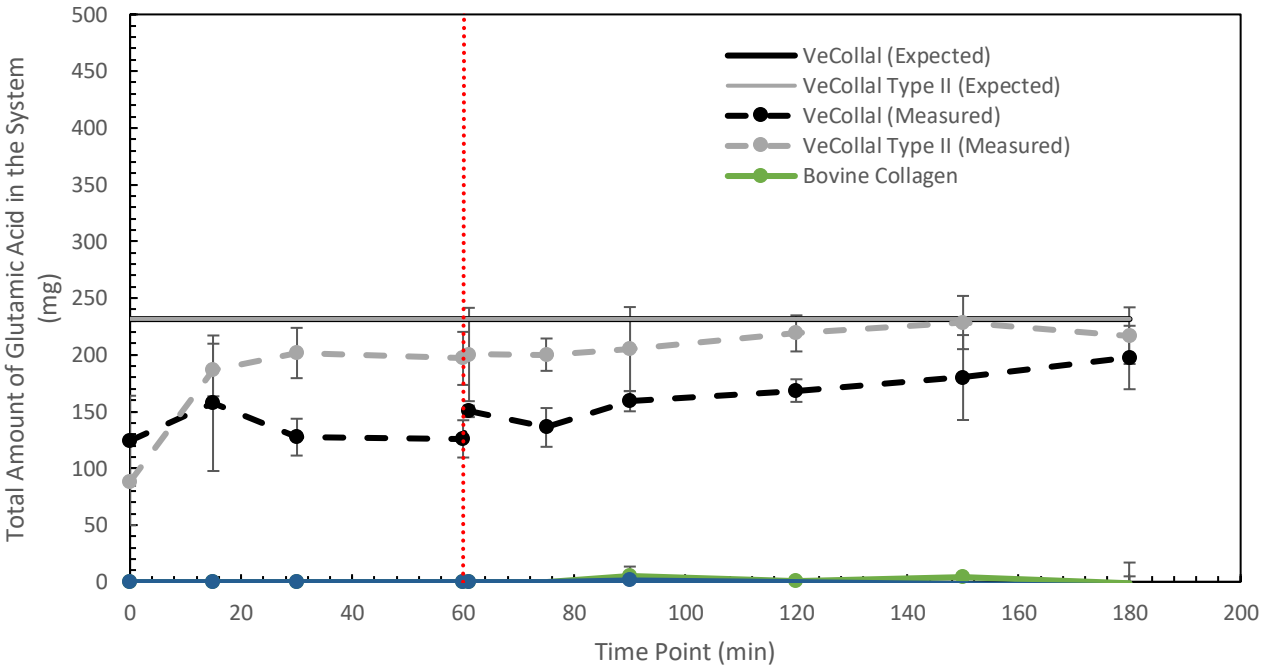

Figure 6. Total mg amount of glutamic acid detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 231.5 mg and 231.683 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little glutamic acid was observed for the animal collagen supplements (<5 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 124.3 mg, which is 53.7% of the expected amount. This rose over the duration of the experiment by 73.3 mg to 197.6 mg, which is 85.3% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 88.2 mg, which is 38% of the expected amount. This rose significantly over the duration of the experiment by 128.7 mg to 216.9 mg, which is 93.6% of the expected amount.

Asparagine

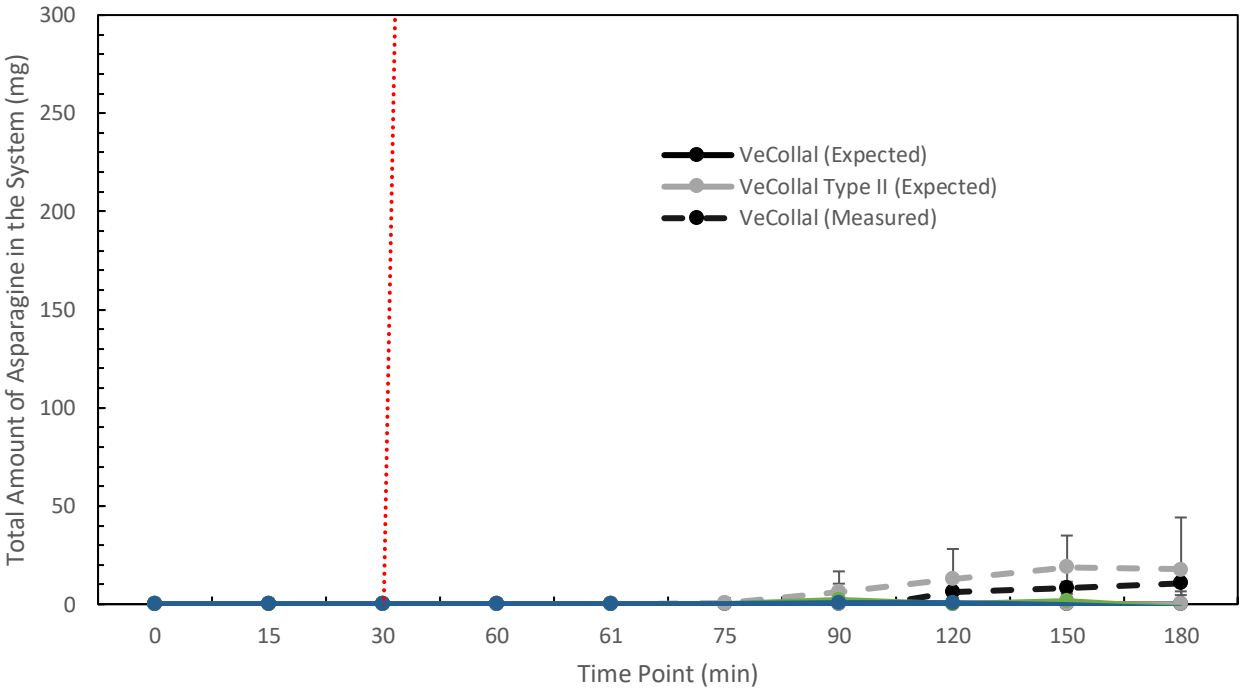

Figure 7. Total mg amount of asparagine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). Asparagine was not present in the ingredient list of either VeCollal formulation, and therefore the expected mount was 0 mg. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little asparagine was observed for the animal collagen supplements (<2 mg)
- For the VeCollal Type I supplement, 10.5 mg of asparagine was detected in the system at 180 minutes.
- For the VeCollal Type II supplement, 17.6 mg of asparagine was detected in the system at 180 minutes.

Serine

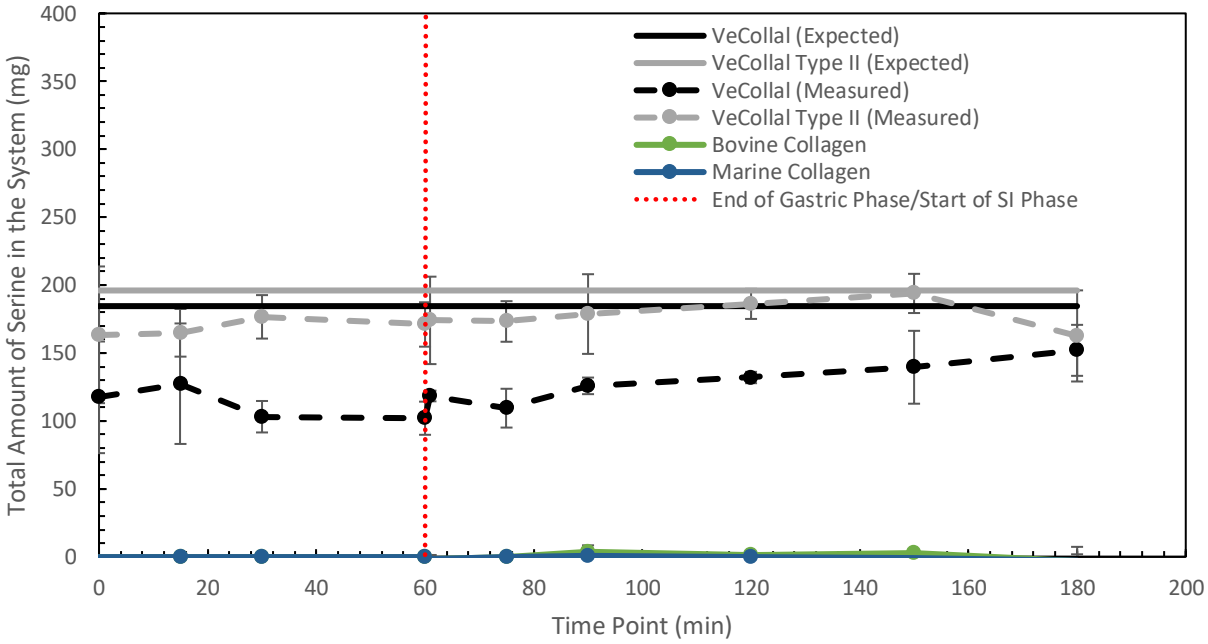

Figure 8. Total mg amount of serine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 184.5 mg and 196.03 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little serine was observed for the animal collagen supplements (<4 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 117.4 mg, which is 63.6% of the expected amount. This rose over the duration of the experiment by 34.6 mg to 152 mg, which is 82.3% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 163.4 mg, which is 83.4% of the expected amount. Little change was observed over the time course, with the amount detectable after 180 minutes being 162.6 mg.

Glutamine

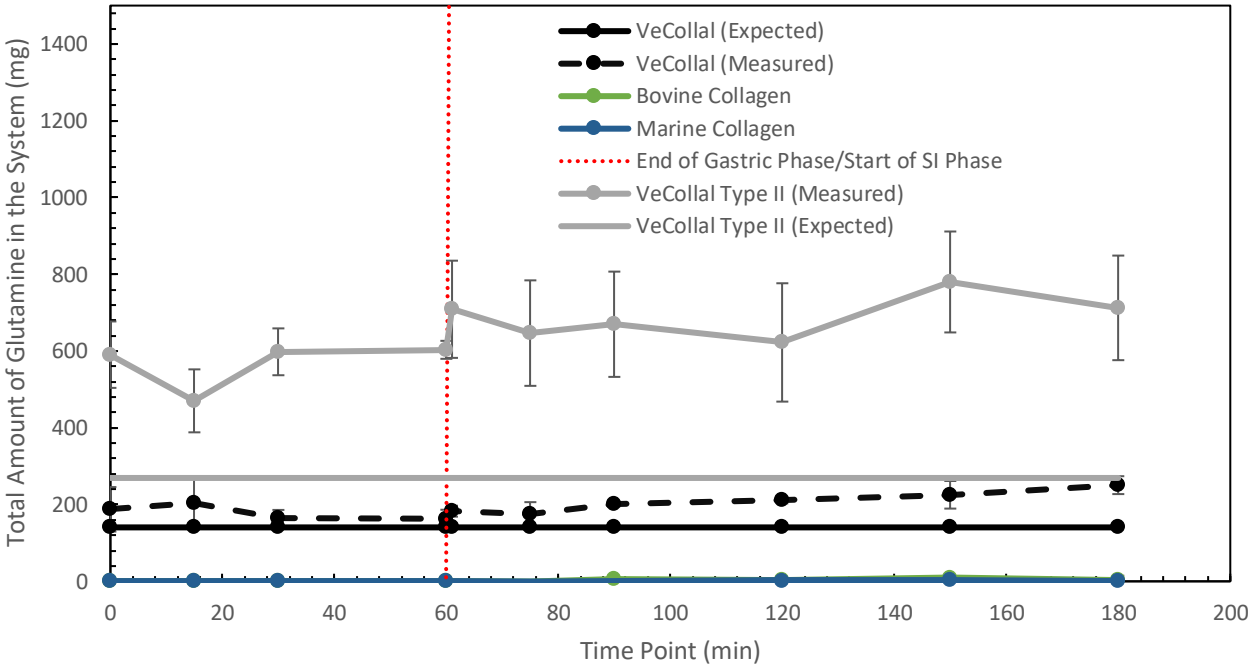

Figure 9. Total mg amount of glutamine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 140.5 mg and 269.3 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by the red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little glutamine was observed for the animal collagen supplements (<10 mg)
- The amount of amino acid detected in the system for the VeCollal Type I supplement was higher than the expected amount throughout the study period.
- Starting at 188 mg, which is 33.8% higher than the expected value, the amount detected in the system rose over the time course to reach a final amount of 250 mg which is 77.9% higher than the expected amount.
- Similarly, though to a much larger extent, the amount of glutamine detected in the system for the VeCollal Type II product was far higher than expected.
- Starting at 590.5 mg, which is 219.3 % of the expected value, the amount detected in the system rose over the time course to reach a final amount of 712.4 mg which is 264.5% of the expected amount.

Histidine

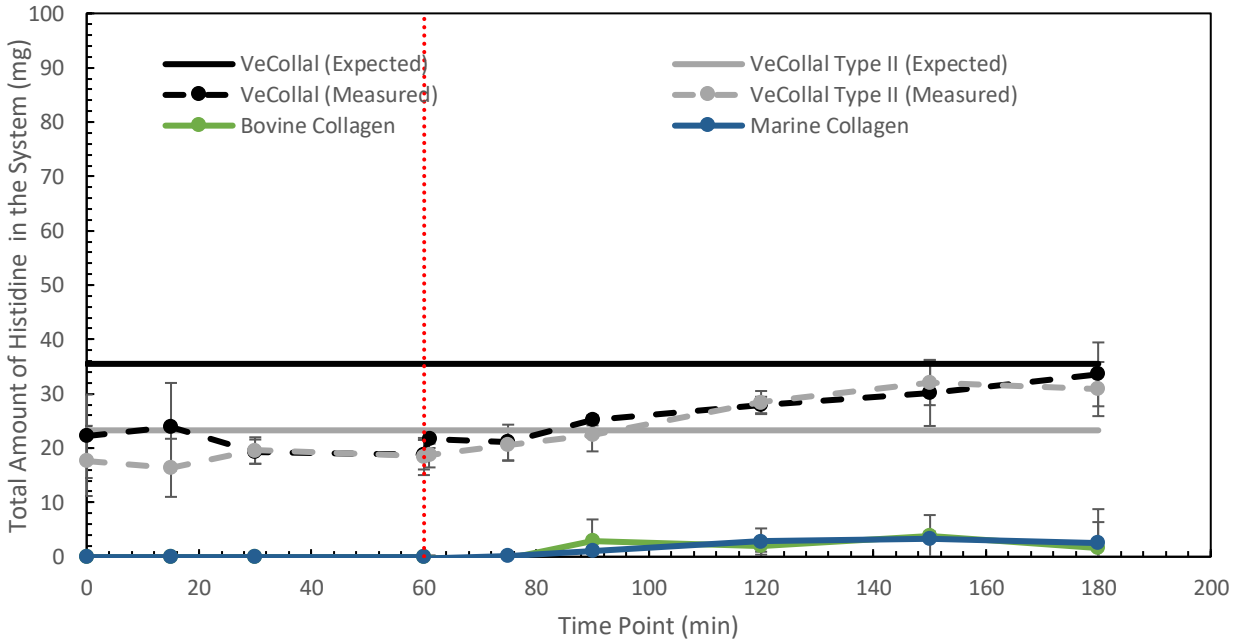

Figure 10. Total mg amount of histidine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 35.5mg and 23.27 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little histidine was observed for the animal collagen supplements (<4 mg)
- The amount of amino acid present for VeCollal Type I began at 22 mg, which is 62% of the expected amount. Overtime this rose to 33.5 mg, which is 95% of the expected amount.
- The amount of amino acid present for VeCollal Type I began at 17.6 mg which is 75.6% of the expected amount. Overtime this rose to 30.8 mg, which is 132.4% of the expected amount.

Glycine

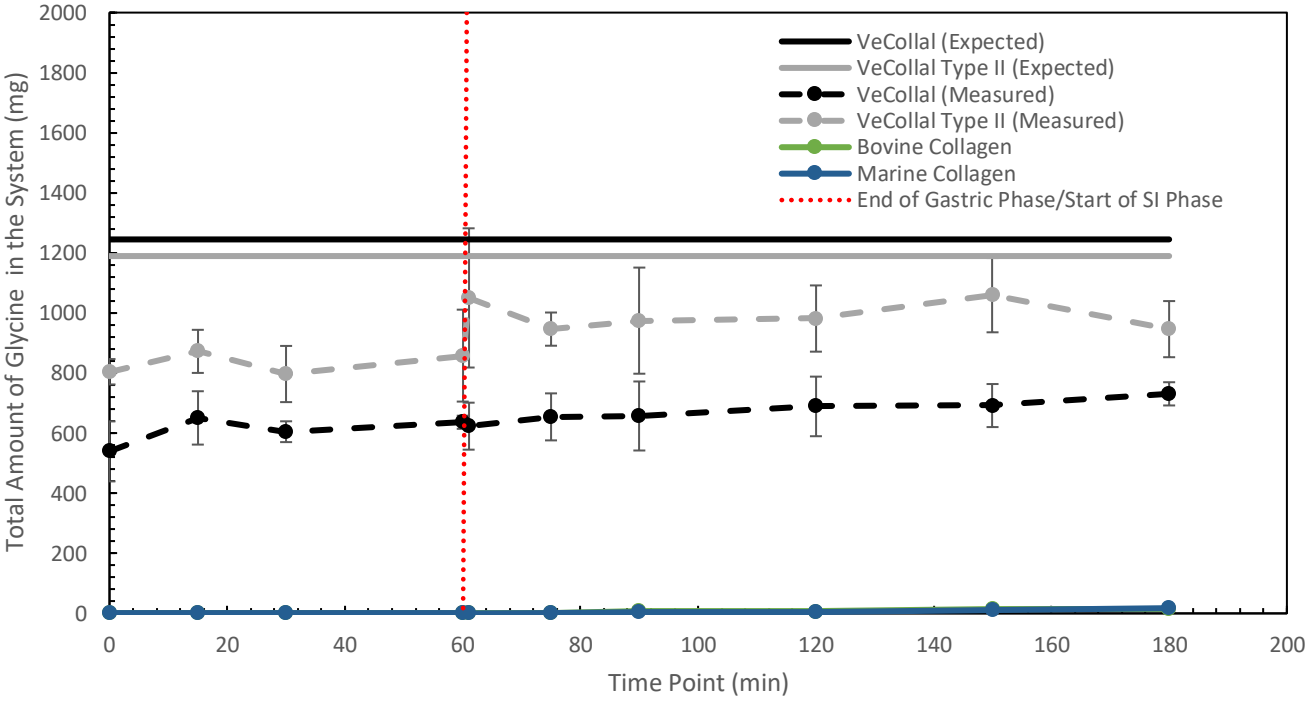

Figure 11. Total mg amount of glycine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 1245 mg and 1189.61 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little glycine was observed for the animal collagen supplements in comparison to the VeCollal samples (<20 mg)
- The amount of amino acid present for VeCollal Type I was much lower than the expected amount throughout the time course. The value at the start of the experiment was 539 mg at the start of the experiment. This is 43% of the expected amount. This increased by 191 mg over time to reach 730 mg - this being only 59% of the expected amount.
- The amount of amino acid present for VeCollal Type II was lower than the expected amount throughout the time course, but not as to the same degree as the type I sample. The value at the start of the experiment was 804.4 mg – this is 67.6% of the expected amount. This increased by 141.9 mg over time to reach 946.3 mg - this being 79.5% of the expected amount.

Threonine

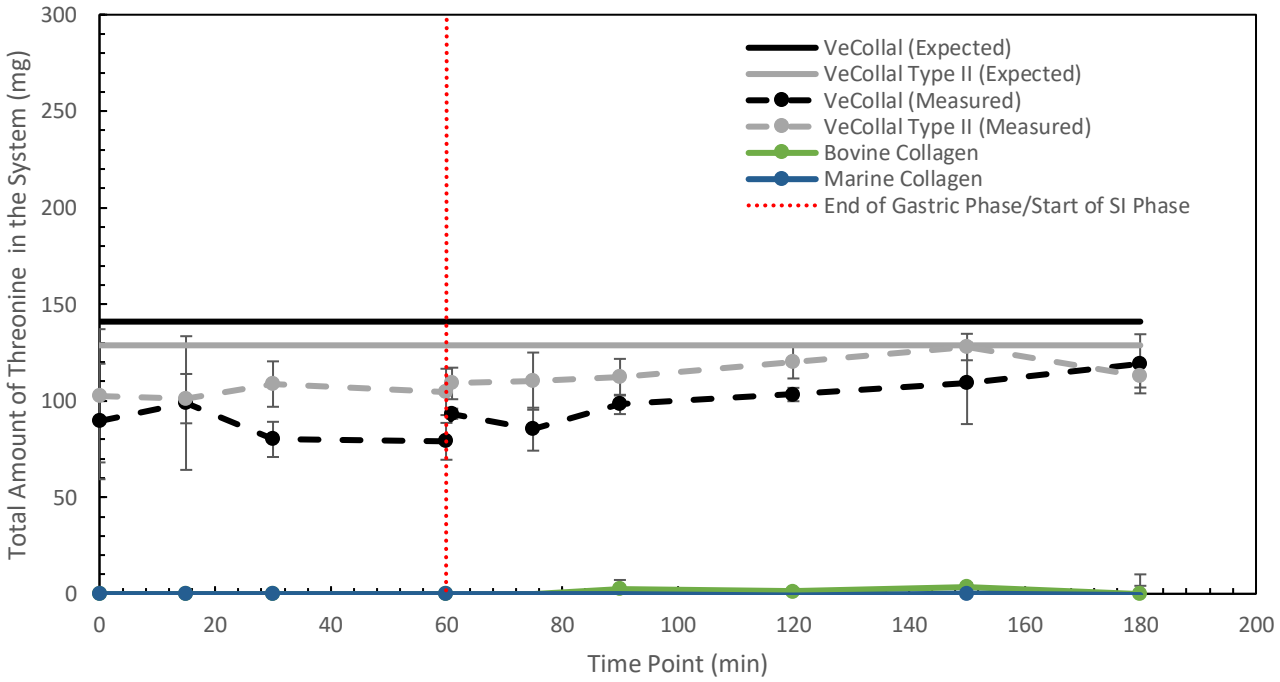

Figure 12. Total mg amount of threonine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 141 mg and 128.7 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase – this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little threonine was observed for the animal collagen supplements (<4 mg).
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 89 mg, which is 63% of the expected amount. This rose over the duration of the experiment by 40 mg to 119 mg, which is 84% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 102.6 mg, which is 79.7% of the expected amount. This rose over the duration of the experiment by 10 mg to 112.6 mg, which is 87.4% of the expected amount.

Arginine

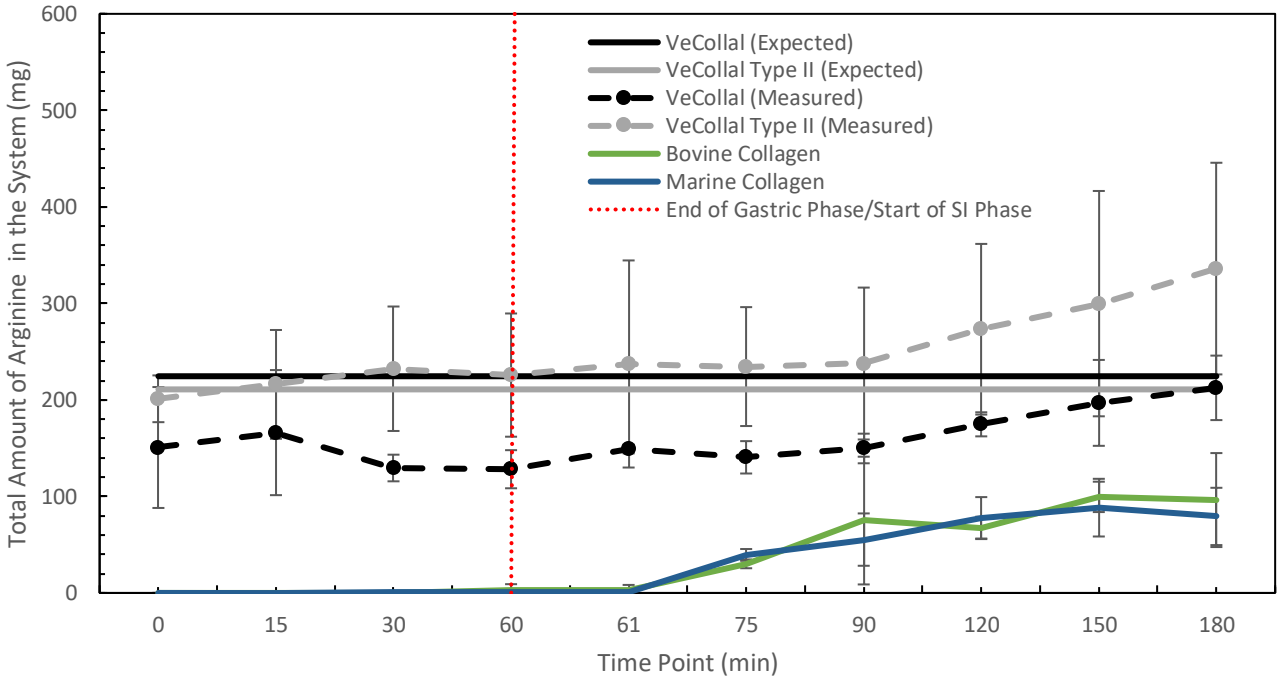

Figure 13. Total mg amount of arginine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 224.5 mg and 210.9 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- No arginine was observed in the gastric phase (0-60 min) of the model gut system when using the animal collagen. However, in the small intestinal phase (61-180) there was a steady increase in the amount of arginine detectable in the system. A total of 96 mg of the amino acid was observed at the end of the experiment for the bovine collage, and 79 mg for the marine collagen.
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 151 mg, which is 67% of the expected amount. This rose over the duration of the experiment by 61 mg to 212 mg, which is 95% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 201.2 mg, which is 95.4% of the expected amount. This rose over the duration of the experiment by 134.9 mg to 336.1 mg, resulting in an amount much higher than the expected amount (159.4% of that expected amount).

Alanine

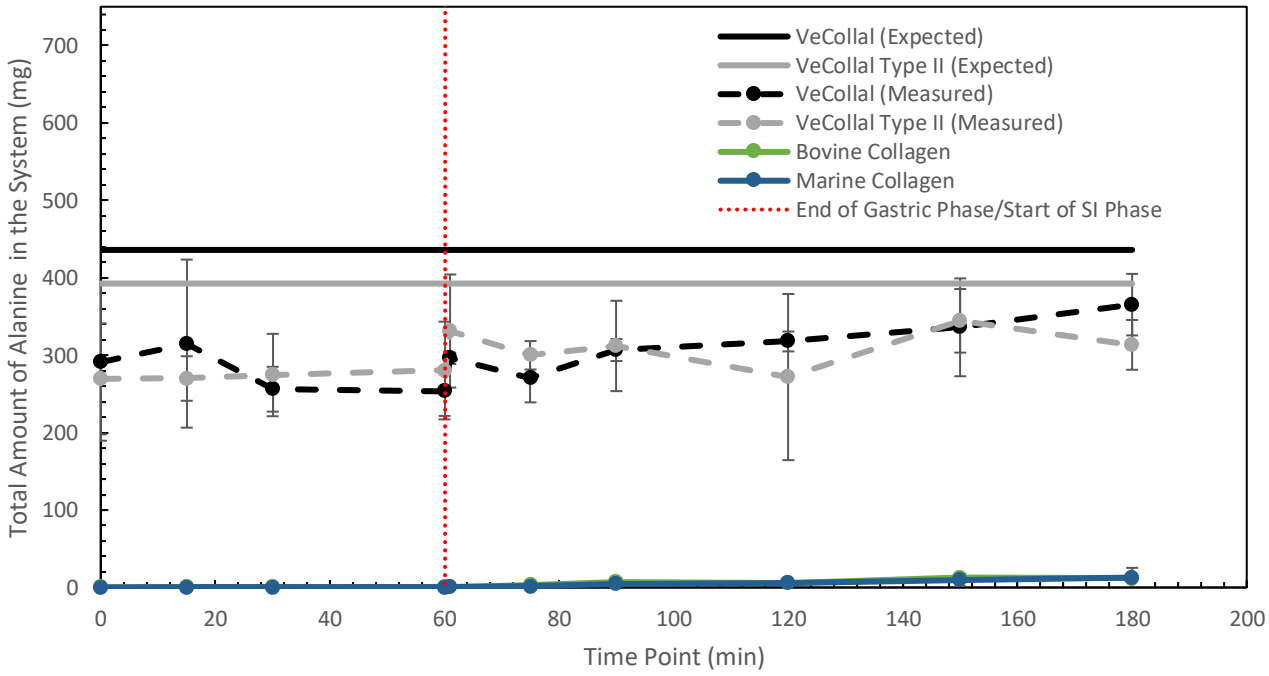

Figure 14. Total mg amount of alanine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 436 mg and 392.6 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little alanine was observed for the animal collagen supplements (<13 mg).
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 290 mg, which is 67% of the expected amount. This rose over the duration of the experiment by 75 mg to 365 mg, which is 84% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 269.4 mg, which is 68.6% of the expected amount. This rose over the duration of the experiment by 43.9 mg to 313.3 mg, which is 79.8% of the expected amount.

Tyrosine

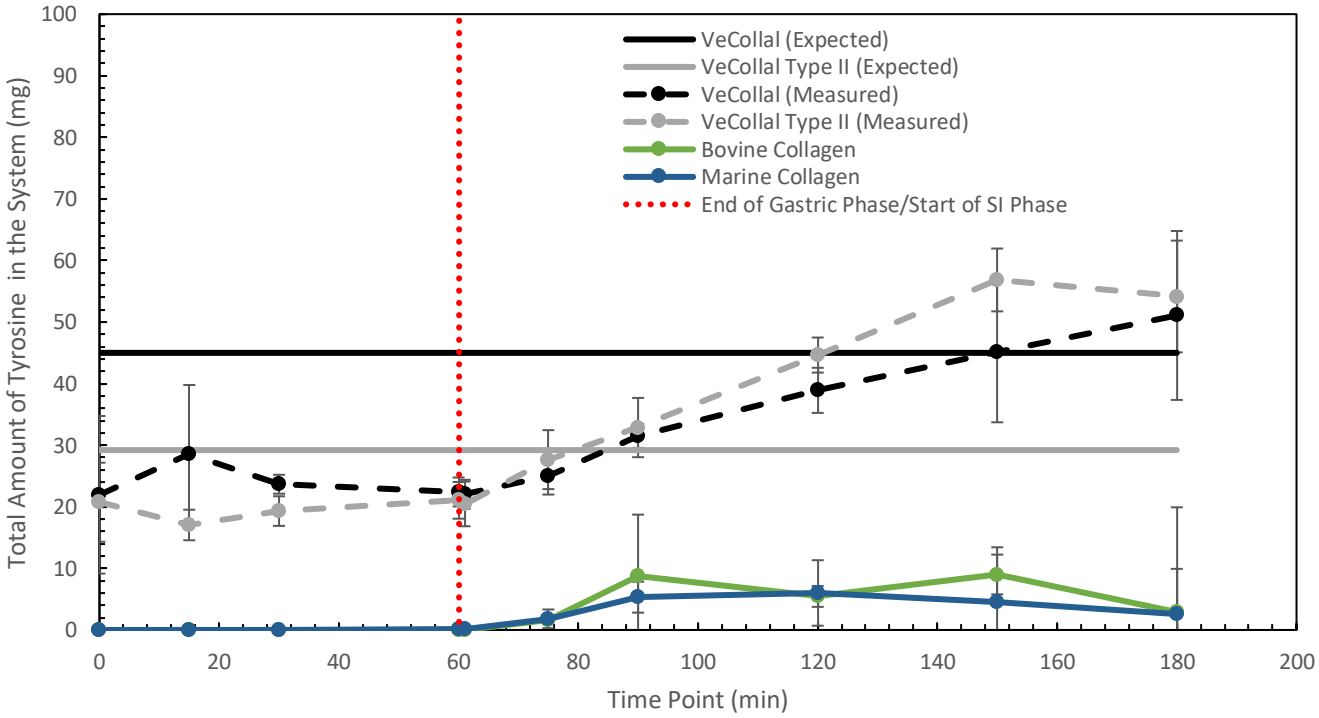

Figure 15. Total mg amount of tyrosine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 45 mg and 29.2 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Up to 10 mg of tyrosine was observed in the animal collagen supplements.
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 21 mg, which is 47% of the expected amount. This rose over the duration of the experiment by 30 mg to 51 mg, which is 113% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 20.7 mg, which is 70.8% of the expected amount. This rose over the duration of the experiment by 33.4 mg to 54.1 mg, which far higher than the expected amount at 183.3%.

Cysteine

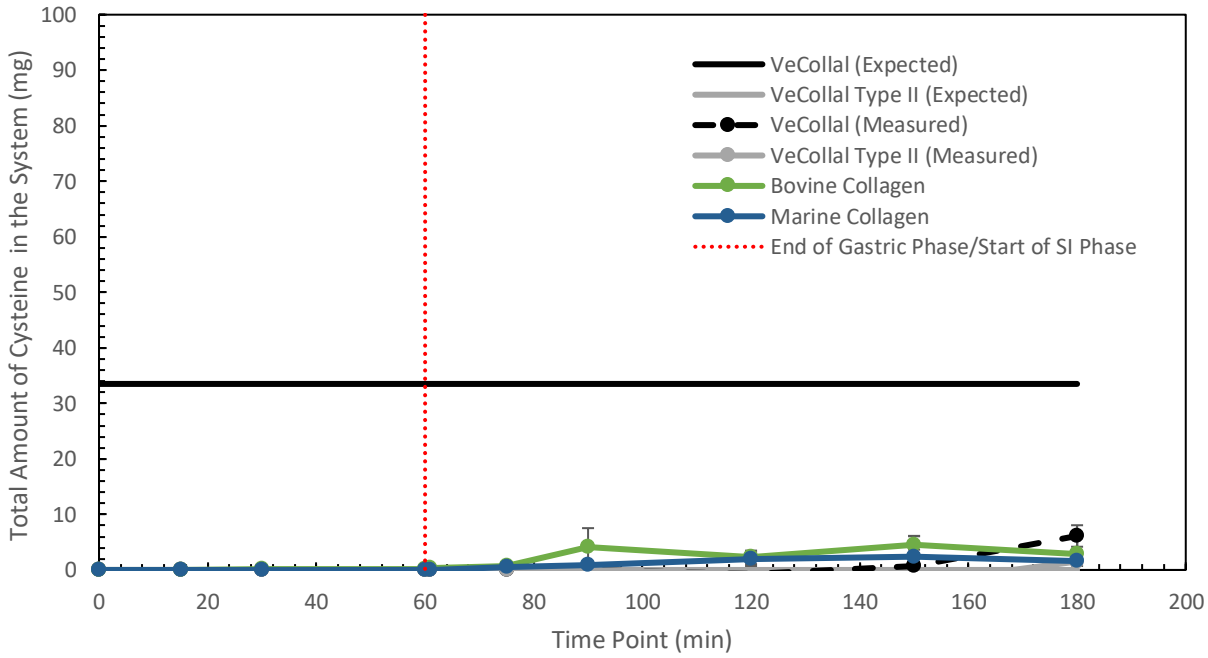

Figure 16. Total mg amount of cysteine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collaged (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I was 33.5 mg. No cysteine was included in the ingredient list of VeCollal Type II. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Similar levels of cysteine were observed for the VeCollal supplements and the animal collagen supplements.
- At the end of the experiment, the animal collagen supplements gave total cysteine amounts of 1.5 and 2.8 for marine and bovine collagen, respectively. Vecollal type I showed 6.1 mg, and VeCollal Type II showed 0 mg.
- No cysteine should be present for VeCollal Type II, as it is not listed in the ingredients.
- VeCollal Type I should contain 30.18 mg of cysteine. This was not observed, and the 6.1 mg that was observed at the end point is 18.2% of the expected amount.

Valine

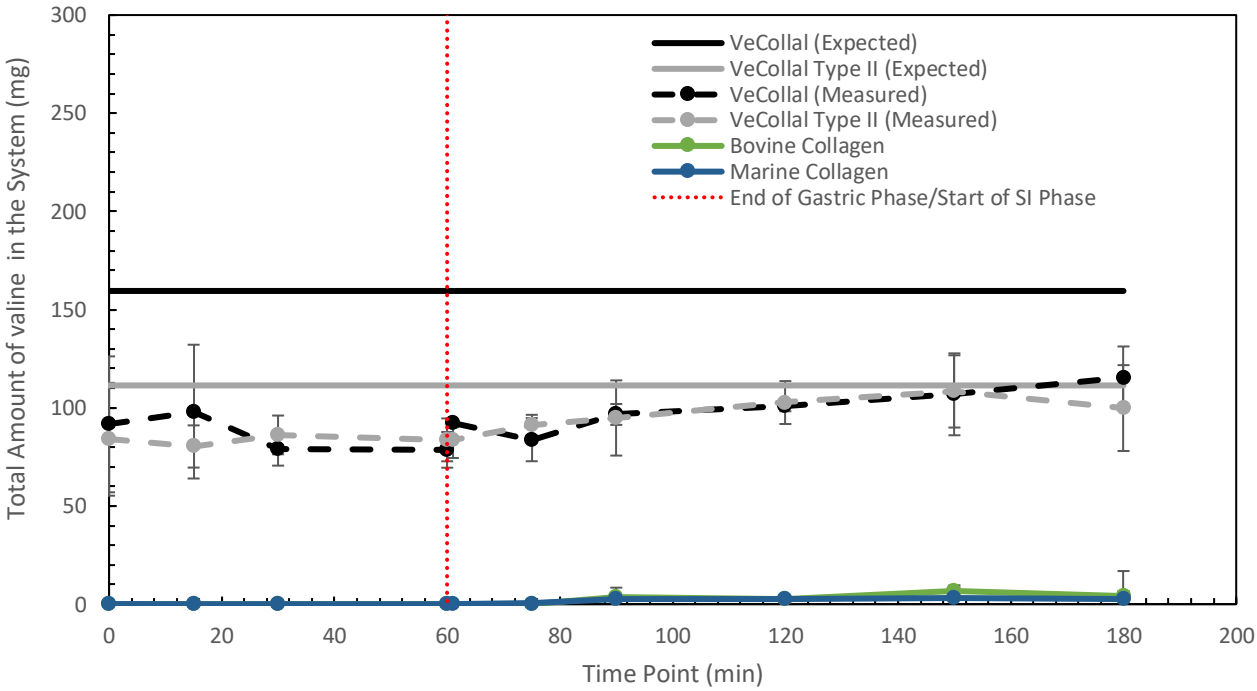

Figure 17. Total mg amount of valine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 159.5 mg and 111.4 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little valine was observed for the animal collagen supplements (<7 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 91.5 mg, which is 57% of the expected amount. This rose over the duration of the experiment by 23.8 mg to 115.3 mg, which is 72.2% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 84 mg, which is 75.4% of the expected amount. This rose slightly over the duration of the experiment by 15.9 mg to 99.9 mg, which is 89.6% of the expected amount.

Methionine

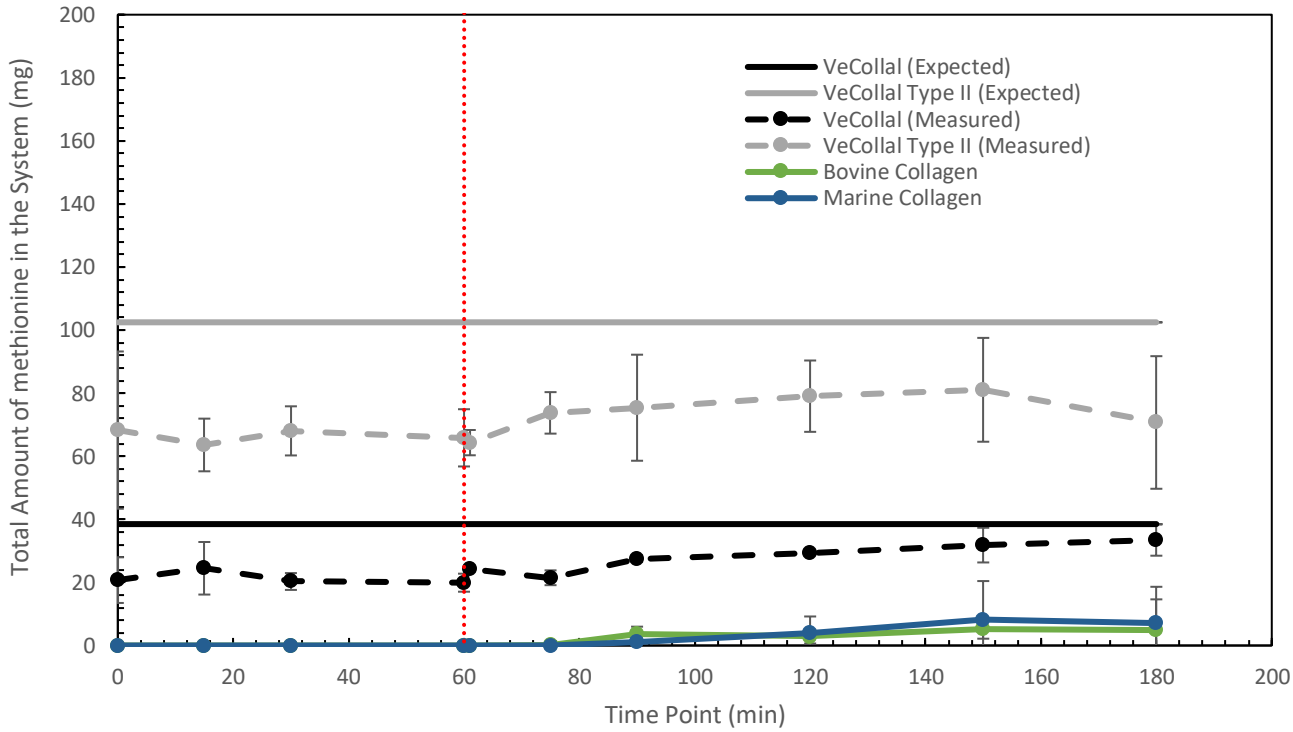

Figure 18. Total mg amount of methionine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 38.5 mg and 102.47 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little methionine was observed for the animal collagen supplements (<9 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 20.7 mg, which is 57.5% of the expected amount. This rose over the duration of the experiment by 12.7 mg to 33.4 mg, which is 87% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 68.3 mg, which is 66.6% of the expected amount. The amount remained similar over the duration of the experiment with a final amount of 70.7 mg, which is 69% of the expected amount.

Tryptophan

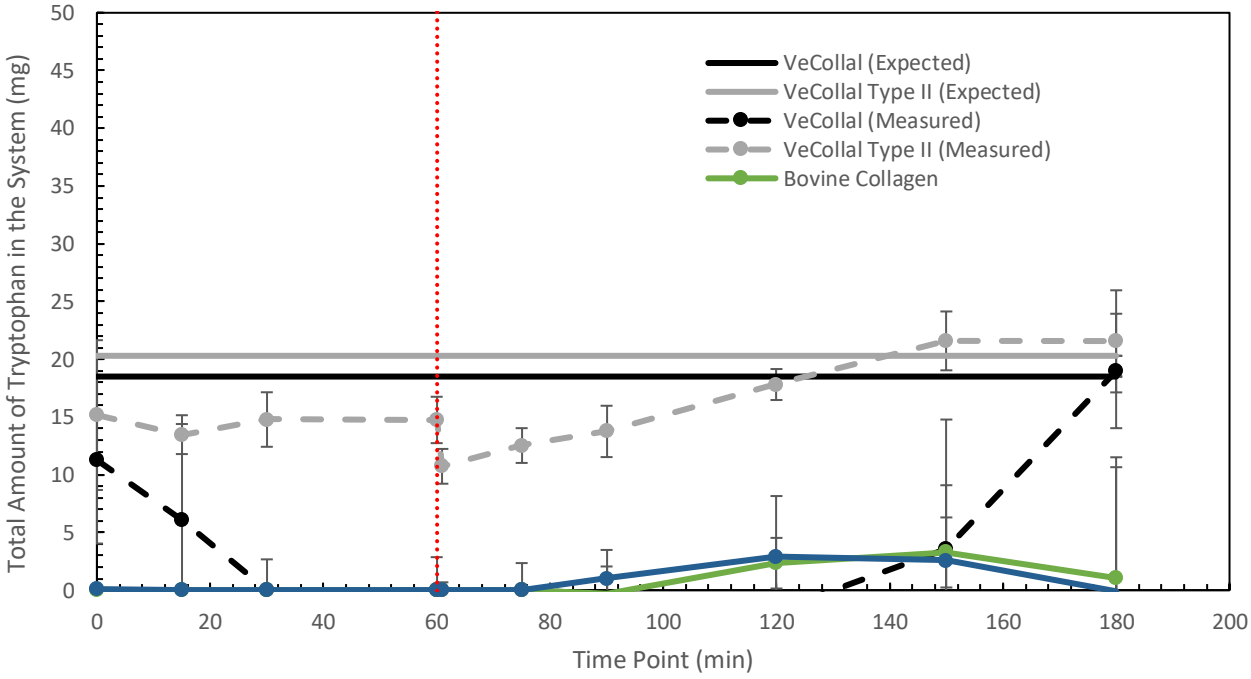

Figure 19. Total mg amount of tryptophan detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 18.5 mg and 20.3 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- No more than 4 mg of tryptophan was observed for the animal collagen throughout the time course.
- The VeCollal Type I supplement gave a tryptophan amount of 11.3 at the start of the experiment. This dropped to 6 mg at 15 minutes. From this point no tryptophan was detected again until the 150-minute mark.
- The Type I VeCollal supplement ended the experiment with 18.9 mg of tryptophan measurable in the system, which is 93% of the expected amount. Though it is apparent that tryptophan was not detectable for most of the experiment.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 15.16 mg, which is 74.6% of the expected amount. The amount rose over the duration of the experiment with a final amount of 21.5 mg, which is 105.9% of the expected amount.

Phenylalanine

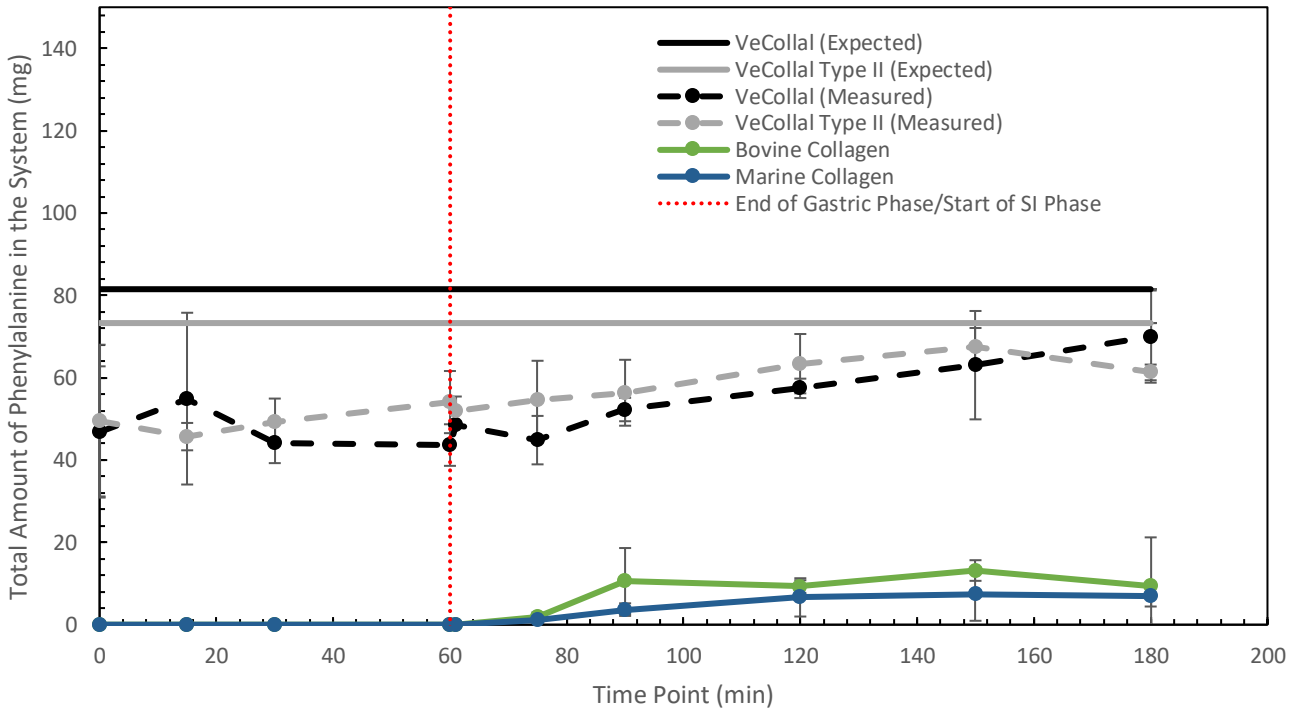

Figure 20. Total mg amount of phenylalanine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 81.5 mg and 73.3 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Up to 14 mg of phenylalanine was observed in the animal supplements but the amounts were always far lower than the VeCollal supplements.
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 46.8 mg, which is 57.4% of the expected amount. This rose over the duration of the experiment by 23.2 mg to 70 mg, which is 85.8% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 49.6 mg, which is 67.7% of the expected amount. This rose over the duration of the experiment by 11.6 mg to 61.2 mg, which is 83.5% of the expected amount.

Isoleucine

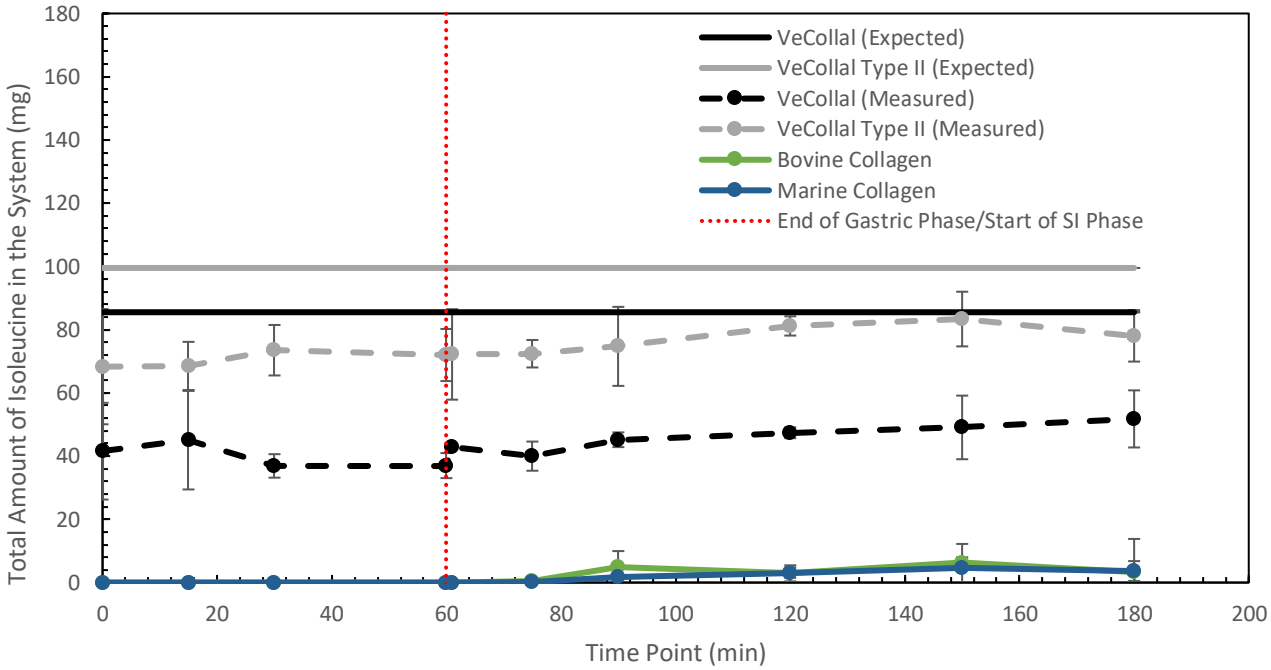

Figure 21. Total mg amount of isoleucine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 85.5 mg and 99.5 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little isoleucine was detected when using the animal collagen supplements (<7 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 41.5 mg, which is 48.5% of the expected amount. This rose over the duration of the experiment by 10.2 mg to 51.7 mg, which is 60.4% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 68.3 mg, which is 68.6% of the expected amount. This rose over the duration of the experiment by 9.7 mg to 78 mg, which is 78.4% of the expected amount.

Leucine

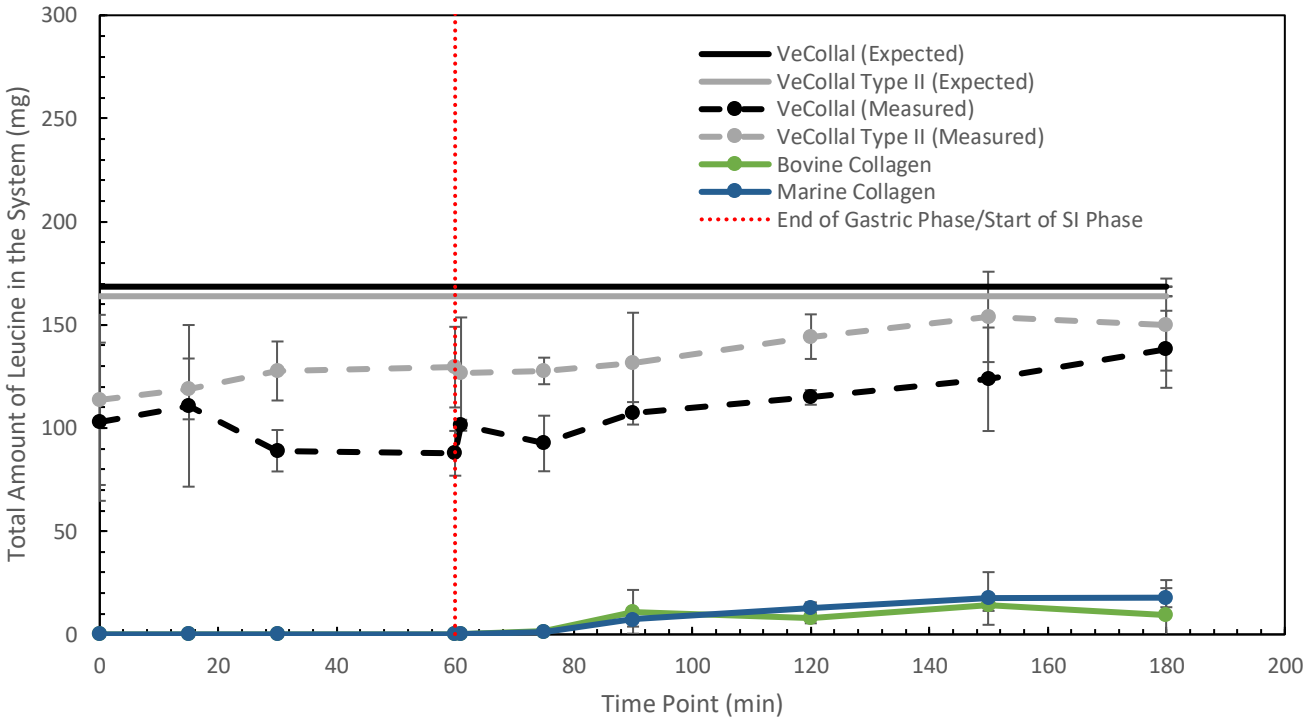

Figure 22. Total mg amount of leucine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 168.5mg and 163.8 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Very little isoleucine was detected when using the animal collagen supplements (<7 mg)
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 103 mg, which is 61% of the expected amount. This rose over the duration of the experiment by 35 mg to 138 mg, which is 82% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 113.6 mg, which is 69.4% of the expected amount. This rose over the duration of the experiment by 36.5 mg to 150.1 mg, which is 91.6% of the expected amount.

Lysine

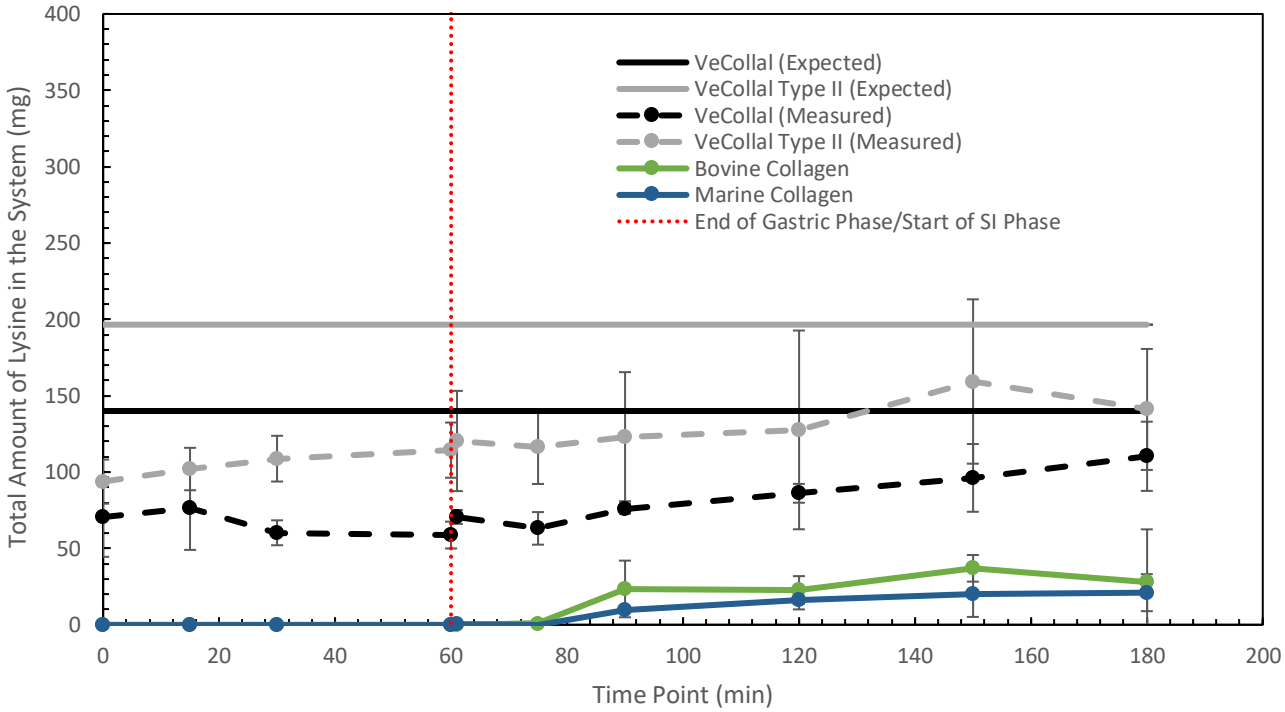

Figure 23. Total mg amount of lysine detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 140 mg and 196.5 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- Like the data for arginine, a higher amount of lysine was detected in the model gut system when using the animal collagen supplements in comparison to the other amino acids studied. Though, while up to 37 mg of lysine was observed for the animal collagen supplements, these values were still much lower than those observed for the VeCollal supplements.
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 70 mg, which is 50% of the expected amount. This rose over the duration of the experiment by 40 mg to 110 mg, which is 79% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 93.8 mg, which is 47.7% of the expected amount. This rose over the duration of the experiment by 47.2 mg to 141 mg, which is 71.7% of the expected amount.

Proline

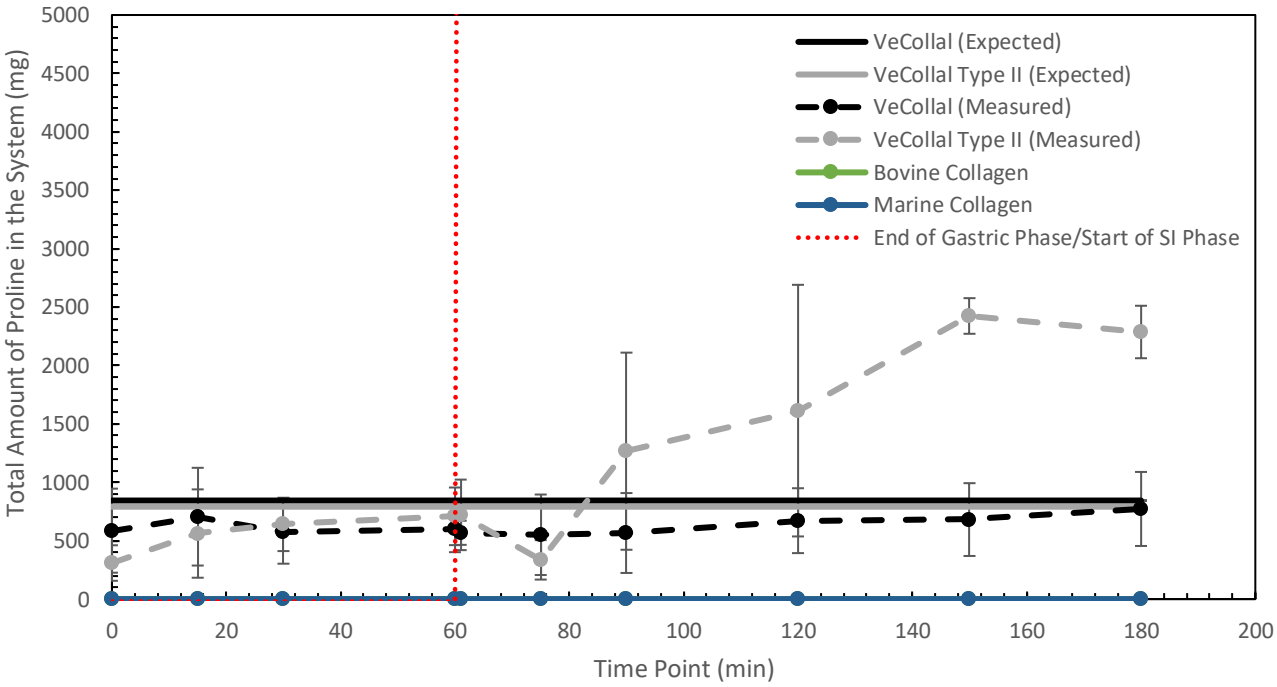

Figure 24. Total mg amount of proline detected in the Aelius Biotech Model Gut System over a time course of 180 minutes after dosing with collagen supplements. The x-axis shows the elapsed time in minutes. The y-axis shows the total mg amount of amino acid in the system. The supplements are identified in the figure as follows: Expected amount for VeCollal Type I (Solid black line); expected amount for VeCollal Type II (solid grey line); measured amount for VeCollal Type I (dashed black line); measured amount for VeCollal Type II (dashed grey line); measured amount for bovine collagen (solid green line); measured amount for marine collagen (solid blue line). The mg amount expected for VeCollal Type I and VeCollal Type II were 844 mg and 719.1 mg, respectively. Minutes 0-60 represent the gastric phase, and minutes 61-80 represent the small intestinal phase - this is shown by a red dotted line. Plotted points show the mean value, and error bars show the standard deviation of the mean.

Key Observations

- No proline was observed at any point when testing the animal collagen supplements.
- The amount of amino acid present for VeCollal Type I at the start of the experiment was 586 mg, which is 69.4% of the expected amount. This rose over the duration of the experiment by 185 mg to 771 mg, which is 91.3% of the expected amount.
- The amount of amino acid present for VeCollal Type II at the start of the experiment was 308.8 mg, which is 42.9% of the expected amount. This rose significantly over the duration of the experiment, particularly in the small intestinal phase, by 1977.2 mg to 2286.5 mg, which is 318% of the expected amount.

Next Steps

- Investigate mucus permeation properties of different formulations and compare to animal collagens to assess any improvements in absorption seen with the liposomal formulations
- Investigate absorption profiles in integrated model of small intestinal digestion, mucus permeation and epithelial absorption. This will help us understand
    - Comparative absorption of Vecollal products compared to animal collagens
    - Impact of liposomal formulation on absorption
    - Impact of brush border enzymes on amino acid profiles
    - Timecourse data of absorption to show if increased availability of free amino acids does as hypothesised lead to increased absorption
- Discuss potential for peer reviewed publication

References

Houghton D, Wilcox MD, Brownlee IA, Chater P, Seal CJ, Pearson JP. Method for quantifying alginate and determining release from a food vehicle in gastrointestinal digesta. Food Chem. 2014 May 15;151:352-7. doi: 10.1016/j.foodchem.2013.11.070. Epub 2013 Nov 20. PMID: 24423543.