CITRULLINE AND THE INTESTINE

THESIS SUBMITTED BY

KONSTANTINOS C. FRAGKOS

OF

THE CENTRE FOR GASTROENTEROLOGY AND NUTRITION

DIVISION OF MEDICINE, UNIVERSITY COLLEGE LONDON

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PH.D.)

SUPERVISOR:

PROF. ALASTAIR FORBES

APRIL 2018

Declaration

I, Konstantinos Fragkos, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Konstantinos Fragkos

April 2018

This thesis is dedicated to the development of greater understanding between health and life sciences and to the opportunity to investigate the complexities of intestinal physiology.

Abstract

Citrulline, a non-protein amino acid, has been playing an important role in scientific research over the last few years. This thesis explores various aspects of citrulline with respect to intestinal disease, short bowel syndrome and intestinal failure. The first important finding was that citrulline as a term has been used at the end of the 19th century-beginning of the 20th century to describe an extract of the *C. colocynthis*, used as a subcutaneous laxative. Also, old sources have revealed that citrulline was first described as an amino acid by Koga and Ohtake (1914) and not by Wada (1930a). From the systematic review and meta-analysis, citrulline levels are strongly positively correlated with small bowel length in short bowel syndrome patients and strongly negatively correlated with intestinal disease severity with regards to enteropathies (coeliac disease, tropical enteropathy, mucositis, acute rejection in intestinal transplantation, but not Crohn's disease). Citrulline cut-off levels have an overall sensitivity and specificity of 80% and citrulline levels compared to controls were reduced by 10 μmol/L. These findings suggest that citrulline is a marker of possible acute intestinal injury or intestinal insufficiency. Next, an original five-by-five cross-over study was designed (Williams design) comparing post-absorptive amino acid concentrations after challenges with citrulline, arginine, glutamine, 3-methyl-hisitidine and placebo. Citrulline was the most potent stimulator for all other amino acids, contrary to beliefs of glutamine challenges. Citrulline challenges could be useful in intestinal failure but also in liver failure where urea cycle pathways including glutamine, arginine and ornithine are implicated. The final study was an investigation of quality of life in short bowel syndrome patients. The quality of life scale is highly reliable in short bowel syndrome patients (Cronbach's alpha > 0.700) and the main causes of low quality of life are fatigue, diarrhoea/increased stomal output, lack of sleep, gastrointestinal symptoms, and muscle pains.

Acknowledgements

I am deeply grateful to Prof. Alastair Forbes for his constant guidance on all aspects of my PhD research until today, without whom all my results would be impossible to achieve. I am also very grateful to the following colleagues of Professor Forbes and faculty members at UCL for playing an advisory and guiding role when needed: Dr George Grimble, Principal Teaching Fellow & Honorary Senior Lecturer at UCL; Dr Richard M. Day, UCL Division of Medicine, for helping me with many aspects of lab techniques and supervising certain projects of myself throughout the PhD; Dr Nathan Davies, based at the Royal Free, and Mr Richard Cohen, based at University College Hospital; and finally, Prof. David Sattelle for his help finalising and submitting the dissertation.

I am also deeply grateful to my father Christos Frangos, for his constant psychological and monetary – in many instances – support throughout this research path I chose; and my mother Jenny, sister Maria-Roza, nephew Alexandros Dimopoulos, my wife Penelope Makrykosta and my son Orestis Fragkos.

Also, acknowledgements are due to all other members of staff, postgraduate research staff and friends who have kindly assisted me with their ideas, discussions, lab assistance or anything else during my research: Prof. Simon Lal, Georgios Bountagkidis, Ioannis Angelopoulos, Nina Parmar, Adeel Hamad, Faris Al-Sultan, Mohammed Eddama, Stephanie Kuku, Bing Wang, Hideo Suzuki, Alistair Windsor, Indira Nathan, Maria Jover-Cobos, Cali Hyde from the Cancer Institute who helped with analyses in the mass spectrometer, Alvin Wong, Peter Berghöfer and other staff at Nycomed, Dr L. Ber from NOW foods (USA), George Kravvas, Bill Batziakas, and last but not least the research participants.

Publications and Presentations Arising from this Thesis

Publications

Fragkos, K.C., & Forbes, A. (2018). A pharmacodynamics study of citrulline challenge testing. *In preparation*.

Fragkos, K.C., & Forbes, A. (2018). Citrulline as a marker of intestinal function and absorption in clinical settings: A systematic review and meta-analysis. *United European Gastroenterology Journal*, *6*(2), 181-191, doi:10.1177/2050640617737632.

Berghöfer, P., Fragkos, K. C., Baxter, J. P., Forbes, A., Joly, F., Heinze, H., et al. (2013). Development and validation of the disease-specific Short Bowel Syndrome-Quality of Life (SBS-QoL™) scale. *Clinical Nutrition*, *32*(5), 789-796, doi:10.1016/j.clnu.2012.12.001.

Fragkos, K. C., & Forbes, A. (2011). Was citrulline first a laxative substance? The truth about modern citrulline and its isolation. *Journal of Japanese History of Medicine*, *57*(3), 275-292.

Presentations

Fragkos, K. C., & Forbes, A. (2017). PWE-101 Citrulline as a marker of intestinal function and absorption in clinical settings: a systematic review meta-analysis. *Gut, 66*(Supplement 2), A178, doi: 10.1136/gutjnl-2017-314472.347.

Fragkos, K. C., & Forbes, A. (2011). Are Citrulline Plasma Levels a Marker of Small Bowel Length and Absorptive Capacity? A Meta-Analysis. Paper presented at the UCL Biomedical Sciences Conference, 28/2-1/3 London, UK.

Fragkos, K., Baxter, J. P., Forbes, A., Joly, F., Heinze, H., Jeppesen, P. B., et al. (2011). PP059-MON Development and Validation of a Short Bowel Syndrome (SBS) Specific Quality of Life Scale. *Clinical Nutrition Supplements*, *6*(1), 137, doi:10.1016/S1744-1161(11)70351-9.

Fragkos, K. C., & Forbes, A. (2011). PP228-SUN Is citrulline a marker of small intestinal absorption? A systematic review and meta- analysis. *Clinical Nutrition Supplements*, *6*(1), 110, doi:10.1016/S1744-1161(11)70281-2.

Fragkos, K. C., & Forbes, A. (2011). OP048 A meta-analysis on the association of citrulline plasma levels and small bowel length. *Clinical Nutrition Supplements*, *6*(1), 20, doi:10.1016/S1744-1161(11)70048-5.

Contents

List of Figures

List of Tables

Abbreviations

3MH/3-MH: 3-Methylhistidine

ADC: Arginine decarboxylase

AMP: Adenosine monophosphate

ANOVA: Analysis of variance

AP-ESI: Atmospheric pressure electrospray ionization

APCI: Atmospheric pressure chemical ionization

ARG/arg: Arginine

ASL: Argininosuccinate lyase

Asp: Aspartate

ASS: Argininosuccinate synthase

ATP: Adenosine triphosphate

AUC: Area under the curve

BMI: Body mass index

C: Celsius

CC: Correlation coefficient

CD: Crohn's disease

CeD: Coeliac disease

CI: Confidence interval

CIT/cit: Citrulline

Cl: Clearance

C_{max}: Maximum concentration

CO_2: Carbon dioxide

CRP: C-Reactive protein

C_t: Last measured concentration

df: Degrees of freedom

DFBETA: difference in regression coefficients when observation deleted

DNA: Deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

EFA: Exploratory factor analysis

ELISA: Enzyme-linked immunosorbent assay

EMA/EMEA: European Medicines Agency

ESI: Electrospray ionization

F: F-test statistics

FBS: Foetal bovine serum

FDA: Food and Drug Administration

FN: False negative

FP: False positive

GFD: Gluten-free diet

GLN/Gln: Glutamine

GLP-2: Glucagon-like peptide-2

GLU/Glu: Glutamate

HILIC: Hydrophilic interaction liquid chromatography

HIV: Human immunodeficiency virus

HPLC: High performance liquid chromatography

HPN: Home parenteral nutrition

HSROC: Hierarchical summary receiver operating curve

IBD: Inflammatory bowel disease

ICU: Intensive Care Unit

IEC: Ion exchange chromatography

IF: Intestinal failure

ISTD: Internal Standard

ITx/IT: Intestinal transplantation

IV: Inverse variance

k_e: Elimination rate constant

LC/MS: Liquid chromatography-mass spectrometry

MANOVA: Multivariate analysis of variance

MD: Mean difference

MOOSE: Meta-analysis of Observational Studies in Epidemiology

MS or MS/MS: Mass spectrometer/mass spectrometry

MS: Mean Square

NEC: Necrotising enterocolitis

nm: nanometre

NO: Nitric oxide

NOS: Nitric oxide synthase

OAT: Ornithine aminotransferase

OCT: Ornithine carbamoyltransferase

ODC: Ornithine decarboxylase

ORN/Orn: Ornithine

P/*p*: *p*-value

P5C: Pyrroline-5-carboxylate

P5CS: Pyrroline-5-carboxylate synthase

PN: Parenteral nutrition

PPi: Pyrophosphate

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

psi: Pounds per square inch

QoL: Quality of life

ROC: Receiver operating curve

RTI: Research Triangle Institute

SBS: Short bowel syndrome

SBSQoL^{TM}: Short bowel syndrome quality of life scale

SBTx: Small bowel transplantation

SD: Standard Deviation

SE: Standard Error

SENS: Sensitivity

SF-36: Short Form 36 questionnaire

SMD: Standardised mean difference

SPEC: Specificity

sROC: Summary receiver operating curve

SS: Sum of squares

SV: Screening visit

$t_{1/2}$: Half life

T_{max}: Time until maximum concentration

TMS: Tandem mass spectrometry

TN: True negative

TOMC: Time point of maximum concentration

TP: True positive

TPN: Total parenteral nutrition

UC: Ulcerative colitis

UCH: University College Hospital

UCL: University College London

UCLH: University College London Hospitals

UPLC: Ultra Performance Liquid Chromatography

V_d: Apparent distribution volume

WMD: Weighted mean difference

ΔC_{max}: Difference in C_{max}

μ: micro

μM: μmol/L

Preface and Overview

Citrulline is a non-protein amino acid produced predominantly by the enterocytes of the intestine. This unique site of production has urged many researchers to examine whether citrulline plasma levels can operate as a reliable marker of enterocyte mass and functionality in conditions of diseased or resected intestine (short bowel syndrome, Crohn's disease, coeliac disease etc.). In this thesis, various aspects of citrulline in relation to the intestine are investigated. The main questions of the thesis are:

1. Is citrulline being used in clinical practice? If so, then how is it being used (e.g. diagnostically, therapeutically, which particular conditions etc.)?
2. How is citrulline related to the intestine?
3. How can existing knowledge provide grounds to utilize citrulline as a marker to reflect intestinal function (absorption and possibly others)?
4. Can citrulline be a surrogate marker of other consequences related to bowel disease?

The thesis is organised around answering these questions. In Chapter 1, original historical details on the history of citrulline are presented, showing that citrulline was first isolated in 1914, contrary to the belief of 1930. The current "citrulline" is product of the watermelon (*C. vulgaris*) and is different from a previous "citrulline" which was a product of the bitter apple (*C. colocynthis*) and was used as a laxative. Next, a brief description of the biochemistry of citrulline is given alongside current measurement techniques. The knowledge about citrulline's biochemical pathways is a result of quite extensive animal studies which have examined citrulline metabolism in developmental biology settings, in experimental medical conditions (massive small bowel resection, transplantation, mucositis, and internal anal sphincter function) and in physiological conditions. Overall, citrulline is part of pathways implicating glutamine and arginine metabolism as well and participates in two important cycles: the urea cycle and arginine-nitric oxide cycle. Important enzymes are ornithine aminotransferase, ornithine carbamoyltransferase, arginase, argininosuccinate synthase, argininosuccinate lyase, and carbamoyl phosphate synthase. A systematic review of animal studies was performed in Chapter 1 and suggests three large fields of citrulline research: biochemistry and developmental

biology of citrulline, clinical significance of citrulline, and citrulline function in colonocytes.

In Chapter 2, I focus my attention on the role of citrulline as a diagnostic marker in human clinical conditions. A systematic review of studies was performed along with a statistical meta-analysis. Overall, plasma citrulline measurements have been used in necrotizing enterocolitis, intestinal transplantation, short bowel syndrome, enteropathies (villous atrophy syndromes, Crohn's disease, and mucositis) and critical illness patients. Citrulline was strongly positively correlated with enterocyte mass. This correlation was clinically significant in short bowel syndrome. In other conditions where short bowel isn't an issue, there is a decrease in average citrulline levels compared to healthy controls and citrulline decrease can be correlated to the degree of disease severity apart from Crohn's disease. Its interpretation should be cautious because its diagnostic accuracy is satisfactory but doesn't exclude absolutely false negative or false positive cases. Factors that influence interpretation are lower population values, combination of intestinal disorders, possible bowel adaptation, measurement timing, inflammation and extent of disease. Citrulline was correlated positively with enteral absorption, albeit moderately. Although a higher plasma citrulline level suggests a more absorptive gut, this interpretation needs to take into consideration other factors such specific macronutrients, condition examined, extent of mucosal disease etc. A cut-off citrulline level at 20 μmol/L has sensitivity and specificity of 80% in all conditions. Limitations of the meta-analysis were presence of heterogeneity and the possibility of publication bias, detection bias and confounding bias.

In Chapter 3, I describe the design and results of the clinical study examining bioavailability of amino acids in healthy volunteers with a view to examine the potential utility of an oral citrulline stimulation test. The five oral challenges were the amino acids citrulline, glutamine and arginine against controls dextrose and 3-methylhistidine in a 5 × 5 crossover design (balanced Williams design). Post-absorptive amino acid levels of citrulline, glutamine, arginine, ornithine and 3-methylhistidine were measured with mass spectrometry and analysed with a crossover ANOVA and bioavailability testing. Overall, citrulline can function successfully as an oral challenge test. This oral challenge test is time dependent with citrulline decreasing after the oral challenge is performed. Interestingly, citrulline was the most potent stimulator for all other amino acids, despite glutamine

challenges being readily considered the most potent stimulators of citrulline production (Peters *et al.*, 2007c; Peters *et al.*, 2008b). This possibly demonstrates more efficient amino acid absorption and that this could be an important step to countering disease-related catabolism or assist in protein anabolism. Hence, citrulline challenges could be useful in intestinal failure but also in liver failure where urea cycle pathways including glutamine, arginine and ornithine are implicated. There were differences in the bioavailability of all five amino acids as shown with statistical testing (ANOVA $p < 0.05$ for citrulline, arginine and ornithine). There was no period, carryover or sequence effect except for 3-methylhistidine which had a significant period effect (possibly due to small concentrations). No treatment was equivalent to treatment with citrulline for producing equivalent post-absorptive concentrations ($p > 0.05$). Results were satisfactory from robustness, despite an apparent variability. Elements that provide robustness are powerful enough design with no carryover effects, acceptable accurate standard curves when analysing data in mass spectrometer, crossover ANOVA is reportedly robust and outlier analysis depicted markedly less influential points in the placebo treatment group in all plasma amino acids measured, except for plasma glutamine.

In Chapter 4, I present results regarding quality of life in patients with short bowel syndrome. This study was an investigation of quality of life in short bowel syndrome patients. The SBSQoL^{TM} scale used is highly reliable in measuring quality of life in short bowel syndrome patients with a high Cronbach's alpha (over 0.700). Four factors were extracted from the 17-item scale: general physical symptoms and activities, daily activities, digestive system symptoms, and stoma related symptoms. The main causes of low quality of life were fatigue, diarrhoea/increased stomal output, lack of sleep, gastrointestinal symptoms, and muscle pains. Lack of sleep has been frequently associated with short bowel syndrome predominantly due to long parenteral fluid infusion times and/or a daily infusion schedule because of large-volume fluid requirements, due to pumps and equipment alarms, fear of catheter dislodgement, nocturia, polyuria, need for extra storage space, and complaints of carrying heavy backpacks (Winkler and Smith, 2014). Physicians should treat these symptoms as much as possible to ease the burden of patients' disease (e.g. loperamide for diarrhoea, paracetamol or stronger pain killers, zopiclone for sleeping). Due to the transitive property of associations,

the findings from this quality of life study suggest that changes of plasma citrulline levels are associated with changes with quality of life in patients with short bowel syndrome, due to the simultaneous association of quality of life and changes in citrulline levels with short bowel syndrome.

Finally in Chapter 5, I describe a summary of the thesis' new findings, along with limitations and future work that could be performed based on this thesis' results. The present thesis creates the potential for future studies in the field. These could involve:

1. A dynamic study with oral citrulline challenges in states of compromised intestine, such as extensive enteropathies (e.g. coeliac disease, mucositis, radiation, Crohn's disease) or short bowel syndrome or intestinal failure.
2. Citrulline challenges in liver failure, since citrulline is part of the urea cycle.
3. Oral challenges in cases of compromised gut during critical illness. This is similar to extensive enteropathy but it is broader since multiple factors can contribute to critical illness enteropathy: endotoxemia, sepsis, catabolism, nitric oxide changes, arginine fluxes, presence of excessive lactate, etc. In Chapter 2 it was shown that citrulline was measured in critical illness but mainly as a static measure, with all the relevant shortcomings. A study in this setting would examine the potential of an oral challenge against single or serial measurements of citrulline.
4. A larger study for quality of life examining quality of life in conjunction with other psychosocial parameters, other quality of life scales, plasma citrulline levels, test re-test reliability, construct validity and responsiveness. Quality of life studies should be able to differentiate quality of life affected by home parenteral nutrition and short bowel syndrome.

Chapter 1
Introduction

Citrulline, a non-protein amino acid, has attracted research groups' interest around the world more predominantly in the last decade. What are those features that make citrulline so special? Why are researchers investigating this amino acid with profound interest? How widespread is citrulline measurement among researchers and practitioners and what findings have been filling the pages of scientific journals regarding citrulline?

These are certain aspects that Chapter 1 of the thesis will try to answer. However, it will take a focused view of citrulline with respect to its role in intestinal physiology and pathology. It will critically review the literature and try to synthesize statistically the literature, where possible, to provide significant outcomes to discuss. Hence, this chapter will first discuss the history of citrulline, then move on to the metabolism of citrulline, important biochemical pathways and measurement methods for citrulline; and finally a systematic review of citrulline and the intestine in animal studies.

1.1 History of Citrulline

"Citrulline was first isolated in 1930" (Moinard *et al.*, 2008). This is a common phrase appearing when we read nutritional and gastroenterology literature regarding citrulline. Nevertheless, some interesting historical sources detail that the amino acid citrulline was discovered much earlier, while there was use of another "citrulline" in the late 19th century.

To begin with, in the *North Carolina Medical Journal*, January 1883 (1883a, p. 44), the following passage can be read:

> The colocynthum purum prepared by Merck […] produces watery stools with moderate tormina[1] […] There is also ***a resinoid substance called citrullin***, extracted from the colocynth fruit, insoluble in water, which, when taken internally in the dose of 5 milligrammes to 1 centigramme, or if administered hypodermically in the same dose […] will produce the desired effect.

[1] Acute abdominal pain

Similar excerpts appeared then in other journals, such as the *Medical Age* (1894) (Figure 1.1) and the *Journal of the American Medical Association* (1909), and a significant gastroenterologist of that time, Ismar Boas (1858-1938) (Hoenig and Boyle, 1988), comments on citrulline's usefulness with difficult cases of constipation (Boas, 1904). To the reader of nutrition and gastroenterological articles, this early mentioning of citrullin[e] causes some surprise. That is because citrulline is generally regarded as having been first discovered in the 1930s (Wada, 1930a) and found to play a key role in the urea cycle. A therapeutic action is not generally attributed to citrulline, so this laxative effect also surprises us. Below I explore the history of the citrullines since, as will become clear, there is more than one.

1.1.1 Early Sources Mentioning the Term "Citrulline"

I performed a systematic review of all readily available texts dating before 1930, which mentioned the term "citrulline". A preliminary analysis had shown us that the synonymic terms "citrullin" and "citrullinum" were also in use. I searched databases with extensive digitization of volumes of rare books including Google Books (http://books.google.com), Internet Archive (http://www.archive.org), and Gallica (http://gallica.bnf.fr). These three databases are the largest online sources of mass digitization projects of books from a large range of American and British universities, and of the National Library of France. The search terms "citrulline", "citrulline" and "citrullinum" for the period 1800-1930. A source of bias might have been introduced, due to the fact that languages which do not use the Latin alphabet were not searched.

Results revealed that 170 books and journal volumes have the word citrulline among their pages. These were all published between 1882 and 1930 and are mainly English, French, and German texts, with very few in Dutch, Swedish, and Italian (Appendix A). Almost all texts mention the results of two main articles, written by Hiller (1882) and Kohlstock (1892), discussing subcutaneous and rectal injection of purgatives.

This citrulline is a resin produced by the pulp of *Citrullus colocynthis*. *C. colocynthis* Schrad (family Cucurbitaceae), also known as colocynth or bitter apple, which is a common weed found in countries of the Middle East and the Mediterranean (Figure 1.2). *C. colocynthis* has been used medicinally since ancient

times (Hatam *et al.*, 1989). The fruits and seeds are used as a purgative and have been suggested to possess antitumor activity (Chaturvedi *et al.*, 2003). From the pulp of *C. colocynthis*, α-elaterin, α-elaterin-2-D-glucopyranoside, citrullol, and an alkaloid with strong purgative action have been isolated (Power and Moore, 1910), while the other parts of this weed contain several more potentially active substances (El Khadem and Abdel Rahman, 1963; Hatam *et al.*, 1989, 1990). When overconsumed, it can cause colitis (Jansen, 1889; Goldfain *et al.*, 1989).

This nineteenth century citrulline is a strong purgative used occasionally in humans. In two articles dealing with subcutaneous and rectal injection of purgatives (Hiller, 1882; Kohlstock, 1892), both authors found that citrulline could be administered subcutaneously or rectally (dose: 5 mg to 20 mg) in cases of chronic constipation, but it was followed by adverse effects of severe pain, oedema and redness of the skin. Rectal injections were tolerated more easily than subcutaneous ones. Its use was most possibly hindered because of a high price or low availability (Bandler, 1915), while it was used more frequently in veterinary medicine (1907). It appears to have passed into oblivion over time possibly reflecting progressively infrequent use of a substance with a poor risk-benefit ratio or a change in its name, making it difficult to track in scientific literature.

1.1.2 The History of Modern Citrulline's Isolation

Modern citrulline is a non-protein amino acid, and in humans its plasma content is derived largely from the amount produced in enterocytes of the small bowel (Windmueller and Spaeth, 1981) (Figure 1.3). Citrulline's first isolation from the juice of the watermelon (*Citrullus vulgaris Schrad)* has generally been attributed to Mitsunori Wada (Kornberg, 2000; Curis *et al.*, 2005; Mandel *et al.*, 2005; Moinard and Cynober, 2007; Moinard *et al.*, 2008), who isolated citrulline and determined its chemical formula in 1930, naming the substance he isolated *citrulline* (Wada, 1930a). In fact, it was isolated much earlier.

The first isolation of citrulline in 1914 was by Yotaro Koga and Ryo Ohtake (Koga and Ohtake, 1914). They isolated a substance from the juice of the watermelon with the chemical formula $C_6H_{13}N_3O_3$ (Figure 1.4 and Appendix B). They did not further elucidate the structure of this new substance nor did they name it. A possible brake on wider dissemination of this new understanding was the fact that the article was

written in Japanese, making it difficult for European and American scientists to grasp its significance.

In 1930, Wada (1930a) was therefore repeating the Koga-Ohtake experiment in the same laboratory (Agricultural Chemical Laboratory, Tokyo Imperial University). He went on to define its chemical formula and structure and to prove that his observations were correct by synthesizing the new amino acid. He named this "citrulline" (Wada, 1930a, b). He further demonstrated its isolation from the tryptic digestion of casein and possibly arginine (Wada, 1933). He published his findings in the German journal *Biochemische Zeitschrift* (Wada, 1930a) and in the *Proceedings of the Imperial Academy* in English (Wada, 1930b). Publishing in German and English was of great significance since it enhanced the swift acceptance of his results.

The first isolation by Koga and Ohtake (1914) was however acknowledged by other researchers over the following years. Koga and Ohtake (1914) were attributed with its isolation from the juice of watermelon, and Wada (1930a) with the first sound study determining the chemical properties and structure of citrulline, as well as coining its name (Wada, 1930a; Vickery, 1941; Kornberg, 2000). Wada 1930a, p. 420) himself acknowledges this contribution to the first isolation of citrulline. "Sixteen years ago in this laboratory, Y. Koga and S. Odake[2] isolated from the juice of watermelon a nitrogenous compound as colorless prisms, different from arginine and Glycocoll betaine… The analysis yielded the empirical formula $C_6H_{13}N_3O_3$. Since then, the structure has not been elucidated".

In 1931, Ackermann (1931, p. 66) indicates that "such a substance had already been isolated from the watermelon, Citrullus vulgaris, by Y. Koga and S. Odake, and its structure had been fully described by Wada". In 1941, Vickery (1941, p. 95) records "In 1914, Koga and Odake described the isolation of a substance $C_6H_{I3}N_3O_3$ from the juice of watermelon. Aside from the fact that it formed a copper salt, little else was recorded. In 1930, Wada prepared the substance again and showed that its properties were best explained on the assumption that it is L-carbamido ornithine". This account is repeated in a later review on the discovery of amino acids (Vickery, 1972).

[2] Wada (1930b) possibly spells Ryo Ohtake's surname as Odake. This spelling is used by all the other authors citing their work after 1930. We have kept the original spelling, according to our translation, but Ohtake and Odake refer to the same individual.

Krebs and Henseleit (1932) demonstrated that citrulline is an intermediate in the mechanism whereby urea is formed in the liver, signifying the importance of this amino acid in nitrogen metabolism (Fearon, 1939), citing first the work by Koga and Ohtake (1914), and then that of Wada (1930a). Fearon (1939, p. 902) notes that "the carbamido-acid citrulline, isolated by Koga & Odake in 1914 from the watermelon, attracted no general attention until Wada established its constitution as α-amino-δ-carbamidovaleric acid". Finally, Impellizzeri *et al.* (1975), while claiming that citrulline has been detected in a variety of plant sources and presumably is universal in plants (because of its role in arginine biosynthesis), cite the work by Koga and Ohtake (1914) as the first to have isolated citrulline from the juice of watermelon.

In conclusion, before 1930, when modern citrulline was first believed to be isolated, there was another use of the term citrulline, signifying a resin produced by *C. colocynthis*. This citrulline is different from modern citrulline. In addition to this, modern citrulline was not isolated in 1930 but surprisingly somewhat earlier. Reviewing the original manuscript indicates that Koga and Ohtake (1914) isolated citrulline for the first time in 1914, even though their work did not lead to the determination of its structure and nature.

1.2 Biochemical and Analytical Properties of Citrulline

Until recently, citrulline had not managed to attract the nutrition community's interest, most probably because citrulline is a non-protein amino acid, and was viewed solely as a metabolic intermediary in the urea cycle. Also, citrulline is almost absent from natural foods, with the watermelon being a notable exception (Curis *et al.*, 2005; Cynober *et al.*, 2010).

1.2.1 Citrulline Metabolism

Citrulline is a colourless solid at ambient temperature and pressure. Its melting point is 222°C. It is an α-amino acid with an asymmetric carbon; hence, it presents two enantiomers. Like most amino acids, its natural form is the L form.

Citrulline is derived from arginine, either directly via a reaction mediated by nitric oxide synthases or indirectly via conversion by arginase into ornithine, which in turn is acted on by ornithine carbamoyltransferase to deliver citrulline. Another

indirect citrulline source is glutamine, via a series of reactions illustrated in Figure 1.5.

Citrulline is metabolized by a unique reaction, catalysed by argininosuccinate synthase, into argininosuccinate, which is immediately converted back to arginine by argininosuccinate lyase (Figure 1.5). Arginase, ornithine carbamoyltransferase, argininosuccinate synthase and argininosuccinate lyase are all expressed simultaneously only in periportal hepatocytes in the Krebs-Henseleit cycle, also known as the urea cycle (Krebs and Henseleit, 1932). Other cells expressing some of these enzymes enable intra- or extra-cellular cycles, as described in Table 1.1, with the kidneys playing the most important function in the degradation of citrulline. These cycles are of physiological significance; for example, up to 20% of nitric oxide production is derived from recycled arginine, while in patients with severe kidney problems such as renal failure, high citrulline plasma levels is a frequent finding (Rabier and Kamoun, 1995; Curis *et al.*, 2005).

1.2.2 Intracellular Cycles

Citrulline recycling into arginine is particularly efficient in macrophages, making citrulline almost as efficient as arginine at producing nitric oxide, especially against a background of low arginine availability such as in inflammatory states. Macrophages not only express both argininosuccinate synthase/ argininosuccinate lyase and nitric oxide synthase but also arginase type II and ornithine decarboxylase. Thus macrophages can synthesize nitric oxide or aliphatic polyamines.

1.2.3 Inter-organ Cycle

The inter-organ exchanges of citrulline are summarized in Figure 1.6. The citrulline interorgan cycle was first described by Windmueller and Spaeth (1981), who established that citrulline was synthesized in the intestine from glutamine and arginine. Later on, a close correlation between glutamine uptake and citrulline release by the small intestine was demonstrated in adults (Crenn *et al.*, 2008). In new-borns, the gut releases arginine and not citrulline, due to high argininosuccinate synthase/ argininosuccinate lyase expression. The reason for this is that breast milk is not sufficiently rich in arginine to meet requirements, coupled with the fact that the kidney is not mature and its arginine production is still low (Köhler *et al.*, 2008). A feature of this inter-organ cycle is the absence of any

significant hepatic uptake or release of citrulline, hepatic citrulline metabolism being strictly compartmentalized to periportal hepatocytes (Rabier and Kamoun, 1995).

This inter-organ cycle plays a key role in the modulation of nitrogen homeostasis according to dietary protein availability (Curis *et al.*, 2005). After a protein meal, the urea cycle is activated by high arginine availability, since arginine is a potent inducer of ureagenesis, both as a cycle intermediary and as an activator of N-acetylglutamate synthase (Figure 1.5). N-acetylglutamate is the allosteric activator of carbamoyl-phosphate synthase. In other words, ureagenesis is activated as more arginine appears in the portal blood. The only other amino acid able to produce citrulline in the gut is glutamine, which is also the only other amino acid able to strongly activate ureagenesis.

Ultimately, arginine in the splanchnic area functions as a sensor of excessive nitrogen intake, since high arginine in the portal blood reflects high protein intake: activation of ureagenesis in this condition prevents excessive, potentially neurotoxic amino acid levels in the general circulation, and provides a way to decrease arginine availability in the general circulation and thereby avoid excessive nitric oxide production.

When protein (i.e. arginine and glutamine) availability is low, derepression of ornithine carbamoyltransferase in the enterocyte leads to release of citrulline (instead of arginine) by the intestine. Since citrulline bypasses the liver, this slows down the urea cycle and avoids nitrogen wastage, as arginine requirements are covered by resynthesis in the kidney. In other words, citrulline is a masked form of arginine that bypasses the liver (arginine synthesized from citrulline represents 60% of the de novo synthesis of arginine in adults).

1.2.4 Analytical Methods to Quantify Citrulline

Various methods can be used to quantify citrulline, either specifically or similar to other amino acids (Curis *et al.*, 2007). Only the most frequently used and recently proposed methods are discussed here.

1.2.4.1 Ion Exchange Chromatography

Ion exchange chromatography with post column derivatization (ninhydrin) is the reference method for amino acid analysis. The dosage is fully automatized and the performance (between-run reproducibility) for citrulline measurement is good, with a coefficient of variation of 4.3 % (Neveux *et al.*, 2003). A *short program* allows specific measurement of citrulline in 30 minutes from run-to-run. The limitation of this method is its relatively low sensitivity, making it inadequate for arteriovenous difference measurement.

1.2.4.2 Reversed Liquid Phase Chromatography

Citrulline concentration can be measured by reversed liquid phase chromatography using various precolumn derivatization agents, in particular o-phtalaldehyde. The between-run reproducibility is not as good as for ion exchange chromatography but the dosage is more sensitive, allowing measurements of arteriovenous differences (Curis *et al.*, 2005).

1.2.4.3 Mass Spectrometry on Dried Blood Spot

Citrulline has recently been measured by hydrophilic interaction chromatography/mass spectrometry/mass spectrometry on dried blood spot specimens to monitor graft function following intestinal transplantation. The advantages of this method are that it is almost non-invasive and requires minimal blood sampling (< 25 µL). Also, hydrophilic interaction chromatography/mass spectrometry/mass spectrometry detection has high sensitivity and a low limit of detection (1.5 µmol/L). Using dry blood spot specimens induces large variations compared to results obtained using plasma, however. For example, patients with a citrulline concentration of 60 µmol/L using the dry blood spot method may have a plasma concentration ranging from 38-90 µmol/L. Similar uncertainty is found for low values (dry blood spot measured at 15 µmol/L equals plasma citrulline levels between 10 µmol/L and 40 µmol/L) (Yu *et al.*, 2005; Englund *et al.*, 2014).

1.2.4.4 Ultra Performance Liquid Chromatography Coupled with Mass Spectrometry

Ultra performance liquid chromatography coupled with mass spectrometry (UPLC-MS/MS) is performed with a SystemSolvent delivery and sample introduction is

performed using a Waters ACQUITY Ultra Performance Liquid Chromatography system (Waters, Milford, MA, USA) equipped with a thermostat for both the sample and column compartments which are maintained at 4°C and 55°C, respectively. A Waters ACQUITY TQ tandem quadrupole mass spectrometer, interfaced with an atmospheric pressure electrospray ionization (AP-ESI) source is used for the analysis. Separation is performed on a Hydrophilic Interaction Liquid Chromatography (HILIC) column, 2.1 mm × 100 mm, packed with 1.7 μm particles designed to withstand 15,000 psi. Mobile phases A and B consist of distilled water containing 0.1% (v/v) formic acid, and acetonitrile with 0.1% formic acid, respectively. The gradient program is as follows: flow rate in all steps at 0.45 mL/minute unless otherwise indicated. Initial: 90% B; 0-2.5 minutes: a gradient to 55% B; 2.5-3.5 minutes: 2% B; 3.5-4.9 minutes: reversion of the mobile phase to 90% B at a flow rate of 0.60 mL/minute; 4.9-5 minutes; 90% phase B. Full loop injection is used to introduce 10 μL of sample into the system.

The atmospheric pressure electrospray ionization is operated in the positive ion mode. Nitrogen is used as the nebulizing and desolvation gas at a flow rate of 100 and 1000 L/hour, respectively; argon at a pressure around 3×10^{-3} mbar will be used as collision gas at a flow rate of 15 mL/hour. The ion source and the desolvation temperature is maintained at 130°C and 400°C, respectively. All aspects of system operation and data acquisition is controlled using Masslynx version 4.1 software with automated data processing using the Quanlynx Application Manager (Waters) (Demacker *et al.*, 2009a; Demacker *et al.*, 2009b).

This section was a general introduction to the biochemistry of citrulline in the body and methods for analytical measurement of citrulline. In the next section, a systematic review of the available literature on citrulline is presented and possible theories that explain the findings are presented.

1.3 Non-Human Studies of Citrulline and the Intestine

For this section, a systematic review of the literature was done. The inclusion criteria for this systematic review were: any empirical study (abstract and full paper) describing investigation of citrulline in relation to the term intestinal function in animals. The only papers that were excluded were those whose object of investigation was not related to intestinal function. There was no restriction to

language of papers and the type of studies. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews were used (Moher *et al.*, 2009).

Electronic database searches were conducted in Google Scholar, Pubmed/Medline, Scopus, and EMBASE with no year limits. Publisher databases were also searched (sciencedirect.com, link.springer.com, Wiley Library Online, Highwire Press, nature.com, Ovid, Cambridge University Press). The search keywords were: citrulline, intestine, gut, non-human populations, mucositis, resection, transplant, radiotherapy, cancer, enterocyte, and colitis. The date of search was up until 1 July 2015. The bibliographies from all included manuscripts and hand searching of relevant gastroenterology and nutrition journals were used to identify further references. The resulting studies (in abstract form) were assessed against the inclusion criteria. When there was insufficient information available in the abstract, the full text was reviewed. Then, data were extracted from the selected studies including: author, year of publication, aim of the study, study design, and results.

The search yielded 226 studies, out of which 80 involved some study protocol examining citrulline metabolism in relation to the intestine or intestinal function in animals other than humans (Figure 1.7). The design and basic findings of all papers are shown in Tables 1.2 and 1.3.

The period of citrulline animal studies begins in the 1980s, when the papers by Windmueller and Spaeth (1981) and others pointed out that plasma citrulline is produced predominantly by the intestine and described basic biochemical pathways. The second period begins in the early mid-1990s with the studies by Wu *et al.* (1994a); Wu *et al.* (1994b), who produced very important clarifications in the pathways of citrulline production and the enzyme activity during initial postnatal stages. Finally, the third period of studies involves the 2000s, particularly after the seminal papers by Crenn *et al.* (2000); Crenn *et al.* (2003) in the significance of citrulline levels for predicting various grades of intestinal failure. The animal models used involve pigs, mice, rats and sheep. The studies can be categorised into the following topics, discussed below.

1.3.1 Biochemistry and Developmental Biology of Citrulline

The majority of non-human studies involve biochemistry, which deal with biochemical pathways in cells, molecules and interorgan crosstalks, and developmental biology, which studies the process by which organisms grow and develop. The basic metabolism of citrulline was described in the previous chapter, but this knowledge was gradually built upon the published results and studies confirming them. Windmueller and Spaeth (1981) described in a model of rats that circulating citrulline was the result of production in the enterocyte; the chemical pathway leading to citrulline's production was also clarified, involving the enzymes ornithine aminotransferase, ornithine carbamoyltransferase, pyrroline-5-carboxylate synthase, and nitric oxide synthase among others, with mainly the amino acids glutamine, ornithine and arginine affecting the production of citrulline. The full classification of papers according to categories is shown in Tables 1.2 and 1.3.

Some interesting papers are described. Recently, a chemical kinetics paper by Bensaci *et al.* (2012) point out that in physiological and pathological conditions of the enterocyte, the most important enzyme affecting citrulline was ornithine aminotransferase with similar results originally described in the past as well (Wu, 1997; Wu *et al.*, 1997; Dekaney *et al.*, 2001, 2003). It is commonly accepted that arginine increases citrulline concentrations through the nitric oxide synthase pathway, with many descriptions going as back as 1976 (Herzfeld and Raper, 1976; Uchiyama *et al.*, 1981; Hurwitz and Kretchmer, 1986; Edmonds *et al.*, 1987). However, a recent study in a rat model of ischaemia and reperfusion injury showed that administering arginine decreased citrulline; this reduction in levels was attributed to the fact that during long term arginine administration, arginine may not be metabolized efficiently in late post-injury stages (Lee *et al.*, 2012).

In parallel with developments in biochemistry, progress has been made in the developmental biology of citrulline and its synthesizing enzymes. There has been a noted difference in citrulline's chemical pathways enzymes during perinatal periods in pigs and chicks as well as before and after weaning. In perinatal mice (Hurwitz and Kretchmer, 1986; de Jonge *et al.*, 1998) and piglets (Wu *et al.*, 1994b; Wu *et al.*, 1995a; Wu *et al.*, 1995b; Wu *et al.*, 1996; Wu *et al.*, 1997), all enzymes necessary for arginine biosynthesis from proline and glutamine, are expressed in the enterocytes of the small intestine, while arginase, the main cytosolic arginine-

catabolizing enzyme, is not detectable prior to weaning (Wu *et al.*, 1995a). In agreement with this, the small intestine plays a prominent role in net arginine production in suckling piglets (Wu *et al.*, 1994a; Wu *et al.*, 1994b; Urschel *et al.*, 2005; Urschel *et al.*, 2006). In rodents, intestinal expression of the enzymes that synthesize arginine from citrulline, argininosuccinate synthetase and argininosuccinate lyase, ceases completely after weaning (de Jonge *et al.*, 1998). In pigs, on the other hand, net synthesis of arginine declines more gradually and is still present at seven weeks of age (Wu, 1997). It has been suggested that enteric arginine synthesis is necessary to cover neonatal requirements, because mammalian milk is a relatively poor source of arginine, whereas its precursors proline and glutamine are abundant (Köhler *et al.*, 2008).

1.3.2 Clinical Significance of Citrulline

Three major themes dominate this field: citrulline levels, metabolism and therapeutic effects in mucositis, intestinal resection and transplantation. There have also been studies in cancer models, endotoxemia and necrotizing enterocolitis. For radiation induced mucositis, there is a universal consensus that citrulline markers correlate with intestinal function markers such as villous depth or villous morphology (Lutgens *et al.*, 2003; Siqueira *et al.*, 2010; Fijlstra *et al.*, 2011). For transplantation, citrulline was examined in pigs as a marker for graft rejection. Plasma citrulline levels failed as a marker in the early diagnosis of acute cellular rejection and became reliable only when advanced mucosal damage was present (Nadalin *et al.*, 2007). Finally, in intestinal resection citrulline decreases massively and arginine becomes an essential amino acid which supplements nitrogen (Wakabayashi *et al.*, 1994; Wakabayashi *et al.*, 1995; Lardy *et al.*, 2004; Osowska *et al.*, 2004; Osowska *et al.*, 2006; Osowska *et al.*, 2008). The other studies on necrotizing enterocolitis, endotoxemia and cancer assess citrulline levels as surrogate markers of intestinal function examining it in conjunction with their other primary objectives.

1.3.3 Citrulline Function in Colonocytes

Citrulline recycling may be responsible for the maintenance of internal anal sphincter relaxation during nonadrenergic, noncholinergic nerve stimulation. Also citrulline has been studied in the context of nitric oxide inhibitors and their use in

colonocytes (Grider and Jin, 1993; Berrino *et al.*, 1995; Shuttleworth *et al.*, 1995; Shuttleworth and Sanders, 1996; Chakder and Rattan, 1997; Rattan and Chakder, 1997; Shuttleworth *et al.*, 1997; Armstrong *et al.*, 2000).

1.4 Summary and Conclusion

Original historical details on the history of citrulline were presented, showing that citrulline was first isolated in 1914, contrary to the belief of 1930. The current "citrulline" is product of the watermelon (*C. vulgaris*) and is different from another "citrulline" of the past which was product of the bitter apple (*C. colocynthis*) and was used as a laxative. Next, a brief description of the biochemistry of citrulline was given alongside current measurement techniques. The knowledge about citrulline's biochemical pathways is a result of quite extensive animal studies which have examined citrulline metabolism in developmental biology settings, in experimental medical conditions (massive small bowel resection, transplantations, mucositis, and internal anal sphincter function) and in physiological conditions. Overall citrulline is a result of glutamine and arginine metabolism and participates in two important cycles: the urea cycle and arginine-nitric oxide cycle. Important enzymes are ornithine aminotransferase, ornithine carbamoyltransferase, arginase, argininosuccinate synthase and lyase and carbamoyl phosphate synthase. A systematic review of animal studies was performed and suggests three large fields of research: biochemistry and developmental biology of citrulline, clinical significance of citrulline, citrulline function in colonocytes. Overall, this information suggests that there is a potential for citrulline (diagnostically or therapeutically) for use in clinical practice and this will be focus of Chapter 2, where a systematic review and meta-analysis of human studies examining citrulline's diagnostic role are presented.

1.5 Tables

Table 1.1. Sites and behaviour of citrulline catabolism into arginine through argininosuccinate synthase/ argininosuccinate lyase (Breuillard *et al.*, 2015; Wijnands *et al.*, 2015).

Organ or cells	***Function***
Kidney	Arginine release to meet tissue requirements
Immune cells	Recycling into arginine for nitric oxide synthesis or polyamine synthesis (mainly in macrophages)
Endothelial cells	Recycling into arginine for nitric oxide synthesis
Hepatocytes	Fuels arginine in the urea cycle
Gut	Only in new-borns (downregulation of argininosuccinate synthase/ argininosuccinate lyase activities at weaning); arginine production to meet requirements (mostly because arginine from the milk fails to do so)

Table 1.2 Animal or cellular studies investigating citrulline in the intestine.

No.	Authors	Settings	Findings	Categorization
1	Beaufrere *et al.* (2014)	• Aim: to investigate whether the effect of glutamine on the increase in intestinal villus height is correlated with an increase in both gut mass and citrulline plasma level in very old rats. • Design: Very old (27 months) female rats were supplemented with oral glutamine (20% of diet protein). • Intestinal histomorphometric analysis of the small bowel was performed. • Amino acids, in particular citrulline, were measured in the plasma, liver and jejunum.	• Total intestine mass was significantly higher in glutamine-supplemented rats than in controls (15%). • Measurement of villus height and crypt depth demonstrated that the difference between villus and crypt was significantly improved in glutamine pre-treated rats compared to controls (~ 11%). • Plasma citrulline also increased by 15% in glutamine-supplemented rats compared to controls.	Clinical Significance (Intestinal Mass)
2	Gutierrez *et al.* (2014)	• Aim: This study compared serial serum citrulline and mucosal adaptive potential after proximal versus distal small bowel resection. • Design: Enterally fed Sprague-Dawley rats underwent sham operation or 50% small bowel resection, either proximal or distal. • Citrulline was measured at operation and weekly for 8 weeks. • At necropsy, histologic features reflecting bowel adaptation were evaluated.	• By weeks 6-7, citrulline in both resection groups significantly decreased from baseline and was significantly lower than the concentration in sham animals. • There was no difference in citrulline between proximal resection and distal resection at any point. Villus height and crypt density were higher in the proximal resection than in the distal resection group.	Clinical Significance (Intestinal Resection)
3	Shim *et al.* (2014)	• Aim: to establish an appropriate and efficient minipig model to study high-dose radiation-induced gastrointestinal syndrome after radiation exposure. • Design: Ileocutaneous anastomosis was performed 3 weeks before irradiation in six male Göttingen minipigs. • Minipigs were locally irradiated at the abdominal area using a gamma source as follows: 1,000 cGy (n = 3) and 1,500 cGy (n = 3). Endoscopic evaluation for the terminal ileum was periodically performed via the ileocutaneous anastomosis tract. Pieces of tissue were serially taken for histological examination.	• Mucosal atrophy and telangiectasia was present in the ileum from day 1 to day 17 after abdominal irradiation. • Microscopic findings were characterized as architectural disorganization, loss of villi and chronic active inflammation. Increase in cyclooxygenase-2 expression was closely correlated with severity of tissue damage and inflammation. • Plasma citrulline level was significantly decreased the day after irradiation and recovered when irradiated mucosa was normalized. • Results showed that citrulline changes were positively correlated with microscopic changes and the endoscopic score in radiation-induced mucosal damage	Clinical Significance (Intestinal Radiation Damage)
4	Cakmaz *et al.* (2013)	• Aim: To measure the plasma levels of diamine oxidase and citrulline in acute mesenteric ischaemia to gain insight into its early diagnosis. • Design: 21 Wistar albino rats were divided into three groups, control group, short-term ischemia group, and prolonged ischemia group. The superior mesenteric artery was occluded for 15 minutes in the short-term ischemia group and for 12 hours in the prolonged ischemia group. • Twelve hours later, the experiment was terminated and plasma diamine oxidase and citrulline levels were measured. Intestinal tissue was evaluated for the histopathological changes.	• Compared to the control group, the short-term and prolonged ischemia groups showed significant increases in the plasma levels of diamine oxidase, whereas the plasma citrulline levels decreased significantly. • Prolonged ischemia caused a larger increase in the plasma diamine oxidase levels and a larger decrease in the plasma citrulline levels compared to the short-term ischemia ($p = 0.011$ and $p = 0.021$, respectively).	Clinical Significance (Ischaemia)

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
5	Fijlstra *et al.* (2013)	• Aim: To compare the quantitative capacity to absorb glucose between rats with methotrexate -induced mucositis and saline-treated controls. • Design: Administration of a physiologically relevant amount of [1-^{13}C] glucose-enriched glucose as a bolus by oral gavage (2 g/kg once) or continuously by intraduodenal infusion (±1.9 g/(kg·h) for 5 hours) to rats with methotrexate-induced mucositis and controls. • Blood [1-^{13}C]glucose concentrations were determined during the experimental period. Steele's one-compartment model was used to calculate the quantitative absorptive capacity, including simultaneous intravenous infusion of [6,6-$^{2}H_2$]glucose • Jejunal histology and plasma citrulline concentrations were assessed.	• Methotrexate-induced mucositis was confirmed by a reduction in villus length and plasma citrulline (both -57 %, relative to controls, $p < 0.01$). • When glucose was administered as a bolus, methotrexate-treated rats only absorbed 15 % of administered glucose, compared with 85 % in controls ($p < 0.01$). • Upon continuous intraduodenal glucose infusion, the median absorptive capacity for glucose in methotrexate-treated rats did not differ from controls (80% versus 93 % of administered glucose respectively, $p = 0.06$). • Glucose absorption differed substantially between individual methotrexate-treated rats (range, 21%-95 %), which correlated poorly with villus length ($rho = 0.54$, $p = 0.030$) and plasma citrulline ($rho = 0.56$, $p = 0.024$).	Clinical Significance (Mucositis)
6	Nakamura *et al.* (2013)	• Aim: To investigate whether dietary glutamate-N is an effective nitrogen source for amino acid synthesis and investigated the fate of dietary glutamate-N using [^{15}N]glutamate. • Design: Fischer male rats were given hourly meals containing [U-^{13}C]- or [^{15}N]glutamate. The concentration and isotopic enrichment of several amino acids were measured after 0-9 hours of feeding, and the net release of each amino acid into the portal vein was calculated.	• Most of the dietary glutamate-C was metabolized into CO_2, lactate, or alanine (56%, 13%, and 12% of the dietary input, respectively) in the portal drained viscera. • Most of the glutamate-N was utilized for the synthesis of other amino acids such as alanine and citrulline (75% and 3% of dietary input, respectively) in the portal drained viscera, and only minor amounts were released into the portal vein in the form of ammonia and glutamate (2 %and 3% of the dietary input, respectively).	Biochemistry
7	Marini *et al.* (2012)	• Aim: To test whether during the neonatal period de novo synthesis is the main source of ornithine for citrulline synthesis • Design: Neonatal piglets were infused intravenously or intragastrically with [U-$^{13}C_6$]arginine, [U-$^{13}C_5$]glutamine, or [U U-$^{13}C_5$]proline during the fasted and fed periods. [ureido-^{15}N]citrulline and [$^{2}H_2$]ornithine were infused intravenously for the entire infusion protocol.	• During fasting, plasma proline (13%) and ornithine (19%) were main precursors for citrulline synthesis, whereas plasma arginine (62%) was the main precursor for plasma ornithine. • During feeding, enteral (27%) and plasma (12%) proline were the main precursors for the ornithine utilized in the synthesis of citrulline, together with plasma ornithine (27%). • Enteral proline and glutamine were utilized directly by the gut to produce ornithine utilized for citrulline synthesis. • Arginine was not utilized by the gut, which is consistent with the lack of arginase activity in the neonate. • Arginine, however, was the main source (47%) of plasma ornithine and in this way contributed to citrulline synthesis.	Biochemistry
8	John-Baptiste *et al.* (2012)	• Aim: To test selected candidate biomarkers with regards to their usefulness as gastric injury biomarkers in this study. • Design: Biomarkers included plasma diamino oxidase and citrulline, faecal calprotectin, bile acids, and miRNA. • p21-activated kinase 4 inhibitor as a preclinical rat model of gastric toxicity • Wistar Han rats	• L-citrulline and miR-194 results appear to correlate well with histopathology findings. • The results for L-citrulline levels in rat plasma detected by mass spectrometry showed a significant dose- and time-dependent decrease with a ~60% decrease on day 3 and ~90% decrease on day 5. • The decrease on day 5 compared with baseline was consistent with an overall reduction in enterocyte mass, which manifested histologically as crypt necrosis and villus atrophy and fusion.	Clinical Significance (Mucositis)

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
9	Batista *et al.* (2012)	• Aim: To assess citrulline effects on gut barrier integrity and bacterial translocation in mice undergoing intestinal obstruction • Design: Mice were divided into 3 groups: sham, intestinal obstruction, and citrulline. The citrulline group received a diet containing 0.6% citrulline; the intestinal obstruction and sham groups were fed a standard chow diet. • Terminal ileum was ligated except the sham group, which only underwent laparotomy.	• The citrulline group presented decreased intestinal permeability and bacterial translocation when compared with the intestinal obstruction group. • Histopathology showed that citrulline preserved the ileum mucosa • The intestinal secretory Immunoglobulin A concentration was higher in the citrulline group. The intestinal obstruction group presented the highest levels of interferon-γ.	Biochemistry Therapeutic significance of citrulline
10	Bensaci *et al.* (2012)	• Aim: To production of mathematical model of the enterocytic glutamine to citrulline conversion in the fasting state • Design: Two different approaches were compared: a standard approach based on the Michaelis-Menten assumptions (King-Altman approach) and an association-dissociation approach based on the kinetic mass action law (Van't Hoff approach) • Cellular scale study	• In both cases, the model correctly predicts the physiological plasma citrulline steady-state, but the two approaches present clear differences for metabolites of enzymes having a complex mechanism, challenging the validity of the King-Altman approach in such cases • When physiopathological scenarios of enzyme activity loss are simulated, both approaches predict a very sharp transition from the physiological citrulline plasma level to the lack of its production: the concentration profiles of these simulations show a clear threshold of which characteristics vary with the involved enzyme. • Ornithine aminotransferase shows the highest sensitivity in the system whatever the approach used	Biochemistry
11	Boutry *et al.* (2012)	• Aim: to examine whether glutamate, glutamine and citrulline concentrations in blood, intestine and muscle are decreased by endotoxemia, and if supplementation with glutamate or glutamine can restore normal concentrations • Design: Endotoxemia induced in rats by an intraperitoneal injection of lipopolysaccharide	• Endotoxemia rapidly but transiently decreased the circulating concentrations of almost all amino acids and more durably of glutamate, glutamine and citrulline in muscle. • Supplementation with glutamate or glutamine failed to restore glutamate, glutamine and citrulline concentrations in plasma and muscles	Clinical Significance (Endotoxemia)
12	Chapman *et al.* (2012)	• Aim: to investigate whether arginine or its precursor, citrulline protects intestinal tight junctions from hypoxia and determined if inducible nitric oxide plays a role. • Design: Neonatal piglet jejunal IPEC-J2 cell monolayers were treated with arginine or citrulline, reversible and irreversible nitric oxide synthetase inhibitors, and were exposed to hypoxia. • Intestinal tight junctions were assessed by serial measurements of transepithelial electrical resistance, flux of inulin-fluorescein isothiocyanate, and immunofluorescent staining of tight junction proteins.	• Arginine and citrulline were protective against hypoxia related damage. • At the final time-point (14 hours) the mean transepithelial electrical resistance ratio (transepithelial electrical resistance compared to baseline) for arginine + hypoxia and citrulline + hypoxia was significantly higher than hypoxia alone • Both arginine and citrulline were associated with decreased inulin flux across hypoxic monolayers and qualitatively preserved tight junction proteins. • Irreversible inhibition of nitric oxide synthases blocked this protective effect.	Biochemistry Therapeutic significance of citrulline
13	Lee *et al.* (2012)	• Aim: To investigate the effects of long-term intra-duodenal supplementation of arginine on intestinal morphology, arginine-associated amino acid metabolism, and inflammatory responses in rats with intestinal ischaemia reperfusion. • Design: Male Wistar rats with or without three hours of ileal ischemia underwent duodenal cannulation for continuous infusion of formula with 2% arginine or commercial protein powder for 7 days • Serological examinations, plasma amino acid and cytokine profiles, and intestinal morphology were assessed	• Arginine supplementation decreased serum cholesterol and increased plasma arginine concentrations. • In rats with intestinal ischaemia reperfusion injury, arginine supplementation significantly decreased serum nitric oxide, plasma citrulline and ornithine, and the mucosal protein content of the ileum. • These results suggest that long-term intra-duodenal arginine administration may not have observable benefits on intestinal morphology or inflammatory response in rats with intestinal ischemia and reperfusion injury.	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
14	Marini (2012)	• Aim: All possible enteral and plasma precursors of citrulline were studied in a mouse model during the postabsorptive and postprandial period using multitracer protocols • Design: Intragastric amino acids: 21 amino acids; Intravenous amino acids: proline, glutamine, arginine, glutamate • Three different models were used to interpret the stable isotope data.	• Dietary and plasma arginine were the main precursors for citrulline synthesis during feeding and plasma arginine during feed deprivation • Contribution of arginine was directly at the site of citrulline synthesis and through plasma ornithine, suggesting that ornithine amino transferase is a pivotal enzyme in this pathway	Biochemistry
15	Fijlstra *et al.* (2011)	• Aim: to determine lactose digestion and absorption of its derivative glucose during mucositis • Design: Wistar rats were injected intravenously with methotrexate or 0.9% Sodium Chloride (controls) • Measurement of plasma citrulline level, harvestation of the small intestine to assess histology, myeloperoxidase level, glycohydrolase activity, immunohistochemical protein, mRNA expression	• During the experimental period, the absorption of lactose-derived glucose was 4.2-fold decreased in methotrexate-treated rats compared with controls • Lactose-derived glucose absorption correlated strongly with villus length (*rho* = 0.86) and with plasma citrulline level (*rho* = 0.81) • Plasma citrulline level correlated with the severity of mucositis as measured by villus length (*rho* = 0.90)	Clinical Significance (Mucositis)
16	Marini *et al.* (2011)	• Aim: To examine if arginase II plays a central role in the supply of ornithine for citrulline synthesis • Design: The contribution of dietary arginine, glutamine, and proline was determined by utilizing multitracer stable isotope protocols in arginase II knockout ($AII^{-/-}$) and wild-type mice.	• The lack of arginase II resulted in a lower citrulline rate of appearance due to a reduced availability of ornithine; ornithine supplementation was able to restore the rate of citrulline production in arginase II knockout to levels comparable with wild-type mice • There were significant differences in the utilization of dietary citrulline precursors. The contribution of dietary arginine to the synthesis of citrulline was reduced from due to the lack of arginase II. • No enteral utilization of arginine was observed in arginase II knockout mice, and the contribution of dietary arginine through plasma ornithine was reduced in the transgenic mice • Dietary glutamine and proline utilization were greater in arginase II knockout than in wild-type mice • Most of the contribution of glutamine and proline was enteral rather than through plasma ornithine	Biochemistry
17	Puiman *et al.* (2011)	• Aim: To examine if enteral arginine is a specific stimulus for neonatal intestinal blood flow and mucosal growth under conditions of total parenteral nutrition or partial enteral nutrition. • Design: Dose dependence and specificity of acute (3 hours) enteral arginine infusion on superior mesenteric artery blood flow in pigs fed total parenteral nutrition or partial enteral nutrition. • Investigation of whether chronic (4 days) arginine supplementation of partial enteral nutrition increases mucosal growth and if this was affected by treatment with the nitric oxide synthase inhibitor, N^G-nitro-l-arginine methyl ester. • A third group of pigs were fed partial enteral nutrition and were infused enterally with citrulline, glutamate, glutamine, or glucose as a control	• Plasma arginine and ornithine concentrations during the basal saline infusion were 80%-100% higher in partial enteral nutrition compared total parenteral nutrition pigs; this may have been due to either the arginine absorbed from the formula or endogenous synthesis by enterocytes. • Plasma concentrations of citrulline were higher, whereas those of glutamine, glutamate, and threonine were lower in partial enteral nutrition pigs than in total parenteral nutrition pigs across all arginine doses • Baseline superior mesenteric artery blood flow was 90% higher in the partial enteral nutrition pigs than in the total parenteral nutrition pigs, but was not affected by acute infusion individually of arginine, citrulline, or other major gut fuels • Supplementing arginine in partial enteral feeding modestly increases intestinal mucosal growth and was nitric oxide independent • Dietary arginine was a better precursor than citrulline for maintaining blood arginine	Biochemistry, Clinical Significance (total parenteral nutrition)

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
18	Fang *et al.* (2010)	• Aim: to determine the effects of DL-2-hydroxy-4-methylthiobutyrate on the first-pass intestinal metabolism of dietary methionine and its extra-intestinal availability. • Design: Barrows (n = 6; aged 35 days; weight 8.6 kg), implanted with arterial, portal, mesenteric and gastric catheters, were fed a diet containing DL-methionine or of DL-2-hydroxy-4-methylthiobutyrate once hourly and infused intramesenterically with 1 % p-aminohippurate and intragastrically with [1-^{13}C]methionine at 7.0 μmol/kg body weight per hour. • Arterial and portal blood samples were taken at hourly intervals until 6 hours of tracer infusion and pigs was then killed for collection of muscle, intestine, liver and kidney samples. One-factorial arrangement	• Over the 6 hours period after the start of feeding, the average concentration of citrulline both in the arterial and portal plasma was higher ($p < 0.05$) in the of DL-2-hydroxy-4-methylthiobutyrate than in the DL-methionine group, and arterial plasma ornithine and taurine concentration was also higher ($p < 0.05$) in the DL-2-hydroxy-4-methylthiobutyrate than in the DL-methionine group	Biochemistry
19	Geng *et al.* (2011)	• Aim: To determine developmental changes in mRNA and protein levels for N-acetylglutamate synthase (a key enzyme in synthesis of citrulline and arginine from glutamine/glutamate and proline) in the small intestine of suckling piglets. • Design: The porcine N-acetylglutamate synthase gene was cloned using the real-time polymerase-chain reaction method. • 24 newborn Landrace × Yorkshire piglets (12 males and 12 females)	• Results indicated that intestinal N-acetylglutamate synthase mRNA levels were lower in 7- to 28-day-old than in 1-day-old pigs. • Immunochemical analysis revealed that N-acetylglutamate synthase protein was localized in enterocytes of the gut. • Notably, intestinal N-acetylglutamate synthase protein abundance declined progressively during the 28-day suckling period.	Developmental Biology
20	Marini *et al.* (2010)	• Aim: To determine if other citrulline precursors can compensate when arginine is absent in the diet • Design: Investigation of contributions of plasma and dietary precursors determined by using multitracer protocols in conscious mice infused i.e. either an arginine-sufficient diet or an arginine-free diet.	• Plasma entry rate of citrulline and arginine did not differ between the 2 diet groups • Entry rate of ornithine was greater in the mice fed the arginine-sufficient diet than the arginine-free diet • Greater utilization of plasma ornithine for the synthesis of citrulline in the mice fed the arginine-free diet than the arginine-sufficient diet • Utilization of plasma arginine did not differ between the 2 diet groups for citrulline synthesis, either through plasma ornithine or at the site of citrulline synthesis • The contribution of dietary proline to the synthesis of citrulline was mainly at the site of citrulline production, rather than through plasma ornithine • Dietary glutamine was utilized only at the site of citrulline synthesis. • Dietary glutamine and proline made a greater contribution to the synthesis of citrulline in mice fed the arginine-free diet but remained minor sources for citrulline production • Plasma arginine and ornithine are able to support citrulline synthesis during arginine-free feeding	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
21	Siqueira *et al.* (2010)	• Aim: To determine serum citrulline concentrations in mice with hepatosplenic schistosomiasis, analyse the morphologic repercussions for the mucosa of the small intestine, correlate citrulline concentrations with morphometric changes in the intestinal mucosa, and evaluate the effect of splenectomy on citrulline concentration • Design: 46 adult female albino Swiss mice were divided into two groups: Control (23 healthy mice) and experimental (23 mice with hepatosplenic schistosomiasis). • Blood samples were collected for the analysis of plasma citrulline before and after splenectomy. • A segment of the jejunum was resected for morphometric analysis.	• The average citrulline concentration in the control group was greater than that in the experimental group both before and after splenectomy ($p < 0.001$). • In the experimental group, the villi had less height and area, and there was a smaller perimeter of the mucosal surface ($p = 0.003$, $p < 0.001$, and $p = 0.001$, respectively) • There was a direct correlation between citrulline concentration and the height and area of the villi ($p = 0.003$ and $p = 0.04$, respectively) • After splenectomy, there was a reduction in the mean citrulline concentration in the experimental group ($p = 0.009$).	Clinical Significance (Mucositis)
22	Berkeveld *et al.* (2009)	• Aim: To determine how, in piglets that have been subjected to intermittent suckling, age at weaning and the duration of the preceding intermittent suckling period contribute to postweaning adaptation through effects on feed intake, growth, and gut characteristics • Design: All piglets had ad libitum access to creep feed from day 7. Litters were subjected to conventional weaning or to 1 of 3 intermittent suckling regimens. In conventional weaning, litters ($n = 29$) had continuous access to the sow until weaning (day 26, day 0 = farrowing). During intermittent suckling, litters had access to the sow between 16:00 and 06:00 hours.	• Weaning reduced plasma citrulline concentrations resulting in reduced concentrations on day 2 and 8 postweaning compared with the values observed at weaning (overall $p = 0.01$ and $p < 0.001$). • Postweaning plasma citrulline concentrations were not different between treatments. • At weaning, plasma citrulline concentrations were negatively correlated to crypt depth (overall $r = -0.36$, $p = 0.022$). At day 2 postweaning, plasma citrulline concentrations were negatively correlated to crypt depth ($r = -0.46$, $p = 0.003$) and small intestinal empty weight ($r = -0.62$, $p = 0.004$). • No correlation was observed between plasma citrulline concentration at day 2 postweaning and the relative growth check, average daily gain, or average daily feed intake during the first 2 days postweaning. • At day 8 postweaning, plasma citrulline was correlated to SI length ($r = 0.37$, $p = 0.017$).	Biochemistry
23	Fu *et al.* (2009)	• Aim: To investigate whether SOM230, a novel, metabolically stable analog with broad receptor affinity, reduces intestinal injury and lethality in mice exposed to total body irradiation. • Design: Male CD2F1 mice were exposed to 7-15 Gy total body irradiation. Twice-daily administration of SOM230 (1, 4 or 10 mg/kg per day) or vehicle was started either 2 days before or 4 hours after total body irradiation and continued for either 14 or 21 days. Parameters of intestinal and hematopoietic radiation injury, bacterial translocation, and circulating cytokine levels were assessed.	• SOM230 prolonged survival and reduced lethality after exposure to total body irradiation • SOM230 preserved the intestinal mucosa surface area after irradiation and attenuated radiation-induced bacterial translocation. • Plasma citrulline levels did not increase significantly after SOM230 administration.	Clinical Significance (Mucositis)
24	Bahri *et al.* (2008)	• Aim: To characterize L-[^{14}C]-citrulline uptake by Caco-2 cells. • Design: Caco-2 cells were cultured in a bicameral insert system. Inhibition studies were conducted in the presence of neutral, cationic, acidic and non-metabolized amino acids.	• Citrulline uptake was pH-independent whereas the uptake rate was reduced in the absence of Na^+. • Kinetic analysis indicated the involvement of Na^+-dependent and Na^+-independent saturable transport components. • Results show that systems involved in citrulline transport are partly different from those involved in arginine transport.	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
25	Berkeveld *et al.* (2008)	• Aim: To investigate whether plasma citrulline or intestinal fatty acid-binding protein concentrations might be used as longitudinal markers for small intestinal function in piglets after weaning. • Selected piglets per litter were either weaned conventionally or remained with the sow. • Plasma citrulline and intestinal fatty acid-binding protein concentrations were measured longitudinally in weaned and unweaned piglets, and related to intestinal absorption values (i.e., plasma mannitol and 3-xylose concentrations in a sugar absorption test)	• The absorption of 3-xylose was not different between treatments ($p = 0.83$). Mannitol absorption, however, was less in the weaned conventionally piglets compared with the piglets on sow ($p = 0.003$), with the nadir on day 4 postweaning. • Weaning also reduced plasma citrulline concentrations in the conventionally weaned treatment compared with the sow treatment ($p < 0.001$). On day 4 and 7 postweaning, plasma citrulline concentrations of conventionally weaned piglets were less ($p < 0.001$ and $p = 0.0013$) than preweaning values. • In the conventionally weaned treatment, plasma citrulline concentrations correlated with plasma mannitol concentrations at day 4 postweaning ($r = 0.89$, $p = 0.008$) and overall ($r = 0.76$, $p = 0.001$)	Biochemistry (Absorption)
26	Bjornvad *et al.* (2008)	• Aim: To investigate whether the potential beneficial metabolic effects of total parenteral nutrition, prior to the start of enteral feeding, could help preterm neonates to resist feeding-induced mucosal dysfunction and inflammation and to investigate whether the previously reported protective effect of colostrum depends on provision of species-specific colostrum • Design: Intestinal mass and necrotizing enterocolitis lesions were first recorded in preterm pigs fed enterally (porcine colostrum, bovine colostrum, or formula for 20-40 hours), with or without a preceding 2- to 3-day total parenteral nutrition period ($n = 435$)	• Mucosal mass increased during total parenteral nutrition and further after enteral feeding to reach an intestinal mass similar to that in enterally fed pigs without total parenteral nutrition • Further studies in 3-day-old total parenteral nutrition pigs fed enterally showed that formula feeding decreased villus height and nutrient digestive capacity and increased luminal lactic acid and necrotizing enterocolitis lesions, compared with colostrum (bovine or porcine, $p < 0.05$). • Formula feeding decreased plasma arginine, citrulline, ornithine, and tissue antioxidants, whereas tissue nitric oxide synthetase and gut permeability increased, relative to colostrum (all $p < 0.05$).	Clinical Significance (Necrotizing enterocolitis)
27	Kerem *et al.* (2008)	• Aim: To evaluate the effects of chlorella crude extract on intestinal adaptation in rats subjected to short bowel syndrome. • Design: Wistar rats weighing 230-260 g with 75% small bowel resection • Randomized and divided into Control group ($n = 10$): where 5% dextrose was given through a gastrostomy tube; Enteral nutrition group ($n = 10$): Isocaloric and isonitrogen enteral nutrition, study group ($n = 10$): chlorella species crude extracts was administrated through a gastrostomy tube • Histopathologic evaluation, intestinal mucosal protein and DNA levels, intestinal proliferation and apoptosis were determined in intestinal tissues, and total protein, albumin and citrulline levels in blood were studied	• In rats receiving chlorella crude extract, villus lengthening, crypt depth, mucosal DNA and protein levels, intestinal proliferation, and serum citrulline, protein and albumin levels were found to be significantly higher than those in control group	Clinical Significance (Intestinal Resection)
28	Mittal *et al.* (2008)	• Aim: To evaluate the effects of three nonsteroidal anti-inflammatory drugs with varying cyclooxygenase selectivities on the small intestinal antioxidant enzyme status and surface characteristics during 1,2-dimethylhydrazine administration. • Design: Male Sprague-Dawley rats were divided into five different groups: Group 1 (control, vehicle treated); group 2 (1,2-dimethylhydrazine treated); group 3 (1,2-dimethylhydrazine + aspirin); group 4 (1,2-dimethylhydrazine + celecoxib); group 5 (1,2-dimethylhydrazine + etoricoxib)	• In comparison to 1,2-dimethylhydrazine-treated group, both celecoxib and etoricoxib showed significant changes. • The celecoxib-treated group registered a significant increase ($p < 0.01$) in the citrulline level, whereas the etoricoxib showed highly significant ($p < 0.001$) increase. The aspirin-treated group showed a nonsignificant rise in the citrulline level	Clinical Significance (Cancer)

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
29	Vissers *et al.* (2008)	• Aim: To investigate how the interorgan pathway for de novo arginine production is affected by the presence of malignant tumour and/or surgical trauma • Design: Controls and methylcholanthrene-sarcoma-bearing mice were studied, both with and without undergoing laparotomy (n = 9-13 per group). One day after laparotomy, amino acid fluxes across the hindquarter, intestine, liver, and kidney were studied (Male FVB mice).	• In methylcholanthrene-sarcoma-bearing mice, decreased de novo arginine production was accompanied by the presence of hepatic citrulline uptake, whereas tumour-bearing mice subjected to surgical trauma showed concomitant decreased intestinal citrulline output.	Clinical Significance (Cancer)
30	Nadalin *et al.* (2007)	• Aim: To investigate the role of plasma citrulline levels as marker of acute cellular rejection after small bowel transplantation. • Design: Eighteen German landrace pigs were used and divided into three groups. Group 1, autologous small bowel transplantation (n = 4) as control; group 2, allogeneic small bowel transplantation without immunosuppression (n = 7) and group 3, allogeneic small bowel transplantation with immunosuppression (n = 7). • Acute cellular rejection was differentiated into indeterminate, mild, moderate and severe	• An acute cellular rejection onset occurred generally from postoperative day 4 both in group 2 and group 3 as mild form and developed differently in the two groups: moderate to severe in group 2 and indeterminate to mild in group 3. • A significant decline of plasma citrulline occurred only in cases of moderate and severe acute cellular rejection, but not in cases of indeterminate and mild acute cellular rejection. • Plasma citrulline level failed as a marker in the early diagnosis of acute cellular rejection and became reliable only when advanced mucosal damage was present.	Clinical Significance (Transplantation)
31	Tsuchioka *et al.* (2006)	• Aim: To ascertain whether simultaneous administration of glutamic acid and taurine to patients on total parenteral nutrition could improve intestinal mucosal atrophy and suppress bacterial translocation. • Design: A 5-day total parenteral nutrition study was conducted in 5-week-old Sprague-Dawley rats. • Commercially available glutamic acid was used for total parenteral nutrition in group G and was enhanced with taurine in group GT. Oral nutrition was provided in controls	• Mucosal thickness and villus height in the small intestine were lower for group with glutamic acid than for controls and glutamic acid/taurine • Arginine and citrulline levels in the groups with glutamic acid/ glutamic acid/taurine were lower than in controls • Citrulline concentration was lower in group with glutamic acid than in groups with glutamic acid/taurine and controls	Biochemistry, Clinical Significance
32	Urschel *et al.* (2006)	• Aim: To determine the most effective arginine precursor in 1-week-old enterally fed piglets. • Design: Piglets were administered either an arginine-deficient (basal) diet or the basal diet supplemented with equimolar amounts of proline, ornithine, citrulline or arginine for 5 days • Daily blood samples were taken and indicators of whole-body arginine status including plasma amino acid, ammonia, and urea concentrations were measured.	• Piglets fed the basal diets supplemented with citrulline and arginine had lower plasma ammonia and urea concentrations ($p < 0.05$) and higher plasma arginine concentrations ($p < 0.0001$) and arginine fluxes ($p < 0.05$) than piglets fed the other 3 diets. • Piglets fed the basal diets supplemented with citrulline and arginine had a lower proline to arginine conversion ($p < 0.05$). • During first-pass splanchnic metabolism, 52% of the dietary arginine was extracted, and this extraction was not affected by whole-body arginine status ($p > 0.05$). • These data indicate that citrulline, but not ornithine or proline, is an effective arginine precursor, and that either citrulline formation or availability appears to limit arginine synthesis in neonatal piglets.	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
33	Boelens *et al.* (2005)	• Aim: To quantify the interorgan relationship of exogenous L-glutamine or glutamine dipeptide, by enteral or parenteral route, contributing to intestinal citrulline and renal de novo arginine synthesis in mice. • Design: Use of a multicatheterized mouse model with Swiss mice (n = 43) in the postabsorptive state. Stable isotopes were infused into the jugular vein or into the duodenum (per group either free L-[2,^{15}N]glutamine or dipeptide L-alanine-L-[2,^{15}N]glutamine, all with L-[ureido-^{13}C-$^{2}H_2$]citrulline and L-[guanidino-$^{15}N_2$-$^{2}H_2$]arginine) to establish renal and intestinal arginine and citrulline metabolism.	• Quantitatively, more de novo L-[2,^{15}N]citrulline was produced when free L-[2,^{15}N]glutamine was given than when L-alanine-L-[2,^{15}N]glutamine was given, whereas renal de novo L-[2,^{15}N]arginine was similar in all groups.	Biochemistry
34	Lardy *et al.* (2004)	• Aim: to understand the metabolic changes underlying early adaptation after massive intestinal resection. • Design: Rats were assigned to either 80% intestinal resection or transection. All animals received the same intragastric nutrition. • On day 8, plasma glutamine turnover was measured. Substrate use was determined on isolated enterocytes that were incubated in the presence of D-[U-^{14}C]glucose (2 mmol/L), L-[U-^{14}C]glutamine (2 mmol/L), L-[U-^{14}C]arginine (1 mmol/L), or L-[1-^{14}C]ornithine (1 mmol/L).	• Both the basal rate of citrulline synthesis and the rate of citrulline synthesis that was stimulated by were similar. • Capacity for L-citrulline generation from 1 mmol/L arginine in the presence of 50 mmol/L NH_4Cl profoundly stimulated L-citrulline production, which suggested that L-citrulline generation from L-ornithine in isolated enterocytes markedly depends on the availability of carbamoylphosphate.	Biochemistry, Clinical Significance (Intestinal Resection)
35	Osowska *et al.* (2004)	• Aim: To test the hypothesis that citrulline, which is not taken up by the liver and is the major precursor of arginine, should be a good candidate to generate arginine and improve nutritional status in massive intestinal resection • Design: Twenty four rats were assigned to four groups: citrulline, arginine, control, and sham. The sham group underwent transection and the three other groups resection of 80% of the small intestine. All rats were fed by enteral nutrition and its composition was as follows: supplementation with citrulline in the citrulline group, supplementation with arginine in the arginine group, and standard polymeric enteral nutrition in the control and sham groups.	• Arginine concentration was higher ($p < 0.05$) in plasma and muscle in the citrulline group than in the three other groups. • Plasma levels of arginine were 110 (±12), 79 (±7), 167 (±22), and 228 (±13) μmol/L in the sham, control, arginine, and citrulline groups respectively. Arginine concentrations in the gastrocnemius were: 0.15 (±0.02), 0.16 (±0.02), 0.40 (±0.05), and 0.94 (±0.20) μmol/g, respectively. Citrulline preserved nitrogen balance in resected rats but not in arginine supplemented rats	Biochemistry, Clinical Significance (Intestinal Resection)
36	Dekaney *et al.* (2003)	• Aim: To measure enzymatic activities of carbamoyl phosphate synthase I, ornithine carbamoyltransferase, and pyrroline-5-carboxylate reductase, in ex vivo preparations, to determine whether functional enzymes exist within the porcine foetal small intestine, throughout gestation, to synthesize both citrulline and proline. To determine cell compartment-specific expression of the enzymes within the small intestine • Design: Activities and mRNA expression patterns of other key enzymes in the arginine biosynthetic pathway in the foetal porcine small intestine from 30 to 110 days of gestation	• The activities of all three enzymes increased from day 30 to day 110 of gestation, and in situ hybridization demonstrates that 1) carbamoyl phosphate synthase I and ornithine carbamoyltransferase genes are expressed in distinct patterns and are confined to the mucosal epithelium and 2) pyrroline-5-carboxylate reductase mRNA is present in mucosal epithelium and lamina propria of the foetal porcine small intestine from day 30 to day 110 of gestation. • The presence of carbamoyl phosphate synthase I and ornithine carbamoyltransferase in conjunction with the presence of ornithine aminotransferase suggests that the foetal porcine small intestine is capable of synthesizing citrulline from pyrroline-5-carboxylate	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
37	Lutgens *et al.* (2003)	• Aim: To investigate whether citrulline can be used for quantifying radiation-induced epithelial cell loss • Design: NMRI mice were subjected to single-dose whole body irradiation. The time course of citrullinemia was assessed up to 11 days after irradiation. A dose-response relationship was determined at 84 hours after irradiation. • Citrullinemia was correlated with morphologic parameters at this time point and used to calculate the dose-modifying factor of glutamine and amifostine on acute small bowel radiation damage	• After whole body irradiation, a time- and dose-dependent decrease in plasma citrulline level was observed with a significant dose-response relationship at 84 hours. • At this time point, citrullinemia significantly correlated with jejunal crypt regeneration ($p < 0.001$) and epithelial surface lining ($p = 0.001$). • A dose-modifying factor of 1.0 and 1.5 was computed at the effective dose 50 level for glutamine and amifostine, respectively. • A linear correlation (Pearson's $r = 0.77$) between plasma citrulline levels and a representative parameter for epithelial cell mass, i.e., the epithelial surface lining	Clinical Significance (Mucositis)
38	Bloomfield *et al.* (2002)	• Aim: To investigate, in the late-gestation ovine fetus: 1) amino acid concentrations in blood and amniotic fluid, 2) the effects of intrauterine growth restriction induced by placental embolization on these concentrations, 3) foetal gut uptake of glutamine in healthy and intrauterine growth restriction foetuses, and 4) the effects of intraamniotic insulin-like growth factor-I treatment on these parameters. • Design: Foetuses (sheep) were randomly assigned to control ($n = 9$), Intrauterine Growth Restriction + saline ($n = 9$), or Intrauterine Growth Restriction + insulin-like growth factor-I ($n = 11$) groups. Intrauterine Growth Restriction was induced by uteroplacental embolization from 114 to 119 days (term = 145 days). Intrauterine Growth Restriction foetuses received daily intraamniotic injections of saline or insulin-like growth factor-I (20 µg/day) from 120 to 130 days.	• The authors described amino acid concentrations in the blood and amniotic fluid of the late-gestation foetal sheep and the changes that occur with Intrauterine Growth Restriction induced by uteroplacental embolization. • They demonstrated for the first time that the foetal gut also uses glutamine as a fuel and releases citrulline, possibly arising from the conversion of glutamine.	Biochemistry
39	Dekaney *et al.* (2001)	• Aim: To determine ornithine aminotransferase enzymatic activity and mRNA expression in the intestine of foetal pigs from 30 to 110 days of gestation. • Design: Foetuses were collected from crossbred (Duroc × Hampshire × Yorkshire) gilts at defined stages of gestation. • Ornithine aminotransferase enzyme activity was determined in homogenates of foetal small intestines from days 30, 35, 45, 60, 90, and 110 of gestation	• Enzymatic activity peaked at day 45 of gestation and increased again between day 60 and 110 of gestation. • At 30 and 35 days of gestation, ornithine aminotransferase mRNA expression was detected throughout the mucosal epithelium of the small intestine. Throughout the remainder of gestation, ornithine aminotransferase expression was notably higher in the villus epithelium than in the crypt epithelium. • The presence of ornithine aminotransferase in the small intestinal epithelium throughout gestation suggests that the porcine small intestine is capable of interconverting ornithine and pyrroline-5-carboxylate during foetal development	Biochemistry, Developmental Biology

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
40	Wu *et al.* (2000)	• Aim: To determine whether a cortisol surge mediates the enhanced expression of intestinal citrulline-synthetic enzymes during weaning. • Design: Jejunal enterocytes were prepared from 29-days-old weanling pigs treated with or without metyrapone (an inhibitor of cortisol synthesis), or from age-matched unweaned pigs. • The mRNA levels and activities of phosphate-dependent glutaminase, pyrroline-5-carboxylate synthase, ornithine aminotransferase, carbamoyl-phosphate synthase I and ornithine carbamoyltransferase were determined	• The mRNA levels for phosphate-dependent glutaminase, pyrroline-5-carboxylate synthase, ornithine aminotransferase and ornithine carbamoyltransferase were 139%, 157%, 102% and 55% higher, respectively, in weanling pigs compared with suckling pigs. • The activities of phosphate-dependent glutaminase and pyrroline-5-carboxylate synthase were 38% and 692% higher, respectively, in weanling pigs compared with unweaned pigs, but the activities of ornithine aminotransferase, carbamoyl-phosphate synthase I and ornithine carbamoyltransferase did not differ between these two groups of pigs. • The effects of metyrapone administration to weanling pigs were as follows: 1) prevention of a cortisol surge, 2) abolition of the increases in both mRNA levels and activity of pyrroline-5-carboxylate synthase, 3) no alteration in the mRNA levels and activities of phosphate-dependent glutaminase and carbamoyl-phosphate synthase I, 4) increases in the mRNA levels for ornithine aminotransferase (216%) and ornithine carbamoyltransferase (39%) and in ornithine aminotransferase activity (30%), and 5) prevention of the increase in intestinal synthesis of citrulline from glutamine.	Biochemistry, Developmental Biology
41	Dillon *et al.* (1999)	• Aim: To determine whether lactate is an inhibitor of intestinal synthesis of citrulline and arginine from proline • Design: Jejunum was obtained from 14-day-old suckling pigs for preparation of enterocyte mitochondria and metabolic studies • Mitochondria were used for measuring proline oxidase activity in the presence of 0-10 mM L-lactate • Enterocytes were incubated at 37°C for 30 minutes in Krebs bicarbonate buffer (pH 7.4) containing 5 mM D-glucose, 2 mM L-glutamine, 2 mM L-[U-^{14}C]proline, and 0, 1, 5, or 10 mM L-lactate	• Kinetics analysis revealed non-competitive inhibition of intestinal proline oxidase by lactate (decreased maximal velocity and unaltered Michaelis constant). • Lactate had no effect on either activities of other enzymes for arginine synthesis from proline or proline uptake by enterocytes but decreased the synthesis of ornithine, citrulline, and arginine from proline in a concentration-dependent manner	Biochemistry
42	Plauth *et al.* (1999a); Plauth *et al.* (1999b)	• Aim: To examine whether the route of glutamine administration and the simultaneous availability of glucose affect intestinal glutamine metabolism. • Design: Net substrate exchange rates of glutamine and its nitrogenous products were measured in the isolated vascularly and luminally perfused rat small intestine (a) as a function of glutamine provision from either the vascular or the luminal or simultaneously from both sides and (b) as a function of simultaneous availability of glucose from various routes	• When glutamine was provided from the lumen, only 19%-32% of absorbed glutamine appeared intact in the venous effluent • Glutamine consumption and the production of citrulline or ammonia decreased when glucose (vascular or luminal perfusate) became available in addition to glutamine. • The authors concluded that glutamine is utilized by the small intestine very efficiently regardless of the route of administration being enteral or parenteral. The two routes can be used interchangeably to provide the intestinal mucosa with glutamine. Glucose and glutamine may partially substitute each other, most likely for the purpose as a metabolic fuel.	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
43	Davis and Wu (1998)	• Aim: To determine the compartmentation and kinetics of urea cycle enzymes in porcine enterocytes • Design: Pigs were offspring of Yorkshire × Landrace sows and Duroc × Hampshire boars • At 60 days of age, the jejunum and liver were dissected from anesthetized pigs • Enterocytes were incubated at 37°C for 30 minutes in the presence of 5 mM glucose, 0.2 mM NH_4Cl and plasma concentrations of all amino acids	• Except for ornithine carbamoyltransferase, V_{max} values of urea cycle enzymes were much lower in enterocytes than in the liver of pigs, and vice versa for their K_m values. • Carbamoyl phosphate synthase I and ornithine carbamoyltransferase were located exclusively in mitochondria, whereas argininosuccinate synthase and argininosuccinate lyase were found in the cytosol. • Arginase isozymes were present in both the cytosol and mitochondria of enterocytes, and differed in their sensitivity to heat inactivation. • Because of a low rate of ureagenesis in enterocytes compared with the liver, intestinal urea cycle enzymes may function primarily to synthesize citrulline. • The co-localization of Carbamoyl phosphate synthase I and ornithine carbamoyltransferase and a high activity of ornithine carbamoyltransferase in enterocyte mitochondria favours the intestinal synthesis of citrulline from ammonia, HCO_3^- and ornithine. • Low activities of cytosolic argininosuccinate synthase and argininosuccinate minimize the conversion of citrulline into arginine and therefore, the recycling of citrulline into ornithine via arginase in postweaning-pig enterocytes. These kinetic properties of intestinal urea cycle enzymes maximize the net synthesis of citrulline from glutamine and explain the release of large amounts of citrulline by the pig small intestine.	Biochemistry
44	de Jonge *et al.* (1998)	• Aim: To study the expression of ornithine cycle enzymes in the rat small intestine during perinatal development. • Design: The spatiotemporal patterns of expression of ornithine aminotransferase, carbamoylphosphate synthetase, ornithine transcarbamoylase, argininosuccinate synthetase, argininosuccinate lyase, and arginase mRNAs were studied by Northern blot analysis and in situ hybridization. • The expression of carbamoylphosphate synthetase and argininosuccinate synthetase protein was studied by immunohistochemistry	• Before birth, the developmentally more mature proximal loops of the intestine expressed the mRNAs at higher concentrations than the more distal loops. • After birth, mRNAs of argininosuccinate synthetase and argininosuccinate lyase, the enzymes that metabolize citrulline to arginine, were detectable only in the upper part of the villi, whereas the other mRNAs were concentrated in the crypts. • The distribution of argininosuccinate synthetase protein corresponded with that of the mRNA, whereas carbamoylphosphate synthetase protein was present in all enterocytes of the crypts and villi. Hepatic arginase mRNA could not be detected in the enterocytes. The spatial distribution of the respective mRNAs and proteins along the villus axis of the suckling small intestine indicates that the basal enterocytes synthesize citrulline, whereas the enterocytes in the upper half of the villus synthesize arginine.	Biochemistry, Developmental Biology
45	Selamnia *et al.* (1998)	• Aim: To determine whether L-citrulline present in the culture medium a precursor of L-arginine in HT-29 cells, newly synthesized L-arginine is used for the production of urea and L-ornithine • Design: human adenocarcinoma cell line HT-29 • Cell incubation in the presence of radioactive precursors	• This study demonstrates that the polyamine precursor L-ornithine in HT-29 cells can (in addition to extracellular source and intracellular synthesis from L-arginine) be provided by the stepwise conversion of L-citrulline • L-arginine derived from L-citrulline is not produced, even in the presence of D-glucose which limits the flux of L-arginine in the arginase pathway, in sufficient quantities to fulfil arginine needs for protein synthesis in an arginine-free culture medium	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
46	Flynn and Wu (1997)	• Aim: To determine whether cortisol plays a role in arginine and glutamine metabolism in enterocytes and, more specifically, whether cortisol regulates metabolic changes in these cells during weaning. • Design: Twenty-eight 21-day-old suckling pigs were randomly assigned to one of four groups (7 animals in each) and received intramuscular injections of vehicle solution (sesame oil) (control group), hydrocortisone 21-acetate (25 mg/kg body weight), RU-486 (10 mg/kg body weight) (a potent blocker of glucocorticoid receptors), or hydrocortisone 21-acetate plus RU-486.	• Compared with control, cortisol administration increased: • 1) the activities of argininosuccinate lyase and arginase and the production of CO_2, ornithine, and proline from arginine • 2) pyrroline-5-carboxylate synthase activity and the formation of glutamate, alanine, aspartate, ornithine, citrulline, proline, and CO_2 from glutamine in enterocytes.	Biochemistry, Developmental Biology
47	Guihot *et al.* (1997)	• Aim: to assess the effects of an elemental diet compared with a complex diet on L-arginine metabolism in rat isolated enterocytes and its modulation by L-glutamine. • Design: Rats were fed the elemental diet or the control diet for 14 days. • Villus enterocytes then were isolated, and metabolic capacities or enzyme activities were assessed	• The incubation of enterocytes isolated from controls with 0.1 mmol/L L-[U-^{14}C]-arginine led to the production of 125 ± 25 pmol L-citrulline/10^6 cells per 30 minutes. This production showed a twofold increase in the presence of 2 mmol/L L-glutamine. • In the lemental diet group, L-citrulline synthesis from L-arginine was markedly lower in the absence or in the presence of L-glutamine. This coincided with lower carbamoylphosphate synthase I activity and carbamoylphosphate content of enterocytes. Other L-arginine and L-glutamine metabolic pathways were not affected. • Similar results were obtained when the elemental diet was administered continuously through a gastric catheter or fed by mouth.	Biochemistry, Developmental Biology
48	Wu (1997)	• Aim: To test that proline is an important substrate for the synthesis of citrulline and arginine in pig enterocytes. • Design: The synthesis of ornithine, citrulline, and arginine from proline was quantified in enterocytes of newborn (0-day-old) pigs, 2- to 21-day-old suckling pigs, and 29- to 58-day-old pigs weaned at 21 days of age. • Mitochondria were prepared from enterocytes for measurement of proline oxidase activity.	• Proline oxidase activity and rates of synthesis of citrulline and arginine from proline were high in enterocytes of new-born pigs and markedly decreased in 7-day-old pigs. • With increasing piglet age from 7 to 21 days, enterocyte proline oxidase activity and rate of conversion of proline into citrulline progressively increased, but the values in 21-day-old pigs remained much lower than in new-born pigs. • The synthesis of ornithine, citrulline, and arginine from 0.5-5 mM proline was concentration dependent and required the addition of glutamine. About 80%-90% of utilized proline carbons were recovered in ornithine plus citrulline plus arginine, with CO_2 being a minor product.	Biochemistry
49	Wu *et al.* (1997)	• Aim: To determine whether endogenous synthesis of arginine plays a role in regulating arginine homeostasis in postweaning pigs. • Design: Pigs were fed a sorghum-based diet containing 0.98% arginine and were used for studies at 75 days of age (28.4 kg body weight). • Mitochondria were prepared from the jejunum and other major tissues for measuring the activities of Δ^1-pyrroline-5-carboxylate synthase and proline oxidase and of ornithine aminotransferase.	• The activities of pyrroline-5-carboxylate synthase, proline oxidase and ornithine aminotransferase were greatest in enterocytes among all of the tissues studied. Incubation of enterocytes with gabaculine resulted in decreases ($p < 0.05$) in the synthesis of ornithine and citrulline from glutamine and proline. • When gabaculine was orally administered to pigs to inhibit intestinal synthesis of citrulline from glutamine and proline, plasma concentrations of citrulline and arginine decreased whereas those of alanine, ornithine, proline, taurine and branched-chain amino acids increased. • Results suggest an important role for endogenous synthesis of arginine in regulating arginine homeostasis in postweaning growing pigs	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
50	Chen *et al.* (1996)	• Aim: to examine the changes in intestinal and circulating amino acid metabolism after massive small bowel resection in rats treated with standard or glutamine-supplemented total parenteral nutrition. • Design: Intestinal amino acid flux, circulating plasma aminogram, mucosal glutaminase activity and protein, and DNA content were measured 7 days after massive small bowel resection in rats receiving either standard or glutamine-supplemented total parenteral nutrition. • Sham-operated rats and rats fed chow after enterectomy served as controls.	• The uptake of glutamine and the release of citrulline by the remaining intestine was significantly decreased, with reduced mucosal glutaminase activity after small bowel resection in the Chow and standard-total parenteral nutrition groups. • Glutamine supplementation resulted in significantly increased gut glutamine uptake compared with standard-total parenteral nutrition group ($p < 0.01$). • Mucosal glutaminase activity, mucosal protein, and DNA content was also increased by glutamine; however, the gut release of citrulline remained unchanged ($p > 0.05$). • The subsequent decrease in circulating arginine in the glutamine-supplemented total parenteral nutrition group compared with the standard-total parenteral nutrition group ($p < 0.05$) was attributed to an insufficient exogenous supply. • These findings show that glutamine-supplemented total parenteral nutrition improves mucosal growth and gut glutamine uptake after small bowel resection. • The intestinal production of citrulline, which remained low in both total parenteral nutrition groups, may lead to an insufficiency of endogenous arginine synthesis. • Both glutamine and arginine may be essential amino acids after small bowel resection.	Biochemistry, Clinical Significance (Intestinal Resection)
51	Flynn and Wu (1996)	• Aim: To test the hypothesis that endogenous arginine synthesis plays an important role in maintaining arginine homeostasis in neonatal pigs. • Design: Gabaculine was used as a suicide inhibitor of ornithine aminotransferase • Four-day-old suckling pigs received oral administration of 0.0 or 0.83 mg gabaculine/kg body weight every 4 hours during a 12-hour period from 6 A.M. to 6 P.M.	• Gabaculine treatment decreased plasma concentrations of ornithine, citrulline, and arginine by 59%, 52%, and 76%, respectively, and increased those of glutamine and proline by 74% and 220%, respectively. • Because pyrroline-5-carboxylate synthase was almost exclusively located in enterocytes of 4-day-old pigs, data suggest that the intestinal production of citrulline plays an important role in endogenous synthesis of arginine.	Biochemistry
52	Wu (1996)	• Aim: To determine a role of pentose cycle in the provision of nicotinamide adenine dinucleotide phosphate for the synthesis of citrulline and proline from glutamine in porcine enterocytes. • Design: Enterocytes from 4-day-old pigs were incubated at 37°C for 0 to 30 minutes in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 2 mM glutamine and 5 mM glucose in the presence of 0, 0.1, or 0.25 mM dehydroepiandrosterone a potent inhibitor of glucose-6-phosphate dehydrogenase which is the key regulatory enzyme of pentose cycle.	• Dehydroepiandrosterone inhibited the synthesis of ornithine, citrulline, arginine, and proline from glutamine in a concentration-dependent manner, but had no effect on the formation of citrulline and arginine from ornithine. • However, dehydroepiandrosterone decreased the synthesis of proline from ornithine by 79%. • Dehydroepiandrosterone had no effect on cellular adenosine triphosphate concentrations.	Biochemistry
53	Wu *et al.* (1996)	• Aim: To determine degradation via arginase, nitric oxide synthase, and arginine decarboxylase in enterocytes of the pig • Design: Arginine degradation was quantified in enterocytes of 0-day-old (new-born) and 4- to 21-day-old suckling pigs and 29- to 58-day-old pigs weaned at 21 days of age.	• Arginine degradation to CO_2, ornithine, or proline was negligible in enterocytes of new-born and suckling pigs and markedly increased in weaned pigs. • In cells from new-born pigs, citrulline generation from arginine was greater than that of ornithine plus CO_2. Citrulline synthesis decreased during the first 2 weeks after birth and increased in weaned pigs to the value similar to that in newborn pigs. • The synthesis of citrulline from arginine decreased by > 88% in the presence of N^G-nitro-L-arginine. • Nitric oxide synthase plays a quantitatively minor role in arginine degradation by enterocytes.	Biochemistry, Developmental Biology

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
54	Dugan *et al.* (1995)	• Aim: To determine the roles of age and diet in the induction of citrulline synthesis from glutamine in enterocytes of weaned pigs. • Design: Enterocytes were prepared from the jejunum of suckling pigs (14-29 days old), 23- and 29-days-old pigs weaned at 21 days of age to either a conventional corn-soybean meal-based or a milk-based diet, and 23-days-old pigs weaned at 21 days of age and food-deprived for 2 days.	• The rate of citrulline production from glutamine was similar in enterocytes from 14-, 21- and 29-days-old suckling pigs, and was 10-fold greater in cells from 29-days-old weaned pigs. • After weaning, enterocytes from 23-days-old pigs fed the milk-based diet had a 33% higher rate of citrulline production from glutamine than cells from age-matched pigs fed a corn-soybean meal-based diet or food-deprived for 2 days. • Findings suggest that the major determinant of the induction of citrulline synthesis from glutamine in enterocytes of weaned pigs may not be age or change in diet, although the extent of the induction may be slightly influenced by diet composition.	Biochemistry
55	Wakabayashi *et al.* (1995)	• Aim: To investigate the effect of intestinal resection and arginine-free diet on rat physiology. • Design: Maintained rats with massively resected small intestine and those with transected intestines on either control or an arginine-free diet	• After 4 weeks, massively resected small intestine rats fed deficient diet lost weight by a mean of 46 g, whereas massively resected small intestine rats fed control diet and transected intestines rats fed control and deficient diet gained 30-96 g. Average nitrogen balance was 150, 60, 110, and -33 mg/day for transected intestines rats fed control and deficient diet, massively resected small intestine rats fed control and deficient diet, respectively. • The concentrations of arginine in skeletal muscle were 654, 163, 230, and 84 nmol/g, respectively, and those in plasma were 133, 50, 103, and 54 μM, respectively. The concentrations of citrulline in massively resected small intestine rats were halved compared with transected intestines rats irrespective of diet.	Biochemistry, Clinical Significance (Intestinal Resection)
56	Wu and Knabe (1995)	• Aim: To quantify arginine synthesis in neonatal pig enterocytes and to elucidate the mechanism involved in the developmental change of intestinal arginine synthesis. • Design: Arginine synthesis was quantified in enterocytes from new-born (0-day-old) and 2- to 7-day-old suckling pigs.	• Arginine was found to be synthesized from glutamine in 0- to 7-day-old pig enterocytes, but the rates of arginine synthesis were three- to fourfold greater in 0- to 2-day-old pigs than in 7-day-old pigs. • The rates of metabolism of glutamine to citrulline were 2.5- to 3.5-fold greater in enterocytes from 0- to 2-day-old pigs than in cells from 7-day-old pigs, as were the rates of conversion of citrulline to arginine. • The activities of all enzymes that synthesize arginine from glutamine, except pyrroline-5-carboxylate synthase and argininosuccinate lyase, increased in enterocytes from 2-day-old pigs compared with 0-day-old pigs • The activities of all these enzymes decreased by approximately 75% in 7-day-old pigs compared with 2-day-old pigs. Arginase activity was negligible in enterocytes from 0- to 7-day-old pigs, thus minimizing intestinal hydrolysis of newly synthesized arginine and maximizing the endogenous provision of arginine.	Biochemistry, Developmental Biology
57	Wu *et al.* (1995a)	• Aim: To determine whether pyrroline-5-carboxylate synthase is deficient in chick enterocytes therefore resulting in the lack of synthesis of ornithine and citrulline from glutamine • Design: Post-weaning pig enterocytes, which are known to contain pyrroline-5-carboxylate synthase and to synthesize both ornithine and citrulline from glutamine, were used as positive controls.	• In chick enterocytes, glutamine was metabolized to NH_3, CO_2, glutamate, alanine and aspartate, but not to ornithine, citrulline, arginine or proline. • Likewise, there was no formation of citrulline, arginine, alanine or aspartate from ornithine in chick enterocytes. • No activity of pyrroline-5-carboxylate synthase or ornithine carbamoyltransferase was found in chick enterocytes, in contrast with cells from post-weaning pigs. • It was also demonstrated that the activity of ornithine aminotransferase in chick enterocytes was only 3% of that in cells from pigs.	Biochemistry, Developmental Biology

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
58	Wu *et al.* (1995b)	• Aim: To quantify glutamine utilization by developing pig enterocytes and to assess the relative importance of glutamine versus glucose as a fuel for these cells. • Design: Glutamine and glucose metabolism was studied in 0- to 21-day-old pig enterocytes. Cells were incubated at 37°C for 30 minutes in Krebs-Henseleit bicarbonate buffer (pH 7.4) in the presence of 2 mM [U-^{14}C]glutamine with or without 5 mM glucose, or 5 mM [U-^{14}C]glucose with or without 2 mM glutamine.	• Glutamine was metabolized to ammonia, glutamate, alanine, aspartate, CO_2, citrulline, ornithine, and proline, whereas glucose was converted to lactate, pyruvate, and CO_2 in pig enterocytes. • By day 14 after birth, the oxidation of glutamine and glucose as well as citrulline production had decreased by 90%-95%. • Arginine synthesis from glutamine occurred in cells from 0- to 7-day-old pigs but not 14- to 21-day-old ones	Biochemistry, Developmental Biology
59	Malo (1994)	• Aim: To evaluate the fate of ornithine as well as the levels of substrates and products of metabolic pathways in normal and ornithine transcarbamylase-deficient mice (sparse-fur) • Design: Concentration of free amino acids was measured in small intestinal mucosa and serum during post-natal development. è	• In control animals, ornithine, alanine and citrulline concentrations in intestinal mucosa increased as a function of age • Major changes were observed in sparse-fur mice: the ornithine level remained low in intestinal tissue, citrulline concentration was significantly decreased in both intestinal tissue and blood, circulating levels of arginine and essential amino acids were drastically reduced in sucklings while plasma glutamine increased after weaning	Biochemistry
60	Wakabayashi *et al.* (1994)	• Aim: To investigate whether the small intestine is or is not the major tissue for the synthesis of arginine and proline • Design: Comparison of effects of feeding arginine- and/or proline-deficient diets with those of a complete diet (Complete) in rats whose small intestine had been massively resected.	• Weight loss, negative nitrogen balance, and markedly reduced arginine concentration in the muscle observed in rats fed arginine deficient and arginine deficient but not proline deficient diet clearly indicate that arginine becomes a strictly essential amino acid in the rats with massive resection of the small intestine. • Plasma glutamine, citrulline in the muscle and plasma, urinary excretion of orotic acid and nitrate (to assess nitric oxide formation from arginine) were also decreased	Biochemistry, Clinical Significance (Intestinal Resection)
61	Wu *et al.* (1994a)	• Design: Arteriovenous differences in the plasma concentrations of amino acids across the jejunum were studied in preweaning (14- to 21-days-old) and post-weaning (29- to 58-days-old) pigs in the postabsorptive state.	• Glutamine was the only amino acid that was extracted by the small intestine in both pre- and post-weaning pigs • The production of citrulline by the jejunum was low in preweaning pigs, but was threefold greater in the post-weaning pigs than in the preweaning pigs • Demonstration of uptake in vivo of glutamine and the release of arginine, alanine, citrulline, glutamate and proline by the small intestine of developing pigs	Biochemistry, Developmental Biology
62	Wu *et al.* (1994b)	• Aim: to quantify citrulline synthesis from glutamine in enterocytes and to elucidate the pathways involved. • Design: The synthesis of citrulline from glutamine was quantified in enterocytes from pre-weaning (14-21 days old) and post-weaning (29-58 days old) pigs. • The cells were incubated at 37 °C for 30 minutes in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 0, 0.5, 2 and 5 mM glutamine.	• The rates of citrulline synthesis were low or negligible in enterocytes from 14-21-day-old pigs, but increased 10-20-fold in the cells from 29-58-day-old pigs • This marked elevation of citrulline synthesis coincided with an increase in the activity of pyrroline-5-carboxylate synthase with the animal's post-weaning growth • Decreases in the activities of phosphate-dependent glutaminase, ornithine aminotransferase, ornithine carbamoyltransferase and carbamoyl-phosphate synthase were observed as the age of the pigs increased • The concentrations of carbamoyl phosphate in enterocytes from pre-weaning pigs were higher than, or similar to, those in the cells from post-weaning pigs. It is possible that the low rate of citrulline synthesis from glutamine in enterocytes from pre-weaning pigs was due to a limited availability of ornithine, rather than that of carbamoyl phosphate	Biochemistry, Developmental Biology

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
63	Blachier *et al.* (1993)	• Aim: to answer whether de novo synthesis of L-arginine from L-glutamine, L-citrulline or L-ornithine is possible in enterocytes isolated from newborn pigs and whether newborn pig enterocytes are able to catabolize exogenous L-arginine into urea and L-ornithine. • Design: The capacity for L-arginine metabolism was studied in villus enterocytes isolated from pigs at birth, after 2-8 days suckling and after weaning	• 40% of L-arginine synthetized de novo from L-citrulline were released into the incubation medium • In enterocytes isolated from post-weaned pigs, no significant production of L-arginine from either L-glutamine or L-ornithine was detected • In contrast, although the L-arginine production from L-citrulline was very low in post-weaned animals, it was significantly enhanced in the presence of L-glutamine, representing 23% of the production measured in suckling animals	Biochemistry, Developmental Biology
64	Vadgama and Evered (1992)	• Aim: To examine the characteristics of citrulline transport across the everted sacs of the rat small intestine. • Design: Male albino (Wistar) rats, 180-240 g body weight, were starved overnight but given water ad libitum.	• Optimal site of citrulline absorption is middle to lower ileum • It shows active transport, and this transport is predominantly Na^+ dependent. • Analog inhibition studies suggest that citrulline may share the neutral brush border system described for the mucosal brush border membranes of the rabbit jejunum or a system analogous to system ASC described for nonepithelial cells and for basolateral membranes of certain epithelia. • The rat small intestine has developed a specific carrier-mediated, Na^+-dependent pathway for citrulline absorption.	Biochemistry
65	Blachier *et al.* (1991a)	• Aim: To examine the metabolic fate of L-arginine in isolated rat enterocytes, with emphasis on its conversion to L-ornithine, L-citrulline and polyamines • Design: Rat enterocytes were isolated from fed female albino rats and exposed to L-arginine	• L-Arginine was converted to L-citrulline either directly in a nicotinamide adenine dinucleotide phosphate-sensitive manner thought to be coupled with the generation of nitric oxide, or indirectly through the sequence of reactions catalysed by arginase and ornithine transcarbamylase. • A large fraction of L-citrulline and L-ornithine generated from exogenous L-arginine was released in the incubation medium. • The oxidative catabolism of L-arginine in enterocytes is quantitatively negligible relative to its conversion to L-citrulline and L-ornithine.	Biochemistry
66	Blachier *et al.* (1991b)	• Aim: To investigate more directly the presence of L-arginine:nitric oxide synthase in isolated pig enterocytes and its possible modulation by D-glucose. • Design: Enterocytes isolated from jejunum of large White pigs weighing 54 ± 4 kg (n = 8) were studies	• Since the production of labelled L-citrulline from L-arginine in pig enterocyte homogenates was markedly increased in the presence of nicotinamide adenine dinucleotide phosphate, it is proposed that ○ The direct conversion of L-arginine to L-citrulline could be stimulated by the production of nicotinamide adenine dinucleotide phosphate from D-glucose in the pentose phosphate pathway.	Biochemistry
67	Edmonds *et al.* (1987)	• Aim: to determine the efficacy of ornithine or citrulline as precursors of arginine • Design: Pigs were individually fed an arginine-deficient, semipurified diet supplemented with 0.3% arginine or an equimolar quantity of ornithine or citrulline. • Pigs were again fed the arginine-deficient, semipurified diet supplemented with 0.3% arginine or four times an isomolar quantity of ornithine.	• Supplemental arginine or citrulline increased rate and efficiency of weight gain, but ornithine addition was without effect. • Free arginine in plasma 3 hours post-prandial was increased by addition of either arginine or citrulline to the basal diet. • Arginine or citrulline addition to the diet increased arginine concentration in muscle tissue, but muscle ornithine was unresponsive to any of the supplements fed. • Arginine addition to the diet increased weight gain and feed efficiency, while ornithine supplementation was without effect. Plasma ornithine was increased by excess ornithine, while plasma citrulline was unaffected by supplemental arginine or ornithine.	Biochemistry

Table 1.2 *(Continued)*

No.	Authors	Settings	Findings	Categorization
68	Hurwitz and Kretchmer (1986)	• Aim: To localize the source of endogenous arginine during the newborn period, to determine the developmental patterns of the urea biosynthetic enzymes in the kidney and intestine of the mouse, and to initiate a study concerned with the fate of the newly synthesized arginine. • Design: Mice of the C57B1/6 strain were maintained on Purine mouse laboratory chow ad libitum. Only timed pregnancies were used for the study of foetuses	• At birth, the arginine-synthesizing enzymes in the kidney of the C57Bl/6 mouse are minimally developed, whereas in the intestine activity of carbamoyl-phosphate synthase is elevated and argininosuccinate synthase and lyase, usually present only in trace quantities in the adult intestine, are markedly increased in the newborn. • The study indicates that, at a time when no other endogenous source of arginine for protein synthesis is available, the intestine of the new-born C57Bl mouse is capable of synthesizing arginine from either citrulline or NH_3 and CO_2.	Biochemistry, Developmental Biology
69	Uchiyama *et al.* (1981)	• Aim: To investigate the activity of N-acetylglutamate synthase in rat small intestinal mucosa. • Design: Wistar or Sprague-Dawley male rats, weighing 250-300 g, were maintained on a commercial laboratory chow containing 24% crude protein for 1-3 weeks before use.	• N-Acetylglutamate synthase, had a high substrate specificity for L-glutamate and acetyl coenzyme A. • Results suggest that a function of the intestinal N-acetylglutamate is to activate carbamoyl-phosphate synthase (ammonia) and to allow citrulline synthesis in the tissue.	Biochemistry
70	Windmueller and Spaeth (1981)	• Aim: To assess the physiologic importance of the intestinal citrulline contribution and to re-evaluate the importance of the liver • Design: Measurement of citrulline uptake and release by isolated, perfused livers and, through surgical means and arteriovenous difference measurements, by various organs of the rat in vivo. • Osborne-Mendel males (260-450 g) were fed NIH-07 open-formula stock diet ad libitum. Fasted rats had their food withheld overnight.	• No significant extraintestinal source of circulating citrulline was found. • Renal citrulline uptake was equivalent to approximately 83% the rate of intestinal release; kidneys, in turn, released arginine equivalent to approximately 75% of the citrulline taken up.	Biochemistry
71	Herzfeld and Raper (1976)	• Aim: To measure in whole upper intestine, or in duodenum, small intestine and colon of adult rats the levels of 11 enzymes, most of them involved in the metabolism of ornithine • Design: The developmental formations in small intestine of arginase, ornithine aminotransferase, and ornithine transcarbamylase were compared with those in liver • Changes with age (late gestation of adult) of the intestinal activities of pyrroline-5-carboxylate reductase, proline oxidase and glutamyl transpeptidase were also described.	• The results suggest that the proximal part of the intestine is well endowed with enzymes involved in the conversion of ornithine to proline as well as to citrulline.	Biochemistry, Developmental Biology
72	Raijman (1974)	• Aim: To investigate the subcellular distribution of ornithine carbamoyltransferase in the liver to establish whether its activity found in soluble fractions of rat and human liver reflects the distribution in vivo or artifacts occurring during the disruption of the tissue and the separation of the subcellular fractions. • Design: Male Wistar rats weighing approximately 125 g • For the measurement of ornithine carbamoyltransferase activity in other tissues, a radioactive assay utilizing [^{14}C]carbamoylphosphate was used.	• Rat liver ornithine carbamoyltransferase appears to be located exclusively in the mitochondria • Only the liver and the mucosa of small intestine contain significant amounts of ornithine carbamoyltransferase • Activity in intestinal mucosa is less than one thousandth of that in liver	Biochemistry

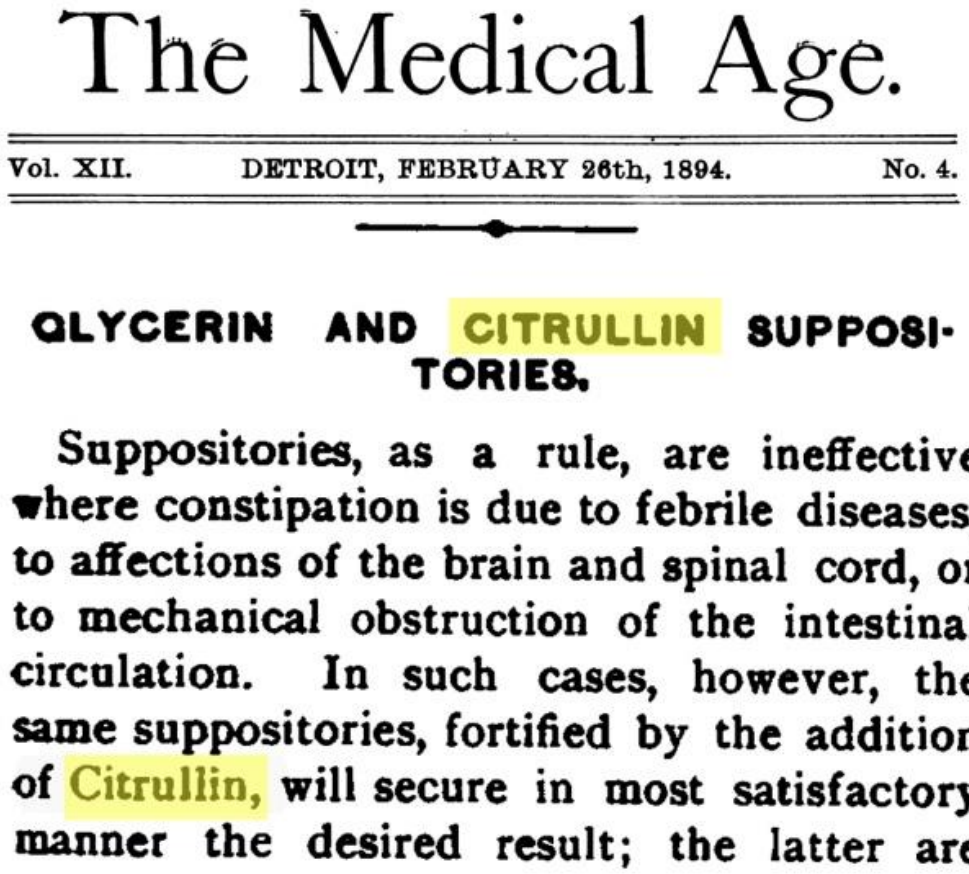
Table 1.3 Citrulline function in colonocytes.

No.	Authors	Settings	Findings
1	Armstrong *et al.* (2000)	• Aim: to assess aminoguanidine, a selective inhibitor of inducible nitric oxide synthase, with regard to its effectiveness as a nitric oxide inhibitor and as a modulator of inflammation in trinitrobenzene sulfonic acid-induced colitis • Design: Colitis was induced in Wistar rats. Selective aminoguanidine and non-selective 1-nitroso-arginine methyl ester inhibitors of nitric oxide synthase were given in the drinking water. • Colonic citrulline and arginine concentrations were assessed using high-performance liquid chromatography. The severity of colitis was assessed by a macroscopic scoring system.	• Both 1-nitroso-arginine methyl ester and aminoguanidine successfully reduced nitric oxide synthesis. There was no evidence of substrate depletion in the colonic wall. Neither of the agents reduced the severity of colonic inflammation. • Oral administration of nitric oxide synthase inhibitors reduced nitric oxide synthesis in the colonic wall.
2	Chakder and Rattan (1997)	• Aim: to further examine the role of L-citrulline recycling in the internal anal sphincter smooth muscle relaxation by the simultaneous determinations of tissue levels of L-arginine. • Design: Studies were performed on the internal anal sphincter smooth muscle strips obtained from opossums (Didelphisvirginiana). • Isometric tension and L-arginine levels of the tissues were measured under basal conditions, in the presence of electrical field stimulation and after treatment with different concentrations of arginase.	• The basal levels of L-arginine were found to be significantly higher in the internal anal sphincter (tonic smooth muscle) than in the rectal (phasic smooth muscle) smooth muscle. • Arginase caused a concentration-dependent attenuation of the internal anal sphincter relaxation caused by electrical field stimulation. L-Citrulline and L-arginine were equipotent in reversing the attenuation. • Both arginase (60 minutes pre-treatment) and continuous electrical field stimulation (tissues collected at the time of maximal recovery of the basal internal anal sphincter tone after the initial relaxation) caused significant decreases in L-arginine levels. • The decreases in the levels of L-arginine were restored by the exogenous administration of either L-arginine or L-citrulline. • The restoration of L-arginine levels by L-citrulline but not by L-arginine was selectively blocked by L-glutamine.
3	Rattan and Chakder (1997)	• Aim: To investigate the possibility of L-citrulline recycling in the maintenance of nitrergic neurotransmission in the opossum internal anal sphincter smooth muscle strips. • Design: Responses to nonadrenergic, noncholinergic nerve stimulation by electrical field stimulation on the basal internal anal sphincter tension were recorded before and after the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine, N^{ω}-nitro-L-arginine plus L-citrulline, or L-arginine. • During continuous electrical field stimulation, when the basal internal anal sphincter tone after the initial relaxation had recovered to almost pre- electrical field stimulation levels, the effects of L-citrulline or L-arginine were examined before and after L-glutamine, which is a putative blocker of L-citrulline uptake.	• Inhibition of nonadrenergic, noncholinergic nerve-mediated internal anal sphincter relaxation by N^{ω}-nitro-L-arginine was reversed by L-citrulline as well as L-arginine. • L-Citrulline and L-arginine caused concentration-dependent relaxation of the internal anal sphincter tone recovered during the prolonged electrical field stimulation. • L-Glutamine blocked the responses of L-citrulline but not of L-arginine. Furthermore, L-glutamine increased the speed of recovery of internal anal sphincter tone during continuous electrical field stimulation. • L-citrulline recycling may be responsible for the maintenance of internal anal sphincter relaxation during frequent short-train and prolonged nonadrenergic, noncholinergic nerve stimulation.

Table 1.3 *(Continued)*

No.	Authors	Settings	Findings
4	Shuttleworth *et al.* (1995); Shuttleworth and Sanders (1996); Shuttleworth *et al.* (1997)	• Aim: Investigation of the contribution of nitric oxide to inhibitory neuromuscular transmission in murine proximal colon and the possibility that citrulline is recycled to arginine to maintain the supply of substrate for nitric oxide synthesis. • Design: Intracellular microelectrode recordings were made from circular smooth muscle cells in the presence of nifedipine and atropine (both 1 μM).	• L-Nitro-arginine-methyl ester (100 μM) selectively abolished the slow component of i.j.ps. The effects of L-Nitro-arginine-methyl were reversed by L-arginine (0.2-2 mM) but not by D-arginine (2 mM). • L-Citrulline (0.2 mM) also reversed the effects of L-Nitro-arginine-methyl, and this action was maintained during sustained exposures to L-citrulline (0.2 mM). This may reflect intraneuronal recycling of L-citrulline to L-arginine. • Higher concentrations of L-citrulline (e.g. 2 mM) had time-dependent effects. Brief exposure (15 minutes) reversed the effects of L-Nitro-arginine-methyl, but during longer exposures (30 minutes) the effects of L-Nitro-arginine-methyl ester gradually returned. In the continued presence of L-citrulline, L-arginine (2 mM) readily restored nitrergic transmission, suggesting that during long exposures to high concentrations of L-citrulline, the ability to generate arginine from citrulline was reduced. • Recycling of L-citrulline to L-arginine may sustain substrate concentrations in support of nitric oxide synthesis and this pathway may be inhibited when concentrations of L-citrulline are elevated.
5	Berrino *et al.* (1995)	• Aim: Nitric oxide synthase activity in rat isolated longitudinal muscle myenteric plexus preparations of the small intestine was determined by measuring the accumulation of ^{3}H-L-citrulline during 30 minutes incubation with ^{3}H-L-arginine • Design: Sprague-Dawley rats were used. The whole small intestine was dissected and longitudinal muscle strips with the longitudinal muscle myenteric plexus attached were prepared	• Intermittent electrical field stimulation caused a threefold increase in ^{3}H-L-citrulline accumulation. • The nitric oxide synthase inhibitor • N^{ω}-nitro-L-arginine methyl ester reduced the spontaneous accumulation of ^{3}H-L-citrulline by 65% and prevented the electrically evoked increase. • During incubation with ^{3}H-L-arginine tissue levels of ^{3}H-L-citrulline in rat isolated longitudinal muscle myenteric plexus preparations, but not accumulation in incubation media may be used as a biochemical marker of the activity of nitrergic intestinal neurons which appear to be inhibited via muscarine receptors.
6	Grider and Jin (1993)	• Aim: Vasoactive intestinal peptide release and L-[^{3}H]citrulline production were examined in ganglia isolated from the myenteric plexus of guinea-pig intestine. • Design: Ganglia were isolated from the ileum of the guinea-pig. • Sheets of longitudinal muscle layer with adherent myenteric plexus were removed from segments of intestine (223 cm long) by tangential stroking.	• The nicotinic agonist l,l-dimethyl-4-phenylpiperizinium stimulated vasoactive intestinal peptide release and L-[^{3}H]lcitrulline production; the latter was considered an index of nitric oxide production. • Both vasoactive intestinal peptide release and L-[^{3}H]lcitrulline production were abolished by tetrodotoxin, hexamethonium, and the nitric oxide synthase inhibitor, N^{G}-nitro-L-arginine. • The pattern of stimulation by nitric oxide and inhibition by N^{G}-nitro-L-arginine implied that vasoactive intestinal peptide release is facilitated by and may be dependent on nitric oxide production. • The study provides the first direct evidence of nitric oxide production from enteric ganglia.

1.6 Figures



The Medical Age.

Vol. XII. DETROIT, FEBRUARY 26th, 1894. No. 4.

GLYCERIN AND CITRULLIN SUPPOSITORIES.

Suppositories, as a rule, are ineffective where constipation is due to febrile diseases, to affections of the brain and spinal cord, or to mechanical obstruction of the intestinal circulation. In such cases, however, the same suppositories, fortified by the addition of Citrullin, will secure in most satisfactory manner the desired result; the latter are

Figure 1.1. Passage from *The Medical Age* 1894;12:115, mentioning citrullin[e] suppositories.

Figure 1.2. Drawing of *C. colocynthis* (Culbreth, 1917, p. 585).

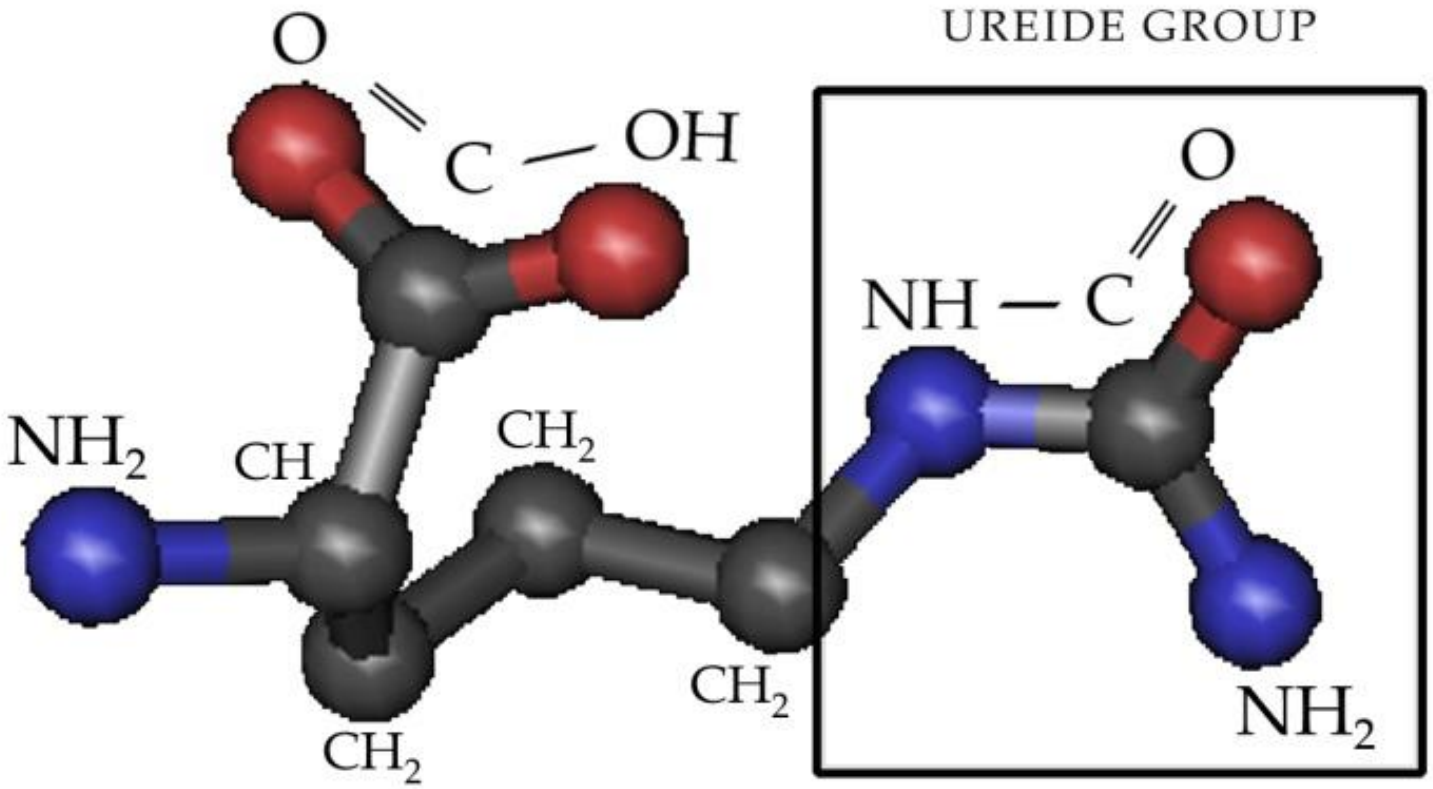
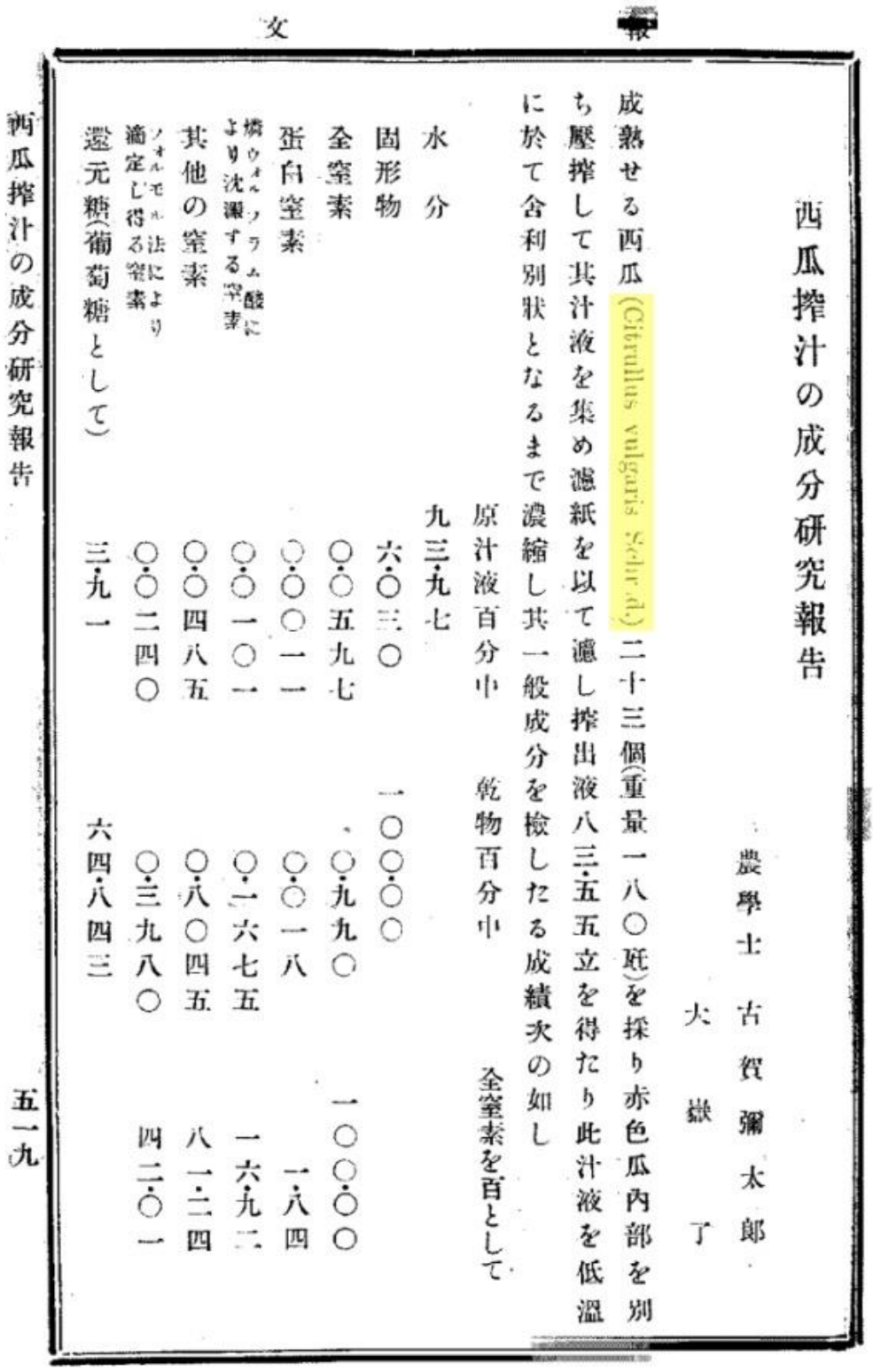


Figure 1.3. Citrulline structure. The characteristic group of citrulline, ureide group (NH_2-CO-NH), is shown in the box.



西瓜搾汁の成分研究報告

農學士 古賀彌太郎
大嶽了

成熟せる西瓜 (Citrullus vulgaris Schr. d.) 二十三個(重量一八〇瓩)を採り赤色瓜内部を別ち壓搾して其汁液を集め濾紙を以て濾し搾出液八三・五五立を得たり此汁液を低溫に於て含利別狀となるまで濃縮し其一般成分を檢したる成績次の如し

	原汁液百分中	乾物百分中	全窒素を百として
水分	九三・九七		
固形物	六・〇三〇	一〇〇・〇〇	
全窒素	〇・〇五九七	〇・九九〇	一〇〇・〇〇
蛋白窒素	〇・〇〇一一	〇・〇一八	一・八四
燐ウォルフラム酸により沈澱する窒素	〇・〇一〇一	〇・一六七五	一六・九二
其他の窒素	〇・〇四八五	〇・八〇四五	八一・二四
ノオルモル法により適定し得る窒素	〇・〇二四〇	〇・三九八〇	四二・〇一
還元糖(葡萄糖として)	三・九一	六四・八四三	

Figure 1.4. The first page from the article by Koga and Ohtake (1914) in the *Journal of the Tokyo Chemical Society*. To be read from right to left and from up to down.

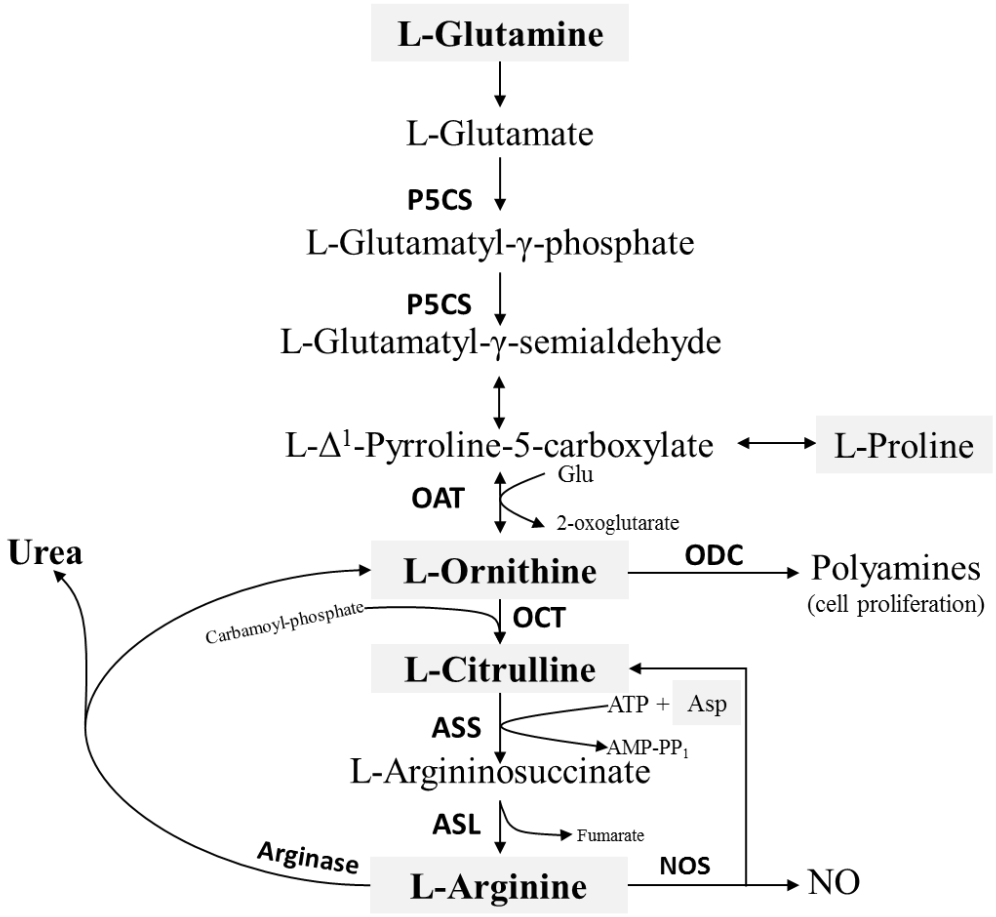


Figure 1.5. Overview of synthesis of L-citrulline and L-arginine from L-glutamine. Catabolism of L-arginine to L-ornithine/urea or L-citrulline/nitric oxide, production of polyamines, and anabolism and catabolism of proline are also shown. L-arginine enters the urea cycle and L-ornithine is reproduced. L-arginine also is converted to citrulline through the effect of nitric oxide synthases. Abbreviations: ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; Asp, aspartate; NOS, nitric oxide synthase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; OCT, ornithine carbamoyltransferase; P5CS, pyrroline-5-carboxylate synthase. Source: Romero *et al.* (2006); Cynober *et al.* (2010).

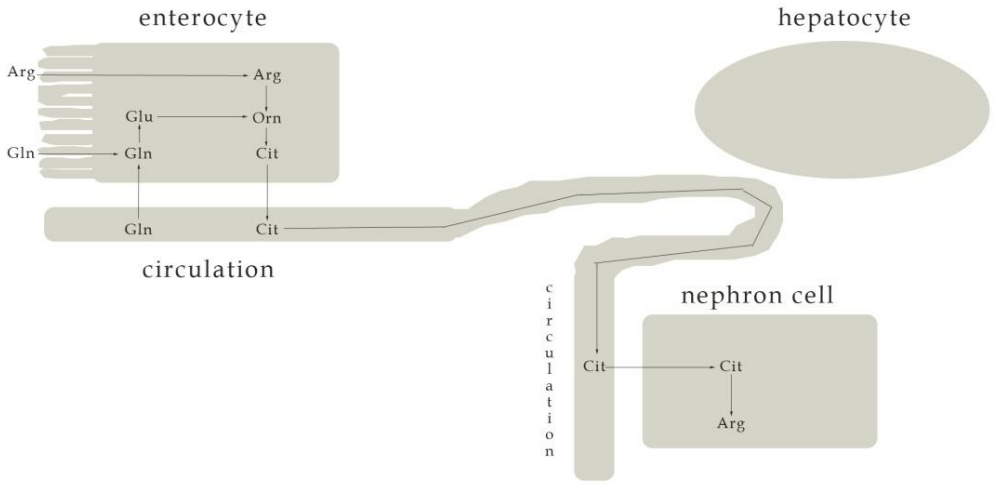


Figure 1.6. The fate of citrulline and the arginine/glutamine-citrulline-arginine cycle. Citrulline is produced at the enterocyte and is circulated to the kidney where it is broken down to arginine. Intestinal ornithine carbamoyltransferase is derepressed when protein intake is low. This process increases citrulline release and decreases arginine release in the portal vein, which increases arginine synthesis by the kidney and decreases liver ureagenesis, respectively. Abbreviations: Arg: arginine, Cit: citrulline, Gln: glutamine, Orn: Ornithine.

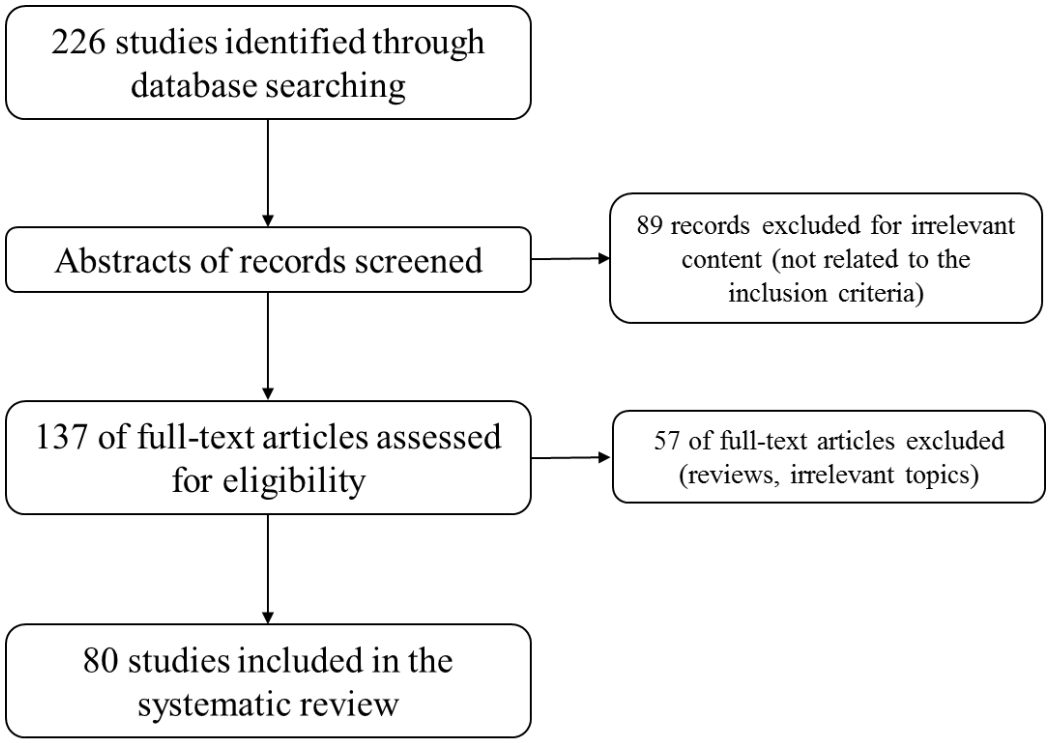


Figure 1.7. Flow chart of the systematic review of animal studies.

Chapter 2
Citrulline and Intestinal Function: Systematic Review and Meta-Analysis

2.1 Introduction

For this section, a systematic review of the literature was done. A comprehensive research in Pubmed/Medline, Scopus and Embase was conducted searching for articles on citrulline and the intestine in human populations. Certain conditions were identified in which citrulline has been used as a marker of intestinal function. However, it is not clear whether citrulline levels reflect intestinal function (notably absorption), enterocyte mass, both or other. The literature has been using this marker interchangeably but at the same time researchers are cautious about attributing to it an absolute interpretation.

Diagnostic markers are commonly used in the context of certain clinical conditions where variations in their levels indicate certain pathophysiological processes. However, certain markers have such a specificity that values outside normal range commonly indicate a limited number of clinical conditions, such that the differential diagnosis is narrow and hence clinical diagnosis of the condition is very likely. For example, in daily clinical practice, it is completely valid and reasonable to deduct that when C-reactive protein is over 5 mg/L – and quite strongly when over 10 mg/L – there is an inflammatory process in the human body (infection or other); it can also be likely deducted that when creatinine and urea surpass their upper normal limits (which differ between hospitals and populations), the patient has a possible acute kidney injury. Does citrulline currently maintain a place in clinical practice such that when it is out of a certain normal range [let it be (x, y) μmol/L], the patient has intestinal failure, insufficiency, malabsorption or dysfunction in general? I will leave this question unanswered for the time being and come back to it once the evidence is presented.

Hence, citrulline's unique metabolism (production at the intestinal enterocyte) and complex findings from various studies compel me to submit the following questions (to myself but to the reader as well) – which I will try to answer at the end of the chapter and thesis. The questions are:

1. Is citrulline a successful indicator of enterocyte mass?
2. Is citrulline a successful marker of enterocyte absorption?
3. What clinical conditions has citrulline been utilized in as a marker?
4. Can a new conceptual theory be postulated regarding citrulline's clinical utility?[1]

I first describe the methods for this research literature synthesis and then move on to categorising the findings in sections for better understanding.

2.2 Methods

2.2.1 Study Eligibility Criteria

The inclusion criteria for this systematic review were: any empirical study (abstract and full paper) describing investigation of citrulline in relation to the term intestinal function. Intestinal function was considered in a very broad term and could mean enterocyte mass, diarrhoea, absorption, or even deranged citrulline levels in correlation with a compromised gut. The only papers that were excluded were those whose object of investigation was not related to intestinal function. There was no restriction to language of papers and the types of interventions could include observational studies, randomized controlled trials, case series and case reports. For the meta-analysis papers had to provide sufficient data to produce an effect measure.

2.2.2 Search Strategy and Terms

PRISMA guidelines and MOOSE checklist for systematic reviews and meta-analyses were used (Stroup *et al.*, 2000; Moher *et al.*, 2009). Electronic database searches were conducted in Google Scholar, Pubmed/Medline, Scopus, EMBASE and Cochrane Library with no year limits. Publisher databases were also searched (Sciencedirect.com, link.springer.com, Wiley Library Online, Highwire Press, Nature.com, Ovid, Cambridge University Press). The search keywords were: citrulline, intestine, gut, bowel, intestinal, mucositis, Crohn's disease, short bowel, radiotherapy, cancer, sepsis, absorption, enterocyte, critically ill, and colitis. The date of search was up until 1 July 2015. The bibliographies from all included

[1] I give a preliminary note that I will introduce the term *possible* acute gut injury as a result of the findings.

manuscripts and hand searching of relevant gastroenterology and nutrition journals were used to identify further references.

2.2.3 Study Selection, Data Extraction and Quality Assessment

The resulting studies (in abstract form) were assessed against the inclusion criteria. When there was insufficient information available in the abstract, the full text was reviewed. Then, data were extracted from the selected studies including: author, year of publication, aim of the study, country, continent, sample size, mean age, male percentage, study design, results. The quality of studies (risk of bias) was assessed with elements from Cochrane Collaboration's tool (Higgins *et al.*, 2008) and the RTI Item Bank for Observational Studies (Viswanathan and Berkman, 2012; Viswanathan *et al.*, 2013), which assess selection, attrition, detection and confounding biases. For the meta-analysis, studies were examined for *p*-values, means and standard deviations, correlation coefficients or other metrics depicting the association of citrulline with intestinal function. Metrics were converted to the standardized mean difference (SMD) (Lipsey and Wilson, 2001; Cooper *et al.*, 2009) or mean difference (MD), where means and standard deviations for groups under comparison were identified (Borenstein *et al.*, 2008); and/or correlation coefficients (CCs). Examples of outcomes included correlations of citrulline levels with small bowel length, with absorption tests/enteral caloric intake, differences in citrulline between tests groups and controls etc. Particularly, studies included satisfied the following criteria: a) for correlations of citrulline with small bowel length, only short bowel syndrome; b) for absorptive marker correlations, all patient groups c) for gastrointestinal disease severity, all patient groups. Where available, diagnostic accuracy data were also collected.

2.2.4 Statistical Analysis

Meta-analysis in medicine is any structured and systematic qualitative and/or quantitative integration of the results of several independent studies on a health problem (Jenicek, 1989). Three main reasons suggest performing a meta-analysis: statistical significance, clinical importance of the effect and consistency of the effects.

Quantitative analysis was performed with Stata 12.0 (StataCorp LP, Texas), Review Manager 5.3 (Cochrane Collaboration, Copenhagen) and SPSS 22.0 (IBM Corp.,

Armonk, NY). SMDs/MDs/CCs were extracted from studies when available. The strength of association was categorized as following: small, SMD = 0.2; moderate, SMD = 0.5; and large, SMD = 0.8; MD has units and reflects the units of the outcome under description; small, CC = 0.1; moderate, CC = 0.3; and large, CC = 0.5 (Cohen, 1988; Faraone, 2008). A random effects model was used to produce a pooled estimate of the SMDs/MDs/CCs. With the random effect model, studies are weighted by the inverse of their variance with tau-squared (τ^2), taking into account the within study variance for estimating the correlation coefficient in each study and the between studies variance (e.g. because of different designs or methods used but also possible biological reasons) (DerSimonian and Laird, 1986). Statistical heterogeneity was assessed using Cochran's *Q* test, which examines the null hypothesis that all studies are evaluating the same effect (Higgins *et al.*, 2003). Statistical significance for heterogeneity was set as $p \leq 0.10$. Heterogeneity was quantified using the I^2 statistic, indicating the percentage of total variation across studies that is due to heterogeneity rather than chance (Higgins *et al.*, 2003). I^2 value of 0% was considered to indicate no observed heterogeneity whilst a value > 50% substantial heterogeneity (Higgins and Thompson, 2002; Huedo-Medina *et al.*, 2006; Bowden *et al.*, 2011; Fragkos *et al.*, 2014). Heterogeneity was further investigated with subgroup analysis and meta-regression. Publication bias was assessed using funnel plots, Egger's test, Begg's test and Rosenthal's number (Rosenthal, 1979; Begg and Mazumdar, 1994; Egger *et al.*, 1997; Fragkos *et al.*, 2016). A funnel plot was created for the clinical measures with more than 10 studies (Sterne *et al.*, 2011). This is a scatter plot of the effect estimates from individual studies against a measurement of the study's sample size or precision. Resemblance of a symmetrical inverted funnel supports that findings are due to sampling variation alone; thus, absence of bias (Sterne *et al.*, 2011).

Regarding CCs, it is common practice not to perform syntheses on the correlation coefficient itself because the variance depends strongly on the correlation. Rather, the CC is converted to the Fisher's *z* scale (not to be confused with the *z*-statistics used with significance tests), and all analyses are performed using the transformed values. The results, such as the summary effect and its confidence interval, would then be converted back to correlations for presentation. The transformation from sample correlation *r* to Fisher's *z* is given by $z = 0.5 \times \ln\left(\frac{1+r}{1-r}\right)$. The variance of *z*

is $V_z = \frac{1}{n-3}$ and the standard error is $SE_z = \sqrt{V_z}$, where *n* is the sample size of the study. We then convert each of these values back to correlation units using $r = \frac{e^{2z}-1}{e^{2z}+1}$.

Regarding diagnostic accuracy data, following the robust construction of the diagnostic 2 × 2 tables, specificity, sensitivity, and 95% CI for each of the included studies was calculated. A hierarchical summary receiver operating curve (HSROC) model was fitted to provide a summary receiver operating curve and to allow derivation of pooled sensitivity and specificity estimates (Harbord *et al.*, 2007). As suggested by the Cochrane Diagnostic Test Accuracy group (http://srdta.cochrane.org/), no analyses of study heterogeneity were performed, as these tests do not account for heterogeneity explained by phenomena, such as positivity threshold effects (Higgins *et al.*, 2008).

2.3 Results

The next sections describe results per condition and/or function. Each section reports separately on condition and / or function with separate meta-analyses, where possible, including reports for quality assessment and other analyses where appropriate. The flow chart for studies is shown in Figure 2.1; from initially 463 studies, 131 were included in the systematic review and 63 in the meta-analyses performed. As a general description of papers, patients in the meta-analyses reached 4292 (mean 68, range 6-847) with mean age 31.6 years old, male percentage 50.9% and BMI 21.9 kg/m^2. Twenty three studies involved children and forty involved adults and the majority of studies were conducted in Europe (45 studies). Mean citrulline value from all studies was 23.2 μmol/L and citrulline was mostly measured with HPLC. Main findings from all studies are shown in Tables 2.1-2.6, grouped by condition.

Regarding overall quality assessment and risk of bias, there was a strong presence of detection bias and almost 50% confounding bias (Figures 2.2 and 2.3). Reporting bias was also an issue that arose from the papers. Because of this fact, all results are presented with the risk of bias figures alongside the forest plots. Before reporting the results of the meta-analyses performed, a short definition of intestinal failure and short bowel syndrome is provided. The reason for this is that many conditions

that will be described include various forms of intestinal failure and the necessary nomenclature is imperative.

Intestinal failure occurs when there is reduced intestinal absorption so that intravenous macronutrient and/or water and electrolyte supplements are needed to maintain health and/or growth (Pironi *et al.*, 2016). If untreated, undernutrition and/or dehydration result. Nutrient/fluid requirements determine whether intestinal failure is termed severe, moderate, or mild. Severe is when parenteral, moderate when enteral, and mild when oral nutritional fluid supplements are needed (Nightingale and Woodward, 2006). A patient may change, due to compensatory mechanisms (for example functional adaptation), from having severe to mild intestinal failure with time. Intestinal failure is classified into three types: Type I, acute, short-term, and often self-limiting condition; Type II, prolonged acute condition, often in metabolically unstable patients, requiring complex multi-disciplinary care and intravenous supplementation over periods of weeks or months; and Type III, chronic condition, in metabolically stable patients, requiring intravenous supplementation over months or years (Pironi *et al.*, 2016). It may be reversible or irreversible, acute or chronic and encompasses five pathophysiological mechanisms (short bowel syndrome, intestinal fistula, intestinal dysmotility, mechanical obstruction, extensive small bowel mucosal disease) from a wide variety of underlying medical and surgical conditions (mesenteric ischemia, Crohn's disease, coeliac disease, immunodeficiency, radiation enteritis, surgical complications, familial polyposis, volvulus, intestinal malformation, necrotizing enterocolitis, primary chronic intestinal pseudo-obstruction, scleroderma, Hirschprung's disease, immunodeficiency and other conditions). These patients may be most appropriately managed in a centre with special interest and expertise in intestinal failure (Nightingale, 2001).

Normal human small intestinal length, measured from the duodenojejunal flexure at autopsy or surgery, varies from about 275 cm to 850 cm, and tends to be shorter in women. After intestinal resection it is important to refer to the remaining length of small intestine measured at surgery or with an opisometer rather than to the amount removed (Nightingale, 2001). In general, nutritional/fluid supplements are likely to be needed if less than 200 cm of small bowel remains.

There are three main types of patient with a short bowel: those who have had a jejunoileal resection and a jejunocolic anastomosis (*jejunum-colon*); those who have had a predominantly jejunal resection, and have more than 10 cm of terminal ileum and the colon remaining (*jejunum-ileum*); and those who have had a jejunoileal resection, colectomy, and formation of a stoma (*jejunostomy*). Jejunum-ileum patients are uncommon and rarely have problems of undernutrition and therefore do not often need nutritional support. When jejunum-ileum patients are seen, with undernutrition or severe diarrhoea after eating/drinking, they are managed in the same way as jejunum-colon patients. Jejunum-colon and jejunostomy patients are most commonly encountered (Nightingale *et al.*, 1992). The most common reasons for a short bowel in adults are Crohn's disease, superior mesenteric artery thrombosis, and irradiation damage (Table 2.7) (Nightingale and Woodward, 2006; Pironi *et al.*, 2016; Pironi *et al.*, 2018). Jejunum-colon patients often appear well after their resection except for diarrhoea/steatorrhea, but in the following months may lose weight and become severely undernourished. Patients with a jejunostomy have problems of dehydration immediately after surgery due to large stomal water and sodium losses. This jejunal output is greatest after food and drink consumption.

Next, the specific conditions where plasma citrulline levels have been described as indicating enterocyte function are reported alongside the meta-analyses' results.

2.3.1 Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis is a medical condition primarily seen in premature infants, where portions of the bowel undergo necrosis (tissue death) (Lin and Stoll, 2006). It occurs postnatally and is the second most common cause of mortality in premature infants. The combination of a genetic predisposition, intestinal immaturity, and an imbalance in microvascular tone, accompanied by a strong likelihood of abnormal microbial colonization in the intestine and a highly immunoreactive intestinal mucosa, leads to a confluence of predisposing factors (Neu and Walker, 2011). The most typical initial signs and symptoms of necrotizing enterocolitis in a preterm infant include feeding intolerance, abdominal distention and bloody stools after 8 to 10 days of age (Lin and Stoll, 2006; Neu and Walker, 2011). Treatment consists primarily of supportive care with bowel rest and any surgery performed may lead up to short bowel syndrome (Lin and Stoll, 2006).

In this context, four studies have assessed citrulline levels in patients with necrotizing enterocolitis (Table 2.1). Risk of bias was low in most. The MD indicated a significant decrease in citrulline levels by -7.8 μmol/L (95% CI [-14.7, 0.9]; I^2 = 97%) compared to control, which indicated a strong decrease when the SMD was analysed -1.44 (95% CI [-2.80, -0.07]; I^2 = 96%) (Figure 2.4). Celik *et al.* (2013) described that the area under the receiver operating characteristic (ROC) curve for citrulline to differentiate NEC from controls was 0.88 (95% CI, 0.77-0.99) and cut-off level of citrulline was 13.15 μmol/l with a sensitivity of 80% and a specificity of 82% but no association with duration of parenteral nutrition was noted. Similarly, Ioannou *et al.* (2012) noted that the area under the ROC curve for plasma citrulline to discriminate neonates with NEC from control neonates was 0.86 (95% CI, 0.77-0.96). The citrulline level that maximized the test's sensitivity and specificity was 17.75 μmol/L, with a sensitivity of 76% and a specificity of 87%.

2.3.2 Intestinal Transplantation

Intestinal transplantation or small bowel transplantation is the surgical replacement of the small intestine for cases of permanent intestinal failure. While intestinal failure can often be treated with alternative therapies such as parenteral nutrition (PN), complications such as intestinal failure associated liver disease and short bowel syndrome may make transplantation the only viable option (Nightingale, 2001; Goulet and Sauvat, 2006). The rarest type of organ transplantation performed, intestinal transplantation is becoming increasingly prevalent as a therapeutic option due to improvements in immunosuppressive regiments, surgical technique, and the clinical management of pre and post-transplant patients (Fishbein, 2009).

Measurement of citrulline levels has sparked the interest of specialists in intestinal transplantation as a possible indicator of rejection – and this is completely reasonable, considering the consequences that may arise. Thirteen studies were identified in the literature search and two groups have published quite extensively in the field: the University of Miami School of Medicine (Pappas *et al.*, 2001; 2002; 2004a; 2004b; Yu *et al.*, 2005; David *et al.*, 2006; 2007; 2008; Ruiz *et al.*, 2010; Hibi *et al.*, 2012) and the Mount Sinai School of Medicine (Gondolesi *et al.*, 2002; 2004; 2006) (Table 2.2).

These studies' focus is the ability of citrulline levels to predict the grade of acute cellular rejection and the cut-off value of citrulline levels that yield a high possibility of acute cellular rejection. There was presence of detection bias and confounding because not all studies assessed possible confounders of associations. Also, there is a strong possibility of reporting bias and attrition bias, because many papers are published in *Transplantation Proceedings*, which publishes short reports from transplant centres (Figure 2.5). The initial studies by the Miami group described a moderate negative correlation of citrulline levels with rejection (Pappas *et al.* (2001) reported CC = -0.59) but in the recent studies by Ruiz *et al.* (2010) and Hibi *et al.* (2012), which include up to around 10,000 plasma citrulline samples, correlation reaches up to a strong -0.977 with acute cellular rejection. The correlation coefficients are shown in Figure 2.5 without meta-analysis due to severe heterogeneity.

Two other trends were noted in the transplantation articles: first, citrulline appears to normalize after a certain amount time post transplantation and this is a significant factor against rejection (Pappas *et al.*, 2002; Gondolesi *et al.*, 2004); Pappas *et al.* (2004a); Pappas *et al.* (2004b); secondly, the cut-off value of citrulline that could very strongly predict rejection. The Miami group have described in two papers that citrulline levels have a very high negative predictive value for moderate or severe acute rejection in these patients (negative predictive value = 99% with cut-off level 13 μmol/L; sensitivity is 96.4% with particularly high specificity in adult patients) (David *et al.*, 2007; David *et al.*, 2008; Hibi *et al.*, 2012) but the Mount Sinai Group did not find that citrulline had satisfactory diagnostic accuracy to discern rejection (Gondolesi *et al.*, 2006).

In conclusion, results suggest the use of citrulline as a measure of graft rejection in intestinal transplantation. However, cautious interpretation is necessary due to sequential measurements (in contrast to single measurements in other conditions) and presence of heterogeneity between studies. Gut function and physiology can be affected by time after surgery, renal function, graft pathology, infection/sepsis, donor and patient anthropometrics post intestinal transplant (Gondolesi *et al.*, 2002; Gondolesi *et al.*, 2004; Gondolesi *et al.*, 2006) with normalization of citrulline not always occurring after treatment of graft versus host disease (Pappas *et al.*, 2004a).

Future research should focus on investigating the predictive power of plasma citrulline measurements against other biomarkers.

2.3.3 Short Bowel Syndrome

Citrulline's unique metabolism which involves production at the intestinal enterocyte has prompted suggestions that plasma citrulline level could be a reliable marker of gut function (Crenn, 2008; Crenn *et al.*, 2008). This led to a hypothesis that citrulline may be a *conditionally* essential amino acid in short bowel syndrome, even if it is not incorporated into proteins. From the literature review, 35 papers and abstracts were identified which included eventually 26 studies (Table 2.3). Quality assessment showed possibilities of reporting, attrition and detection bias (Figures 2.2 and 2.3). Several outcomes were analysed in the short bowel syndrome group of studies.

2.3.3.1 Citrulline and Residual Small Bowel Length

Twenty one studies were analysed and the random effects analysis of correlation coefficients produced a pooled effect of 0.68 (95% CI 0.37-0.85, range 0.26-0.99), which indicates a strong correlation. In addition there was evidence of publication bias (funnel plot asymmetry, Egger's test $p = 0.001$ but Begg's test $p = 0.156$, Failsafe number = 5286) and high heterogeneity ($Q = 764.98$, df = 20, $p < 0.001$; $I^2 = 97\%$, $\tau^2 = 0.99$). See Figure 2.6 for the forest plot of this outcome and Figure 2.7 for the funnel plot.

Subgroup analyses were performed to explore heterogeneity (Figure 2.8):

1. When correlations were analysed by country, Spain, the USA and Italy had reduced I^2 (0%, 4% and 56.4% respectively). In the French group of papers, heterogeneity remained high ($I^2 = 98.6\%$).
2. When correlations were analysed by continent, America and Asia had no heterogeneity (I^2 was 4% and 0% respectively) while heterogeneity remained high in Europe ($I^2 = 97.9\%$).
3. When correlations were analysed by population, the adults group had still high heterogeneity $I^2 = 98.2\%$ while the paediatric group had less heterogeneity $I^2 = 62\%$.

4. When correlations were analysed by citrulline measurement method, heterogeneity remained high in all groups (HPLC, IEC and mass spectrometry).

Finally, we performed metaregression with four continuous variables that could possibly affect this association: male percentage, mean age, mean BMI, mean citrulline concentration and mean small bowel length. No variable had significant effect on the mean correlation and the model was not significant (Table 2.8). Speculating on the causes of heterogeneity, most likely the heterogeneity in France is due to measurement methods between institutions and unlikely to be ethnic anatomical variations between different countries. Although both children and adult studies had heterogeneity, this was higher in adults. Adult studies were predominantly performed in European countries, whilst most studies from the US were performed in children.

2.3.3.2 Citrulline between SBS Patients and Healthy Controls

Twelve studies were analysed and the random effects model showed that citrulline levels were decreased by -12 μmol/L (95% CI [-16.3, -7.7]) (SMD -1.34 (95% CI [-1.77, -0.91]); there was heterogeneity (MD: Q = 134.69, df = 11, $p < 0.001$; I^2 = 92%, τ^2 = 49.75; SMD: Q = 56.39, df = 11, $p < 0.001$; I^2 = 80%, τ^2 = 0.44) but no publication bias (symmetric funnel plot, Egger's test p = 0.606, Begg's test p = 0.537, Failsafe N = 853) (Figure 2.4 and Figure 2.9B).

2.3.3.3 Citrulline Levels in PN Dependent vs PN Independent Patients

Twelve studies were analysed comparing levels of citrulline in patients who needed parenteral nutrition against patients who were weaned off PN. The random effects model showed that citrulline levels were decreased by -13.3 μmol/L (95% CI [-17.6, -9.0]) (SMD -1.58, 95% CI [-2.09, -1.08]); there was heterogeneity (MD: Q = 97.28, df = 11, $p < 0.001$; I^2 = 89%, τ^2 = 46.89; SMD: Q = 52.10, df = 11, $p < 0.001$; I^2 = 79%, τ^2 = 0.57) but no publication bias (symmetric funnel plot, Egger's test p = 0.174, Begg's test p = 0.451, Failsafe N = 753) (Figure 2.10A and Figure 2.9D).

Sixteen studies described diagnostic accuracy results and these were meta-analysed with the hierarchical summary receiver operating characteristic (ROC) model by Harbord *et al.* (2007). The details of each study are shown in Table 2.9, which shows individual sensitivities, specificities, citrulline cut-off levels and values for

true positives, false positives, false negatives and true negatives. The results are shown in in Table 2.10, forest plots for sensitivity and specificity are shown in Figure 2.11 while the sROC curve is shown in Figure 2.12. Overall, citrulline levels have a sensitivity of 82.5% and specificity 82%. Since thirteen studies compared citrulline levels at a cut-off level of 20 μmol/L to discern among short bowel syndrome patients who needed PN or not, the meta-analysed sensitivity and specificity reflect mostly that. Any heterogeneity is present due to different cut-off levels and comparison groups. Healthy patients with normal intestinal mucosa function and normal renal function have a citrulline level between 30 and 50 μmol/L with a median of 40 μmol/L (Rabier and Kamoun, 1995; Curis *et al.*, 2005). Although this range for plasma citrulline levels mainly comes from studies in Western Europe and North America, a Chinese study on 33 healthy Chinese subjects found a mean plasma citrulline level of 16.87 ± 5.97 μmol/L (range 19-54) measured with HPLC (Liu *et al.*, 2004). This finding is very interesting, becomes it creates a paradox in the multiple findings of the high diagnostic accuracy of citrulline level at 20 μmol/L.

2.3.3.4 Teduglutide and Citrulline Levels

There have been four studies studying the effect of teduglutide – a GLP-2 analogue which acts as a growth factor in patients with short bowel syndrome – on citrulline levels (Gilroy *et al.*, 2009; Jeppesen *et al.*, 2009; Buchman *et al.*, 2010; 2011; 2012; Naimi *et al.*, 2013; Seidner *et al.*, 2015). Three studies compared citrulline levels in patients who received teduglutide against patients who received placebo in Crohn's disease (Buchman *et al.*, 2010) and short bowel syndrome (Gilroy *et al.*, 2009; Jeppesen *et al.*, 2009; 2011; 2012; Naimi *et al.*, 2013; Seidner *et al.*, 2015). The random effects model showed that citrulline levels in teduglutide versus placebo were increased by 12.4 μmol/L (95% CI [5.5, 19.3]) (SMD 1.02, 95% CI [0.47, 1.58]) and there was heterogeneity (MD: Q = 13.13, df = 2, p = 0.001; I^2 = 85%, τ^2 = 31.01; SMD: Q = 7.41, df = 2, p = 0.02; I^2 = 73%, τ^2 = 0.18) (Figure 2.10B). Four studies provided citrulline levels of patients who received teduglutide at the end of treatment compare to their baseline (Gilroy *et al.*, 2009; Jeppesen *et al.*, 2009; Buchman *et al.*, 2010; 2011; 2012; Naimi *et al.*, 2013; Seidner *et al.*, 2015). The random effects model showed that citrulline levels in teduglutide at end of treatment versus baseline were increased by 15.3 μmol/L (95% CI [12.5, 18.2])

(SMD 1.21, 95% CI [1.00, 1.43]); there was no heterogeneity (MD: Q = 3.57, df = 3, p = 0.31; I^2 = 16%, τ^2 = 1.41; SMD: Q = 2.05, df = 3, p = 0.56; I^2 = 0%, τ^2 = 0.00) (Figure 2.10C).

Historically, short bowel syndrome was the first pathological situation to be studied in conjunction with citrulline levels due to its innate experimental nature, created by the removal of a significant amount of the anatomical and functional mass of intestine. Since Crenn *et al.* (2000) published their study, there have been at many more studies studies performed in adults (see Table 2.3). All of these studies have found a strong and significant positive correlation, with CCs ranging from 0.26 to 0.99, between postabsorptive plasma citrulline concentration and remnant small bowel length. It appears that citrulline concentration reflects the overall small bowel function, including small bowel excluded from the digestive circuit; hence, determination of citrulline concentration can be used preoperatively as a reliable biomarker of the probability of parenteral nutrition weaning at a 20 µmol/L threshold (Crenn *et al.*, 2000).

2.3.4 Enteropathies

2.3.4.1 Villous Atrophy Syndrome

In chronic villous atrophy (e.g., coeliac disease, tropical sprue, and various infectious enteritides), citrulline is decreased (less than 20 µmol/L) in patients with proximal destructive lesions of villous architecture and severely decreased in patients with extensive (proximal and distal) impairment of intestinal mucosa (Curis *et al.*, 2007).

The first study to associate citrulline levels with enterocyte damage from coeliac disease was by Crenn *et al.* (2003). Their main results showed that plasma citrulline concentration was lower (p < 0.001) in patients with villous atrophy (24 ± 13 µmol/L) than in healthy subjects (40 ± 10 µmol/L) and patients with anorexia nervosa (39 ± 9 µmol/L). Three thresholds were individualized: less than 10 µmol/L for patients with diffuse total villous atrophy (n = 10), 10-20 µmol/L for patients with proximal-only total villous atrophy (n = 12), and 20-30 µmol/L for patients with partial villous atrophy (n = 10). Plasma citrulline concentration was correlated to the severity and extent of villous atrophy (r = 0.81; p < 0.001) while ROC curves indicated that plasma citrulline concentration was the best biological variable to

predict villous atrophy. In these patients, a citrulline value below 10 μmol/L is highly predictive of the need for at least one period of PN. The patients who received PN had invasive small bowel lymphoma, refractory sprue with cryptic lymphoma, and extensive small intestinal CD4 T-cell infiltration all with severe malabsorption and diffuse destructive mucosal lesions.

Similar results were recently reported in patients with human immunodeficiency virus enteropathy or severe intestinal infectious disease (Crenn *et al.*, 2009a). In this situation, plasma citrulline levels can be considered as an indicator of evolution of the intestinal disease. Patients with only mild enterocyte involvement, i.e., partial proximal villous atrophy, have a normal or moderately decrease in citrulline concentration. The results by Crenn *et al.* (2009a) were verified by Papadia *et al.* (2010). In their study, postabsorptive fasting serum citrulline was measured in 150 tropical enteropathy patients, 44 of whom had HIV infection, using reverse phase HPLC. Absorptive capacity and permeability were measured after intrajejunal instillation of 5 g lactulose, 1 g L-rhamnose, 0.5 g D-xylose, 0.2 g 3-O methyl D-glucose with assay by thin-layer chromatography. In human immunodeficiency virus (HIV) positive patients, the median serum citrulline was significantly lower (median 19 μmol/L) than in HIV negative patients (median 27 μmol/L; $p < 0.001$). There were statistically significant correlations ($p < 0.005$) between citrulline and crypt depth; villous height/crypt depth ratio; Shenk-Klipstein score; and xylose absorption, only in the HIV positive. Thus, serum citrulline concentration appears to be a quantitative biomarker of small bowel mass integrity in HIV positive enteropathy.

Eleven studies were used in meta-analyses in this category which included cases that had coeliac disease or other enteropathy (tropical, HIV etc.) (Table 2.4). Meta-analyses firstly compared citrulline levels in diseased patients vs controls, then those who had received gluten free diet (GFD) compare to those who hadn't, and finally association of citrulline levels with disease severity. Severity of disease was categorised broadly and included either histological diagnoses, worsening symptoms or any other metric reported by authors which indicated severity of the enteropathy. Severity is to be considered as a scale by which higher values indicate more severe disease and lower values indicate less severe disease. The random effects model showed that citrulline levels in coeliac disease/enteropathy patients

compared to control were decreased by -9.5 μmol/L (95% CI [-12.9, -6.1]) (SMD -0.99, 95% CI [-1.30, -0.67]); there was heterogeneity (MD: Q = 77.52, df = 9, p < 0.001; I^2 = 88%, τ^2 = 23.08; SMD: Q = 41.51, df = 9, p < 0.001; I^2 = 78%, τ^2 = 0.19) but no publication bias (symmetric funnel plot, Egger's test p = 0.247, Begg's test p = 0.283) (Figure 2.4 and Figure 2.9C). Citrulline levels were compared in coeliac disease patients who had received GFD vs those who hadn't in five studies (Crenn *et al.*, 2003; Hozyasz *et al.*, 2006; Miceli *et al.*, 2008; Blasco Alonso *et al.*, 2011; Ioannou *et al.*, 2011); the random effects model showed that citrulline levels were decreased by -8.2 μmol/L (95% CI [-10.4, -5.9]) (SMD -1.08, 95% CI [-1.42, -0.75]) in those patients who hadn't received GFD; there was no heterogeneity (MD: Q = 4.08, df = 4, p = 0.39; I^2 = 2%, τ^2 = 0.15; SMD: Q = 2.47, df = 4, p = 0.65; I^2 = 0%, τ^2 = 0.00) (Figure 2.10D).

2.3.4.2 Crohn's Disease

Papadia *et al.* (2007) first studied the levels of plasma citrulline in patients with Crohn's disease (CD). They studied 55 CD patients according to diagnosis, small bowel length, and degree of bowel inflammation. Their results were very interesting in the fact that they showed a positive relationship between citrulline and small bowel length for lower citrulline values (p < 0.001), but this relationship tailed off for higher citrulline values. Citrulline concentrations correlated with small bowel absorptive capacity for carbohydrate, while the presence of intestinal inflammation didn't affect plasma citrulline concentrations. (Buchman *et al.*, 2010) investigated CD patients who had received teduglutide to induce remission and measured plasma citrulline as surrogate marker of mucosal healing. Their results suggest a potential link between improved mucosal healing and increase in plasma citrulline, indicating that citrulline could function as a surrogate marker of intestinal absorption.

Citrulline levels were compared between Crohn's disease patients and controls in two studies (Papadia *et al.*, 2007; Diamanti *et al.*, 2011a). The random effects model showed that citrulline levels in patients vs controls was decreased by -9.7 μmol/L (95% CI [-12.6, -6.7]) (SMD -1.19, 95% CI [-1.63, -0.75]); there was no heterogeneity (MD: Q = 0.02, df = 1, p = 0.90; I^2 = 0%, τ^2 = 0.00; SMD: Q = 0.00, df = 1, p = 0.99; I^2 = 0%, τ^2 = 0.00) (Figure 2.4).

2.3.4.3 Acute Mucosal Enteropathy and Cancer Treatments

Acute mucosal enteropathy can cause a significant loss of enterocytes, as experienced clinically by secondary lactose intolerance. Plasma citrulline concentrations are reduced in these situations, for example, in adenovirus enteritis and in all infectious intestinal diseases with high cytopathic effect (Gondolesi *et al.*, 2006). The normalization of citrullinemia is often rapid, after 1-3 weeks. Chemotherapy in haematology (e.g. as part of conditioning regimens for bone marrow transplants) and oncology induces a decrease in citrulline levels, which relates with the known cytokinetic renewal of intestinal mucosa with a nadir 5-8 days after treatment initiation (Blijlevens *et al.*, 2004; Lutgens and Lambin, 2007). Citrullinemia is more sensitive and more specific than the sugar-based permeability test for detecting chemotherapy-induced gut damage in patients with haematological malignancies (Blijlevens *et al.*, 2004). After bone marrow transplantation, the decrease in citrullinemia appears to be a risk factor for infections (predominantly septicaemia and intestinal infections) (Blijlevens *et al.*, 2004). This suggests that, during antineoplastic therapies, citrullinemia could be used to monitor the intestinal mucosa toxicity. Mucositis and epitheliitis can be treated or prevented in part by keratinocyte growth factor. In a mouse model, recombinant human keratinocyte growth factor treatment allowed maintenance of the citrulline level at a normal value during chemotherapy (Lutgens *et al.*, 2003; Lutgens *et al.*, 2004). Acute radiation enteritis induced by total body irradiation or fractioned localized irradiation can be monitored by citrullinemia that correlates to dose received and volume of bowel in the field of radiation (Lutgens *et al.*, 2003; Lutgens *et al.*, 2004; Vanclée *et al.*, 2005).

Fourteen studies were used in meta-analysis in this category which included cases of patients who had received chemoradiation for bone marrow transplants, cancer or other malignant disorder (Table 2.5). The most common theme that these papers employ is the use of citrulline levels to diagnose and monitor gastrointestinal toxicity related to treatment or mucositis. Generally, most papers described that:

1. Citrulline decreases in the initial phase of treatment and then increases while the initial toxicity related to treatment seem to reside.
2. Gastrointestinal toxicity involves diarrhoea, pain, vomiting and mucositis.

3. Citrulline decrease is related to higher doses of chemoradiation treatment and is usually inversely correlated with severity of gastrointestinal toxicity. Meta-analysis was performed on this outcome since 14 studies were recognised.

The random effects model showed that citrulline levels were negatively correlated with severity of gastrointestinal toxicity with a moderate correlation of -0.41 (95% CI [-0.52, -0.29]) (Figure 2.13); there was no heterogeneity (Q = 24.4, df = 13, p = 0.03; I^2 = 47%, τ^2 = 0.03) and no publication bias (symmetric funnel plot, Egger's test p = 0.009, Begg's test p = 0.102) (Figure 2.9F).

2.3.5 Critical Illness Patients

Twenty five studies were diagnosed investigating citrulline levels in patients with critical illness (Table 2.6). The majority of studies involve patients in intensive care unit (ICU) settings which attempt to correlate citrulline levels with severity of condition or other sepsis markers. No meta-analyses were performed on these studies due to different study designs with heterogeneous patient populations not allowing data extraction. The following comments can be made:

1. Citrulline appears decreased in most studies and is related to critical illness and markers of sepsis or inflammation.
2. This decrease in citrulline doesn't necessarily mean that there is intestinal dysfunction since in inflammatory responses as severe as critical illness, nitric oxide and arginine are depleted through inflammatory pathways hence leading to the reduction of citrulline (Luiking *et al.*, 2009). This is also corroborated by the fact that citrulline levels increase once critical condition is overcome.
3. Citrulline seems to act as a negative inflammatory marker as is albumin in inflammatory or septic conditions. Its use as a marker of intestinal compromise should be cautious.

2.3.6 Citrulline Levels: An Overall Assessment

2.3.6.1 Diagnostic Accuracy

In this section a meta-analysis of citrulline's diagnostic accuracy is performed in all patient groups (26 studies) with hierarchical summary ROC analysis (Harbord *et al.*, 2007). The overall sensitivity of citrulline levels appeared to be satisfactory

80% (95% CI 69%-87%) (Figure 2.11; Table 2.10); specificity was 84% (95% CI 77%-89%) (Figure 2.11); and the diagnostic odds ratio was 20.03. The sROC indicated overall satisfactory diagnostic accuracy of citrulline levels (Figure 2.14).

2.3.6.2 Citrulline Levels in Diseased Patients versus Controls

The random effects model showed that citrulline levels in patients versus controls (30 studies) was decreased by -11.2 μmol/L (95% CI [-13.8, -8.6]) (SMD -0.53, 95% CI [-0.69, -0.36]); there was no heterogeneity (MD: Q = 56.07, df = 29, p = 0.002; I^2 = 48.3%, τ^2 = 23.77; SMD: Q = 92.64, df = 29, p < 0.001; I^2 = 68.7%, τ^2 = 0.12) (Figure 2.4). No publication bias was observed (symmetric funnel plot, Egger's test p = 0.969, Begg's test p = 0.0.986) (Figure 2.9A).

2.3.6.3 Citrulline Levels as a Marker of Intestinal Disease Severity

Citrulline levels were described in association with disease severity in 28 studies. The random effects model showed that citrulline levels were negatively correlated with severity of disease with a moderate correlation of -0.56 (95% CI [-0.70, -0.37]) (Figure 2.13); there was heterogeneity (Q = 582.68, df = 27, p < 0.001; I^2 = 95%, τ^2 = 0.38) but no publication bias (symmetric funnel plot, Egger's test p = 0.356, Begg's test p = 0.722) (Figure 2.9E). Interestingly, only in Crohn's disease, citrulline is not associated with disease severity (Figure 2.13).

2.3.6.4 Citrulline and Absorptive Function

Fourteen studies reported an association of citrulline levels with the level of enteral absorption. Absorption was assessed with the D-xylose absorption test, oral or enteral nutrition tolerance, and nutrient absorption tests with bomb calorimetry and measuring oral/enteral intake in comparison to faecal and other loses. The random effects model showed that citrulline levels were positively correlated with enteral absorption with a moderate correlation of 0.47 (95% CI [0.27, 0.62]) (Figure 2.15) but there was heterogeneity (Q = 59.26, df = 13, p < 0.001; I^2 = 78%, τ^2 = 0.13).

Overall, there are fewer results concerning the relationship between citrulline and absorptive function. Crenn *et al.* (2000) suggested a positive relationship between citrullinemia and percentage of fat (r = 0.53) and nitrogen (r = 0.47) absorbed. Rhoads *et al.* (2005) determined in children that plasma citrulline concentration enables estimation of the percentage of enteral calories that a short gut can tolerate

without diarrhoea. Luo *et al.* (2007) however, did not find any significant relationship between citrulline and macronutrient (nitrogen, fat, carbohydrate, calories), fluid, and electrolyte (sodium, potassium, phosphorus, magnesium) absorption expressed in percentage of amount ingested in adult short bowel syndrome patients. Thus, it is likely that plasma citrulline concentrations cannot reflect equally the various aspects of gut function. Citrulline reflects the integrity of the intestinal epithelial cells with a predominant site of production at the proximal jejunum, whereas absorption is a complex integrated function related to small bowel mucosa, biliopancreatic secretions, digestive motility, gut lumen, and colonic absorption (Curis *et al.*, 2007; Papadia *et al.*, 2007; Peters *et al.*, 2008b). In addition, the absorption process is variable in capacity and location according to the nutrients considered. Hence, citrulline concentration might be an indicator of the functional enterocyte metabolic mass but less exclusively of the digestive and absorptive function.

2.4 Summary of Findings, Discussion, and Conclusion

The present study is the first meta-analysis on the association of citrulline with gut function. Although citrulline appears to be a strong marker of enterocyte mass, its correlation with intestinal absorption is weaker. A systematic review of studies was performed along with a statistical meta-analysis. Overall plasma citrulline measurements have been used in necrotizing enterocolitis, intestinal transplantation, short bowel syndrome, enteropathies (villous atrophy syndromes, Crohn's disease, and mucositis) and critical illness patients. Citrulline was strongly positively correlated with enterocyte mass. This correlation was clinically significant in short bowel syndrome. In other conditions where short bowel is not an issue, there is a decrease in average citrulline levels compared to healthy controls and citrulline decrease can be correlated to the degree of disease severity apart from Crohn's disease. Its interpretation should be cautious because its diagnostic accuracy is satisfactory but doesn't exclude absolutely false negative or false positive cases. Factors that influence interpretation are lower population values, combination of intestinal disorders, possible bowel adaptation, measurement timing, inflammation and extent of disease. Citrulline was correlated positively with enteral absorption, albeit moderately. Although a higher plasma citrulline level suggests a more absorptive gut, this interpretation needs to take into consideration other factors such

specific macronutrients, condition examined, extent of mucosal disease etc. A cut-off citrulline level at 20 μmol/L has sensitivity and specificity of 80% in all conditions. Limitations of the meta-analysis was presence of heterogeneity and the possibility of publication bias, detection bias and confounding bias.

Hence, returning to the questions posed in the introduction:

1) Is citrulline a successful indicator of enterocyte mass?

Yes, citrulline was strongly positively correlated with enterocyte mass. This correlation was clinically significant in short bowel syndrome. In other conditions where short bowel isn't an issue, there is a decrease in average citrulline levels compared to healthy controls and citrulline decrease can be correlated to the degree of disease severity apart from Crohn's disease. Its interpretation should be cautious because its diagnostic accuracy is satisfactory but doesn't exclude absolutely false negative or false positive cases. Factors that influence interpretation are lower population values, combination of intestinal disorders, possible bowel adaptation, measurement timing, inflammation and extent of disease.

2) Is citrulline a successful marker of enterocyte absorption?

Yes, citrulline was correlated positively with enteral absorption, albeit moderately. Although a higher plasma citrulline level suggests a more absorptive gut, this interpretation needs to take into consideration other factors such specific macronutrients, condition examined, extent of mucosal disease etc.

3) What clinical conditions has citrulline been utilized in as a marker?

Citrulline has been examined in short bowel syndrome, intestinal transplant, coeliac disease, Crohn's disease, other enteropathies, necrotizing enterocolitis and critical illness. In all conditions except critical illness, the gut is compromised and in this case a decrease in citrulline seems to be attributed mainly to this. However, in critical illness the interpretation of a low citrulline as a marker of intestinal dysfunction should be treated with caution – in a similar manner that a low albumin in a critical ill patients needs to be cautiously interpreted as malnutrition. The availability of nitric oxide and arginine during septic and inflammatory states is decreased hence decreasing citrulline and in this context citrulline could be a negative inflammatory marker – without of course excluding the possibility of acute illness enteropathy. The mechanism of the enteropathy in this condition would be a

combination of inflammatory and possibly infective triggers leading to colitis or malabsorption and related sequelae.

4) Can a new theory regarding citrulline's utility be suggested?

I am going to advocate that this can be done fairly confidently. The synthesis of literature has shown that citrulline concentration is decreased compared to controls in circumstances of intestinal compromise; it has a sensitivity and specificity of 80% in all conditions; it is negatively correlated with disease severity in intestinal enteropathies (except Crohn's disease); it is positively correlated with small bowel length in short bowel syndrome; and it is moderately correlated with enteral absorption in various conditions. I would like to introduce the term ***likely acute gut failure (similar to intestinal insufficiency)*** at this point and hence propose that interpretation of citrulline levels leads to diagnoses of possible acute gut failure.

Limitations of the present meta-analysis stem from various sources of heterogeneity and possibility of publication bias, detection bias and confounding bias. It was a pattern in the present review that many studies didn't analyse confounding factors such as other amino acids, renal function (citrulline's pathways involve a renal component) and inflammatory state. Hence, for future studies it is suggested that nitric oxide, full amino acid profiles (or at least arginine, glutamine and ornithine) are measured when citrulline is measured, renal function and inflammatory state.

Heterogeneity was a frequent observation in this study and the source was not always found. One needs to take into account different measuring methods for citrulline (IEC, HPLC, TMS, ELISA) which were used, sample preparation, population parameters, different scales for documenting disease severity, absorption, small bowel length and so on. Each biological method and clinical assessment scale has inherent variability which is added up when a meta-analysis is performed and this leads to heterogeneity. The random effects models performed takes heterogeneity into account and thus the results presented are less burdened from it when interpreted – meaning that when significant results are reported with meta-analysis despite increased heterogeneity, this is indicative of a truly strong effect.

Another interesting addition of this study is the study of diagnostic accuracy for citrulline. There were various thresholds for discerning a high from low citrulline level but the level of 20 μmol/L seems to be most prevalent. It also indicates that

under these levels, clinical alarms need to be triggered – taking into account the confounding factors of course.

In the next chapter, the clinical experiments performed at UCL for investigating citrulline physiology in a pharmacodynamics study are described.

2.5 Tables

Table 2.1 Necrotizing enterocolitis studies.

No	Authors	Settings (sample and design)	Main Results
1	Becker *et al.* (2000)	• Aim: to determine whether premature infants who have necrotizing enterocolitis have deficiencies in glutamine and arginine • 4-month prospective cohort study of serum amino acid and urea levels in premature infants • Serum amino acid and urea levels were measured by high-pressure liquid chromatography and enzymatic methods • Control (n = 32), necrotizing enterocolitis (n = 13) (comparable for birth weight, gestational age, and Apgar scores)	• Days 7, 14, 21: Median values of glutamine: 37 % to 57 % lower in the necrotizing enterocolitis group compared to control group ($p < 0.05$) • Days 7 and 14: Median values of arginine, glutamine, alanine, lysine, ornithine, and threonine were decreased by 36 % to 67 % ($p < 0.05$) in the necrotizing enterocolitis group • Citrulline levels were decreased in the necrotizing enterocolitis group compared to control ($p < 0.05$)
2	Ioannou *et al.* (2012)	• Plasma citrulline levels were measured prospectively in 17 preterm neonates with necrotizing enterocolitis stage II during the entire course of the disease • Serial citrulline determinations in 24 healthy preterm neonates on 2, 7, 14, 21 and 28 days of life, served as reference values	• In healthy preterm neonates plasma citrulline levels showed a progressive increase in relation to age • In neonates presenting with necrotizing enterocolitis, mean citrulline levels were significantly lower as compared to controls' citrulline levels (day of life 7: 16.85 ± 4.2 vs 20.5 ± 4.5 μmol/L, $p < 0.05$; day of life 14: 18 ± 4.2 vs 23.5 ± 4.3 μmol/L, $p < 0.01$; day of life 21: 17 ± 2.5 vs 30 ± 5.7 μmol/L, $p < 0.01$) • Optimal citrulline cut-off distinguishing necrotizing enterocolitis patient from controls: 17.75 μmol/L (sensitivity 76%, specificity 87%) • Plasma citrulline at presentation correlated inversely with the duration of parenteral nutrition ($r=-0.49$, $p<0.05$)
3	Celik *et al.* (2013)	• Plasma citrulline levels of neonates with a gestational age less than 32 weeks and ≤ 1500 g who developed necrotizing enterocolitis stage II/III were measured by high-performance liquid chromatography • 36 preterm infants including 20 with necrotizing enterocolitis and 16 controls	• Median citrulline levels of necrotizing enterocolitis and control groups were 8.6 and 20.18 μmol/L ($p < 0.05$), and cut-off level of citrulline was 13.15 μmol/L with a sensitivity of 80% and a specificity of 82% • Median arginine levels of necrotizing enterocolitis and control groups were 22.02 and 39.89 μmol/L ($p < 0.05$), and cut-off level of arginine was 28.52 μmol/L with a sensitivity of 70% and a specificity of 75% • Blood sampling day, gender, and parenteral or enteral nutrition did not affect amino acid levels
4	Englund *et al.* (2014)	• Aim: To determine whether citrulline concentrations measured in neonatal dried blood spots could predict necrotizing enterocolitis • National Danish registries were retrospectively searched to identify 361 babies born between 2003 and 2009, diagnosed with necrotizing enterocolitis and a valid citrulline concentration • Control group: 1083 healthy new-borns (three controls for every new-born with necrotizing enterocolitis, matched for birthweight and gestational age)	• Neonatal dried blood spots were collected between 2 and 21 days of life, with a median of 8 days • Necrotizing enterocolitis was not associated with low citrulline concentration ($p = 0.73$)

Table 2.2 Intestinal transplantation studies.

No	Authors	Settings (sample and design)	Main Results
1	Pappas *et al.* (2001)	• Aim: To investigate impact of acute cellular rejection of intestinal allografts on serum citrulline levels • Sample: healthy volunteers (n = 6), patients who underwent small bowel transplant (n = 7) • Concurrent measurement of serum citrulline levels with characterization of acute cellular rejection • Rejection confirmed by biopsy and graded by standardized criteria	• Controls vs post-transplantation samples: significantly higher mean citrulline concentrations at any rejection grade • Mean concentrations declined significantly as rejection severity increased • Statistically significant overall downward trend ($p < 0.05$) • In sequential measurements, citrulline levels increased significantly over time with declining severity of rejection • Significant increase in mean citrulline concentrations between post-transplant days 3-16 and 52-60 ($p < 0.01$)
2	Gondolesi *et al.* (2002)	• Aim: To investigate impact of acute cellular rejection of intestinal allografts on serum citrulline levels • Concurrent measurement of plasma citrulline levels with histopathological diagnosis from biopsy [acute cellular rejection (normal, indeterminate, mild, or moderate), viral enteritis (cytomegalovirus or adenovirus), and for other miscellaneous histological diagnoses • Sample: 9 consecutive intestinal transplant recipients • Thirty-two citrulline measurements	• Mean citrulline levels o overall: 17.5 ± 13.3 μmol/L (range, 0.8 to 68 μmol/L) o normal biopsies:26 ± 15.7 μmol/L o indeterminate biopsies: 11.9 ± 7.7 μmol/L o mild rejection: 15.4 ± 7.5 μmol/L o moderate rejection: 5.5 ± 0.7 μmol/L o viral enteritis: 9.3 ± 5.85 μmol/L o functional bowel biopsies (n = 22) vs dysfunctional bowel biopsies (n = 10): 19.3 ± 13.6 μmol/L vs 7.8 ± 4.7 μmol/L; p = 0.0001 • Pearson correlation coefficient between citrulline levels and rejection: -0.425 ($p = 0.05$) • Spearman's rho correlation coefficient between citrulline levels and rejection: -0.52 ($p < 0.01$)
3	Pappas *et al.* (2002)	• Aim: To investigate impact of rejection of intestinal allografts on serum citrulline levels • 10 pre-transplant samples, 11 control specimens, 49 post-transplant samples from 7 patients along with 1 pre-transplant serum sample from each patient and 6 samples from healthy controls, 83 sequential serum samples from 11 patients (5 children, 6 adults), median follow-up 26 days • All samples obtained within 3 days of biopsy	• Pre-transplant specimens vs healthy controls: significant difference in mean citrulline ($p < 0.01$) • Mean citrulline levels declined significantly with increasing acute cellular rejection in post-transplant period • Mean citrulline levels: pre-transplant: 20.1 ± 10.3 μmol/L vs control: 40.0 ± 7.3 μmol/L ($p < 0.01$)
4	Gondolesi *et al.* (2004)	• Aim: To analyse plasma citrulline in intestinal transplant recipients without rejection or other histological abnormalities • Sample: 40 patients • Plasma citrulline measured with high performance liquid chromatography Beckman amino acid analyser (within 24 h of protocol or clinically indicated endoscopic biopsy procured > 6 and < 360 days post-transplant) • Measurements included for analysis corresponded to normal (or minimally abnormal) biopsies that remained so for 7 days • Criteria met in: 145 samples, 10 adults and 14 children	• Mean citrulline levels: o overall: 34.0 ± 19.9 μmol/L o between 6 and 30 days post-transplant: 22.2 ± 13.2 μmol/L o between 30 and 60 days post-transplant: 34.9 ± 17.2 μmol/L ($p = 0.001$) o between 60 and 90 days post-transplant: 43.6 ± 15.8 μmol/L ($p = 0.001$) o stable until end of first year • Plasma citrulline lower in 13 patients with body surface area ≤ 1 m^2 vs 11 patients with body surface area ≥ 1.1 m^2 ($p = 0.0001$) • Plasma citrulline increased linearly during first 120 days in both body surface area groups ($r = 0.573$, $r = 0.512$; $p = 0.0001$)

Table 2.2 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
5	Pappas *et al.* (2004a)	• Aim: To compare serum citrulline concentrations with biopsy-based grades of rejection • Sample: 26 isolated intestinal and multivisceral transplant recipients • Other factors recorded: patient and donor age and sex, ischaemia time, serum creatinine, type of transplant. • Straight-line fitting of citrulline levels over time (stepwise linear regression)	• Median time-to-achieve normal citrulline (≥ 30 μmol/L): 79 days post-transplant (n=21) • Significantly higher maximum grade of rejection after 14 days post-transplant linked to longer time-to-achieve normal citrulline ($p < 0.00001$) and not receiving a multivisceral transplant ($p = 0.0005$) • Normalization of citrulline levels did not occur in some cases with moderate-to-severe rejection
6	Pappas *et al.* (2004b)	• Aim: To compare serum citrulline concentrations with biopsy-based grades of rejection • Sample: 26 isolated intestinal and multivisceral transplant recipients • Serum citrulline concentrations determined by ion exchange chromatography and compared to biopsy-based grade of acute cellular rejection. • Other factors recorded: patient and donor age and sex, ischemia time, serum creatinine • Straight-line fitting of citrulline levels over time (stepwise linear regression)	• Time to achieve normal citrulline (>30 μmol/L): 1-730 days post-transplant (n = 21) with increasing citrulline levels over time • Longer time-to-achieve normal citrulline: independent predictor of maximum acute cellular rejection ($p < 0.0001$) and average acute cellular rejection ($p = 0.0059$) 14 days post-transplant.
7	Yu *et al.* (2005)	• Aim: To investigate correlation between plasma and dried blood spot specimen citrulline concentrations after intestinal transplantation • Plasma and dried blood spot samples were analysed by hydrophilic interaction chromatography tandem mass spectrometry • Correlation analysed by type of surgery, sonication time, dried blood spot citrulline levels, the time interval between the blood sample collection and assay date	• Very strong linear correlation between the plasma and dried blood spot citrulline concentrations ($r = 0.87$, $p < 0.001$) • Correlation was maintained when evaluating only intestinal transplant recipients • Sonication time, citrulline concentrations, length of time to assay date: no effect on strength of correlation ($p > 0.05$)

8	David *et al.* (2006)	• Aim: To determine whether serum citrulline level within 30 days of acute rejection could predict rejection episode
		• Comparison of mean citrulline level determined within 30 days of the start of an acute rejection episode against mean citrulline level during a rejection-free period
		• Sample: 22 patients who experienced 37 episodes
			• Mean serum citrulline levels:
			  ○ Mild rejection (12 episodes): 15.0 ± 2.3 μmol/L (prior) vs 18.8 ± 2.4 μmol/L (rejection-free periods) ($p = 0.17$)
			  ○ Moderate to severe rejection (25 episodes): 12.4 ± 1.1 μmol/L (prior) vs 18.8 ± 2.0 μmol/L (rejection-free periods) ($p = 0.002$)

9	Gondolesi *et al.* (2006)	• Aim: To determine sensitivity and specificity of plasma citrulline as diagnostic tool for allograft injury • 403 citrulline samples within 24 h of intestinal biopsy in 49 patients • Correlation of citrulline with mucosal damage and histopathological diagnoses	• Significant mucosal damage vs intestines with no or mild injury: plasma citrulline 22.9 ± 15.4 vs 38 ± 23.2 μmol/L ($p < 0.0001$) • Sensitivity and specificity of the test were 80% and 58.1% for rejection, and 56.5% and 66% for viral enteritis
10	David *et al.* (2007)	• Aim: to determine citrulline cut-off levels for diagnosis of acute rejection and predictors of citrulline levels post-transplant • Dried blood spot citrulline samples from 57 intestinal transplant recipients at or beyond 3 months post-transplant • Stepwise linear regression was performed to determine significant predictors patients' citrulline levels	• Seven significant predictors of lower citrulline levels were identified: presence of mild, moderate, or severe acute cellular rejection, presence of bacteraemia or respiratory infection; paediatric age; and time from transplant to sample ($p < 0.00001$) • A cut-off level citrulline 13 μmol/L had high sensitivity for detecting moderate or severe acute cellular rejection negative predictive value were high (96.4%, 99%, respectively). Specificity was 54% to 74% in children and 83% to 88% in adults.

Table 2.2 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
11	David *et al.* (2008)	• Aim: To determine the significant value of citrulline level in the post-transplant setting, which would correlate with complications of rejection and infection • 2,135 dried blood spot citrulline samples were obtained from 57 small intestine transplant recipients three months or more after post-transplant	• A cut-off level citrulline 13 μmol/L had high sensitivity for detecting moderate or severe acute cellular rejection (96.4%) • Specificity was high (54%-74% in children and 83%-88% in adults), and the negative predictive value was >99%
12	Ruiz *et al.* (2010)	• Aim: To evaluate the correlation of plasma citrulline and rejection episodes in intestinal transplantation • From January 2007 until present, citrulline was measured from small bowel patients and examined for correlation with rejection status of the graft as defined by graft biopsies • 5195 citrulline samples were analysed	• Average serum citrulline levels decreased significantly when patients presented a rejection episode o No rejection: 17.38 μmol/L o mild rejection, 13.05 μmol/L o moderate rejection, 7.98 μmol/L o severe rejection, 6.05 μmol/L
13	Hibi *et al.* (2012)	• Aim: To investigate the association between citrulline levels acute cellular rejection to identify a cut-off point of citrulline that predicts acute cellular rejection beyond 3 months postransplant in the paediatric patient population. • 13,499 citrulline samples were prospectively collected from 111 consecutive paediatric intestinal/multivisceral transplant recipients. 2,155 were obtained concurrently with intestinal biopsies (1995-2011) • 185 acute cellular rejection episodes observed among 74/111 patients (median follow-up: 4.4 years).	• Citrulline levels were inversely proportional to the severity of acute cellular rejection. • Negative predictive values for any type of acute cellular rejection (cut-off, 20 μmol/L) and moderate/severe acute cellular rejection (cut-off, 10 μmol/L) were 95% and 99%, respectively. • When patients were divided according to graft size, diagnostic accuracy using the same cut-off was identical. • Subgroup analysis by the timing of citrulline measurement prior to biopsy varying from 1 to 7 days demonstrated comparable results.

Table 2.3 Short bowel syndrome studies.

No	Authors	Settings (sample and design)	Main Results
1	Crenn *et al.* (1998); Crenn *et al.* (2000)	• 57 patients post-absorptive citrulline concentration was measured and parenteral nutrition dependence was used to define permanent (n = 37) and transient (n = 20) intestinal failure • Absorptive function, studied over a 3-day period, was evaluated by net digestive absorption for protein and fat • Relations between quantitative values were assessed by linear regression analysis and cut-off citrulline threshold, for a diagnosis of intestinal failure by linear discriminant analysis	• Short bowel syndrome vs controls (n = 51): 20 ± 13 vs. 40 ± 10 μmol/L ($p < 0.001$) • Citrulline levels were correlated to small bowel length ($p < 0.0001$, $r = 0.86$) and to net digestive absorption of fat, but not to body mass index and creatinine clearance • A cut-off level of 20 μmol/L classified short bowel patients with permanent intestinal failure with high sensitivity (92%), specificity (90%), positive predictive value (95%), and negative value (86%) and was a more reliable indicator (odds ratio 20.0, 95% CI 1.9-206.1) than anatomic variables (odds ratio 2.9, 95% CI 0.5-15.8) to separate transient from permanent intestinal failure
2	Pita *et al.* (2003); Pita *et al.* (2004)	• Sample: 13 short bowel syndrome patients (7 men; 60.2 ± 15.2 years) • Groups according to remnant bowel length (Group A: 61-150 cm, n =6; Group B: > 60 cm, n =7) • Plasma urea-cycle amino acids, ammonium and urinary orotic acid were determined	• Regarding citrulline, Group B levels were significantly lower vs controls ($p < 0.001$) • Comparisons between patient groups showed higher arginine in Group A ($p < 0.05$) and non-statistically lower citrulline in Group B

3	Kábrt *et al.* (2003)	• Sample: adult patients with short bowel syndrome (n = 20)
		  ○ 10 on long-term parenteral nutrition
		  ○ 10 not on parenteral nutrition
		  ○ Controls: 9 normal subjects
		• Nutritional assessment with anthropometry and laboratory parameters
		• Post-absorptive plasma concentrations of amino acids determined by ion exchange chromatography
			• Total amino acids and non-essential amino acids were same in all groups.
			• Essential amino acid/non-essential amino acid and branched-chain amino acid/total amino acid ratios were significantly lower in the short bowel syndrome patient group than in the normal controls.
			• Concentration of citrulline was significantly lower only in the group of short bowel syndrome patients who had to remain on total parenteral nutrition.

4	Gong *et al.* (2005, 2007)	• Aim: To investigate the significance of serum citrulline in evaluating the remnant small bowel enterocytes mass and absorptive function in short bowel patients • Serum citrulline concentrations were determined using high-performance liquid chromatography in 22 short bowel syndrome patients and 33 healthy controls • Five-hour urine D-xylose excretion and digestive protein absorption were measured using high-performance liquid chromatography and micro-Kjeldahl method	• Serum citrulline levels were significantly lower in short bowel syndrome patients compared with healthy controls • In short bowel syndrome patients, citrulline correlated with remnant small bowel length ($r = 0.82$, $p < 0.001$), surface area ($r = 0.86$, $p < 0.001$), 5-h urine D-xylose excretion ($r = 0.56$, $p = 0.007$), and digestive protein absorption ($r = 0.48$, $p = 0.046$). • Citrulline level in six patients receiving rehabilitation therapy correlated with intestinal protein absorption ($r = 0.79$, $p = 0.063$) and urine D-xylose excretion ($r = 0.81$, $p = 0.053$).
5	Rhoads *et al.* (2005)	• Aim: To determine whether serum citrulline levels correlate with total parenteral nutrition independence in children with short bowel syndrome • Study design: serum amino acid profiles over a 24-month interval from all infants with short bowel syndrome 3 weeks to 4 years of age. • Remaining small intestine length was recorded at surgery, and % of enteral calories tolerated was determined in 24 infants with short bowel syndrome and 21 age-matched controls	• In patients with short bowel syndrome, serum citrulline correlated linearly with tolerated enteral calories ($r = 0.85$, $p < 0.001$) and bowel length ($r = 0.47$, $p < 0.03$) • A citrulline cut-off level of19 μmol/L had 94% sensitivity and 67% parenteral nutrition independence. • Mean citrulline levels: • short bowel syndrome weaned off parenteral nutrition:30 ± 2 μmol/L • short bowel syndrome subsequently weaned off parenteral nutrition: 20 ± 2 μmol/L • short bowel syndrome parenteral nutrition: 11 ± 2 μmol/L • Controls: 31 ± 2 μmol/L

Table 2.3 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
6	Luo *et al.* (2007)	• Aim: To examine whether plasma citrulline and glutamine concentrations are biomarkers of residual small intestinal length and nutrient absorptive functions in adult short bowel syndrome patients • Sample: 24 patients on parenteral nutrition in a double-blind, randomized trial of individualized dietary modification ± recombinant human growth hormone • intestinal absorption studies and plasma measurements of citrulline and glutamine were performed	• Residual small bowel length was positively correlated with baseline plasma citrulline ($r = 0.467$, $p = 0.028$) • No significant correlations between absolute citrulline and glutamine concentrations and the percent absorption of nutrient substrates at any time point were observed. • No correlation between the change in citrulline and glutamine concentration and the change in % nutrient absorption was observed
7	Nion-Larmurier *et al.* (2007)	• Twenty-three patients who had a bowel resection and a provisional ileostomy were studied in the month before and months after recovery • Basal citrulline levels were measured before and after restoration of continuity on 16 operated patients and prospectively in 7.	• Citrulline levels (mean ± SD) before recovery were 20.9 ± 8.6 μmol/L (n = 23) • Citrulline levels correlated to the length of bowel length ($r = 0.83$; $p = 0.002$)
8	Papadia *et al.* (2006a); Papadia *et al.* (2006b); Papadia *et al.* (2007)	• Sample: (a) Crohn's disease with massive small bowel resection leaving < 50 cm (n = 6), (b) Crohn's disease with 50-150 cm remaining (n = 9), (c) Crohn's disease with no resection but active inflammation (n = 7), (d) Crohn's disease without resection or active inflammation (n = 9), (e) mesenteric infarction with resection leaving < 50 cm (n = 6), (f) mesenteric infarction leaving 50-150 cm (n= 6), (g) active coeliac disease (n = 6), (h) healthy volunteers (n = 6). • Post-absorptive fasting plasma citrulline was measured using reverse-phase, high performance liquid chromatography. Absorptive capacity and permeability were also measured after oral sugar-mix ingestion	• Plasma citrulline strongly correlated with small bowel length ($p < 0.0001$) and xylose absorption ($p < 0.001$) • No correlation was found with C-reactive protein, permeability, albumin, sedimentation rate, white cell count, or platelet count. • Citrulline levels in Crohn's disease and mesenteric infarction with small bowel length 50-150 cm vs less than 50 cm: 21.0 vs 9.2 μmol/L ($p < 0.0004$), respectively

9	Peters *et al.* (2007a, 2007b); Peters *et al.* (2007c); Peters *et al.* (2008b)	• Aim: to explore diagnostic value of fasting citrulline concentrations to detect decreased intestinal energy absorption in patients with recently diagnosed coeliac disease (n=15), refractory coeliac disease (n = 9)and short bowel syndrome (n = 16)
		• Fasting plasma citrulline concentrations were determined by high performance liquid chromatography in 40 adult patients and 21 healthy subjects.
		• Intestinal energy absorption capacity using bomb calorimetry was determined
			• Mean citrulline levels:
			  ○ Refractory celiac disease vs healthy subjects: 28.5 ± 9.9 vs 38.1 ± 8.0 μmol/L, $p < 0.05$
			  ○ Coeliac disease vs healthy subjects 28.5 ± 9.9 vs 38.1 ± 6.4 μmol/L, $p < 0.05$
			• Mean intestinal energy absorption capacity:
			  ○ Short bowel syndrome patients vs healthy subjects: 64.3 ± 18.2 vs 90.3 ± 3.5%, $p < 0.001$
			  ○ Refractory celiac disease vs healthy subjects: 64.3 ± 18.2 vs 82.3 ± 11.7%, $p < 0.01$
			  ○ Coeliac disease vs healthy subjects 64.3 ± 18.2 vs 89.2 ± 3.4%, $p < 0.001$
			• No relation was observed between fasting plasma citrulline concentration and intestinal energy absorption capacity ($r=0.09$, $P=0.56$, area under the ROC curve 0.50)

Table 2.3 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
10	Parekh *et al.* (2008)	• Sample: 49 healthy controls with an intact gastrointestinal tract and no known metabolic or digestive diseases and 30 short bowel syndrome (< 200cm small bowel) patients dependent on parenteral • Venous post-absorptive plasma amino acid concentrations were measured in all subjects after an 8 hour fast	• Mean citrulline levels: Short bowel syndrome patients vs healthy controls: 21.4 vs 33.2 μmol/L p = 0.0002 • Area under the ROC curve was 0.82 (95% CI: 0.71, 0.93) and a citrulline cut-off of 20 μmol/L had 100% specificity and 56.6% sensitivity. • Citrulline increased by 1.65 μmol/L with every 5 year increase in age (p = 0.044) • Citrulline increased by 4.9 μmol/L for every 50cm increase in small bowel length (p = 0.018) • Citrulline decreased by 9 μmol/L for every 1000 kcal/day increase in parenteral nutrition (p < 0.0001)
13	Santarpia *et al.* (2008)	• Sample: 25 patients with short bowel syndrome after at least 18 months since last digestive circuit modification; 24 of them were again evaluated 1 year later. • Ten patients were weaned off parenteral nutrition and 15 were dependent on parenteral nutrition. • Fifty-four healthy volunteers (28 women and 26 men) served as controls.	• Five amino acids (citrulline, leucine, isoleucine, valine and tyrosine) were significantly lower in all short bowel syndrome patients versus controls, whereas glutamine was significantly higher. • Only serum citrulline measured was significantly related to small bowel length.
14	Bailly-Botuha *et al.* (2009)	• Prospective plasma citrulline assays were performed in 31 children with short bowel syndrome • Median age was 16 months and median follow-up was 14 months	• Plasma citrulline at inclusion showed a positive correlation with residual short bowel length. • Follow-up values correlated negatively with intestinal failure severity. • Plasma citrulline increased over time during or after weaning from parenteral nutrition (from 15.8 ± 11.5 μmol/L to 19.3 ± 3.8 μmol/L) but remained stable and low in patients who continued on parenteral nutrition (6.5 ± 3.0 μmol/L at inclusion and 7.7 ± 6.0 μmol/L at last follow-up).
15	Fitzgibbons *et al.* (2009)	• Aim: To evaluate the relationship between citrulline and parenteral nutrition independence in children with short bowel syndrome • Sample: Retrospective review of all patients in a multidisciplinary paediatric intestinal rehabilitation clinic with a recorded citrulline between January 2005 and December 2007 (n = 27)	• Citrulline levels correlated positively with bowel length (r = 0.73; p < 0.0001) and were a strong predictor of parenteral nutrition independence (p = 0.002; area under the ROC curve = 0.88; 95% CI 0.75-1.00). • Optimal citrulline cut-off point distinguishing patients who reached parenteral nutrition independence was 15 μmol/L (sensitivity = 89%; specificity = 78%).
16	Gong *et al.* (2009)	• Aim: To evaluate long-term clinical significance of enteral nutrition in weaning adult short bowel patients off parenteral nutrition undergoing intestinal rehabilitation therapy • Sample: 61 adult patients with small bowel length 47.95 ± 19.37 cm were retrospectively analysed	• Nutritional and anthropometric parameters, urine 5-hr D-xylose excretion and serum citrulline levels all increased significantly after intestinal rehabilitation therapy and on follow-up compared with baseline
17	Picot *et al.* (2010)	• Twenty-six patients with small bowel disruption and double enterostomy were treated with chyme reperfusion • Faecal wet weight, nitrogen and fat absorption, parenteral nutrition delivery and citrulline were measured before and after the initiation of chyme reperfusion with a median follow-up of 30 days.	• Chyme reperfusion decreased the intestinal wet weight output and parenteral nutrition dependence • Chyme reperfusion was associated with a rise in net nitrogen and fat digestive absorption and citrulline (17.0 ± 10.0 vs 31.0 ± 12.0 μmol/L, p = 0.0001). • Before the initiation of chyme reperfusion, citrulline levels correlated positively with the absorptive post-duodenal small bowel length (r = 0.39, p = 0.04), but not with the total post-duodenal small bowel length (r = 0.11, P = 0.60).

Table 2.3 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
18	Noto *et al.* (2008a); Noto *et al.* (2008b); Diamanti *et al.* (2010); Diamanti *et al.* (2011b)	• Sample: Between March 2005 and March 2010, 28 short bowel syndrome patients on parenteral nutrition • Citrulline levels and enteral intake determinations were measured on inclusion and at 6-month intervals	• Citrulline significantly correlated with the residual duodenum-jejunum length ($r^2 = 0.22$, $p = 0.0113$) and with enteral intake ($r^2 = 0.20$, $p = 0.016$, $r^2 = 0.48$, $p = 0.0001$) • Baseline citrulline over 10 μmol/L and a longitudinal increase >25% provided a weak association with bowel adaptation (likelihood ratios 2.6 and 2.4, respectively), unlike residual small bowel length ≥ 20 cm and the presence of > 50% of the colon.
19	Khan *et al.* (2011)	• Sample: Serum citrulline was measured in 19 subjects with short bowel syndrome; 10 females and 17 were on parenteral nutrition • Age: 7 months to 21 years; Bowel length: 5 to 150 cm, and percentage of parenteral nutrition providing 0-100% of caloric intake.	• Citrulline levels decreased with increased parenteral nutrition intake (r = 0.69) • Citrulline levels correlated with bowel length (r = 0.73)
20	Pironi *et al.* (2005); Pironi *et al.* (2011); Pironi *et al.* (2012)	• Sample: Nineteen healthy subjects and 93 short bowel syndrome patients were studied, 67 on home parenteral nutrition and 26 stable on oral diet	• Mean citrulline levels: • Healthy subjects: 37 μmol/L • Short bowel syndrome patients on oral diet: 33 μmol/L • Short bowel syndrome patients on home parenteral nutrition 20 μmol/L ($p < 0.001$). • Citrulline cut-off of 14 μmol/L had sensitivity 49%, specificity 100%, $p < 0.001$; for distinguishing between short bowel syndrome on parenteral nutrition vs oral diet
21	Raphael *et al.* (2011)	• Design: Open-labelled pilot study in a limited access program for cisapride. • Indications were short bowel syndrome with underlying dysmotility and difficulty advancing enteral feeds despite standard therapies and without evidence of anatomic obstruction. • Patients received cisapride 0.1 to 0.2 mg/kg per dose for 3 to 4 doses per day.	• Ten patients were enrolled in a multidisciplinary paediatric intestinal rehabilitation program. • Median (IQR) residual bowel length was 102 (85-130) cm. Median (IQR) citrulline level was 14.5 (10.5-31.3) μmol/L. • Seven patients improved in enteral tolerance during treatment and 2 were weaned completely from parenteral nutrition.
22	Suzuki *et al.* (2012)	• Design: To measure plasma citrulline in six patients with intestinal dysfunction who were in the acute and chronic phase for more than 6 months.	• Four patients out of six could be withdrawn from total parenteral nutrition, and their plasma citrulline level increased up to 15 μmol/L • Two patients, who could not be withdrawn from parenteral nutrition, showed very low levels of plasma citrulline throughout the treatment course (under 15 μmol/L)
23	Amiot *et al.* (2013)	• Sample: 268 non-malignant short bowel syndrome patients • Home parenteral nutrition dependence and survival rate were studied with univariate and multivariate analysis.	• Home parenteral nutrition dependence was significantly decreased with an early (<6 months) plasma citrulline concentration >20 μmol/l, a remaining colon >57% and a remnant small bowel length >75 cm
24	Pinto Costa *et al.* (2013)	• Sample: Case-control study, 11 patients with short bowel syndrome, 13 patients submitted to malabsorptive bariatric surgery and 11 healthy controls. • Plasma levels of amino acids were determined, before and after a stimulation test with oral L-glutamine, by ion exchange chromatography.	• Citrulline levels were lower in short bowel patients (28.6 ± 11.3 vs 35.5 ± 11 in operated obese vs 32.2 ± 6.6 μmol/L in controls; $p > 0.05$) and lower than 25.5 μmol/L in 54.5% of them • Relative variation of citrulline levels at the 80th minute of test was lower in short bowel patients with high predictive capacity of a short bowel ≤ 50 cm (area under ROC curve = 0.823; $p = 0.038$).

Table 2.3 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
25	Vecino Lopez *et al.* (2013)	• Plasma citrulline concentration was determined by chromatography in 57 patients (age 0.5-18 years) admitted to the Intestinal Rehabilitation Unit with intestinal failure. • Group I: short bowel syndrome totally dependent on parenteral nutrition • Group II: short bowel syndrome under mixed enteral-parenteral nutrition • Group III: Intestinal failure weaned off parenteral nutrition after a rehabilitation period • Group IV: small bowel transplanted patients weaned off parenteral nutrition and on a normal diet	• Mean plasma citrulline levels: o Group I (n = 15): 7.1 ± 4.1 μmol/L o Group II (n = 11): 15.8 ± 8.9 μmol/L o Group III (n = 13): 20.6 ± 7.5 μmol/L o Group IV (n = 25): 28.8 ± 10.1 μmol/L • Values were significantly lower in group I compared to groups II-IV ($p < 0.001$), and in group II compared to groups III-IV ($p < 0.001$). Citrulline was correlated with remnant small bowel length ($r = 0.85$, $p< 0.05$). • In group IV citrulline levels decreased >50% in 3 patients who developed moderate-severe rejection, and in one patient who developed viral enteritis
Teduglutide studies			
1	Buchman *et al.* (2010)	• Design: Subjects with moderate-to-severe Crohn's disease randomized to placebo or 1 of 3 doses of teduglutide (0.05, 0.10, or 0.20 mg/kg daily) (n = 100) • Primary outcome: the percentage of subjects in each group that responded to treatment, defined as a decrease in Crohn's Disease Activity Index score	• Mean baseline Crohn's Disease Activity Index score was 290.8 ± 57.6, similar across groups • Plasma citrulline was similar across groups at baseline, but increased substantially over time in all teduglutide groups when compared with placebo at week 8
2	Jeppesen *et al.* (2011); Seidner *et al.* (2015)	• Sample: 83 patients randomised to receive subcutaneous teduglutide 0.10 mg/kg/day (n = 32), 0.05 mg/kg/day (n = 35) or placebo (n = 16) once daily • Responders were subjects who demonstrated reductions of ≥ 20% in parenteral volumes from baseline at weeks 20 and 24	• Three teduglutide-treated patients were completely weaned off parenteral support. • Villus height, plasma citrulline concentration and lean body mass were significantly increased with teduglutide compared with placebo
3	Gilroy *et al.* (2009); Jeppesen *et al.* (2009); Jeppesen *et al.* (2012); Seidner *et al.* (2015)	• 24-week study of short bowel syndrome patients who were given subcutaneous teduglutide (0.05 mg/kg/d; n = 43) or placebo (n = 43) once daily. • Parenteral support was reduced if 48-hour urine volumes exceeded baseline values by ≥ 10% • The primary efficacy end point was number of responders	• There were significantly more responders in the teduglutide group (27/43) than the placebo group (13/43, $p = 0.002$). • At week 24, the mean reduction in parenteral support volume in the teduglutide group was 4.4 ± 3.8 L/week compared with 2.3 ± 2.7 L/week in the placebo group ($p < 0.001$). • Teduglutide increased plasma concentrations of citrulline, a biomarker of mucosal mass.
4	Naimi *et al.* (2013)	• Sample: Eight short bowel syndrome patients (5 Females, 60 ± 7 years; remnant small bowel 111 ± 62 cm) • Design: open-label, sequential study comparing continuous GLP-2 vs three times per day GLP-2 • Post-absorptive plasma citrulline, reflecting enterocyte mass, was measured by high performance liquid chromatography.	• Both GLP-2 dosing regimens reduced diarrhoea and increased wet weight absorption • Significant increases in plasma citrulline (continuous GLP-2: 7.5 ± 7 μmol/L and three times per day GLP-2: 12.7 ± 8 μmol/L; $p = 0.001$) suggesting intestinotrophic effects in relation to GLP-2 treatment, are followed by increases in relative absorption of energy, carbohydrate and fat.

Table 2.4 Coeliac disease, Crohn's disease and enteropathy studies.

No	Authors	Settings (sample and design)	Main Results
1	Crenn *et al.* (2003)	• Aim: To evaluate citrulline as a marker of severity and extent of villous atrophy in patients without intestinal resection. • Sample: o 42 patients with coeliac disease and 10 patients with non-celiac villous atrophy disease were studied by plasma postabsorptive citrulline and biological dosages, biopsies of proximal (duodenojejunal) small bowel and distal ileum (n = 25), or measurement of vitamin B12 absorption (n = 4). o 9 patients were re-evaluated after following a gluten-free diet for 1 year o Controls: 51 healthy subjects and 10 severely malnourished patients with anorexia nervosa with no intestinal mucosal abnormalities	• Plasma citrulline concentrations: Villous atrophy vs healthy subjects: 24 vs 40µmol/L, $p < 0.001$ • Three cut-offs identifies:<10 µmol/L for patients with diffuse total villous atrophy, 10-20 µmol/L for patients with proximal-only total villous atrophy, and 20-30 µmol/L for patients with partial villous atrophy • Plasma citrulline concentration was correlated to the severity and extent of villous atrophy ($r = 0.81$; $p < 0.001$) and to albumin levels ($r = 0.47$; $p < 0.01$). • Receiver operating characteristic curves indicated that plasma citrulline concentration was the best biological variable to predict villous atrophy • Following a 1-year gluten-free diet, plasma citrulline concentration increased in histologically responsive but not in unresponsive patients
2	Hozyasz *et al.* (2006)	• Aim: To determine amino acid concentrations in coeliac disease patients on gluten-free diet and gluten-containing diet • Sample: 61 patients with coeliac disease • Whole blood citrulline were determined in dried blood spots by tandem mass spectrometry	• Mean citrulline levels were higher in patients on strict gluten-free diet comparing to those newly diagnosed (32.2 ± 8.7 vs 24.9 ± 5.7 µmol/L; p=0.025)
3	Papadia *et al.* (2006a); Papadia *et al.* (2006b); Papadia *et al.* (2007)	• See Table 2.3 for details	
4	Peters *et al.* (2007a, 2007b); Peters *et al.* (2007c); Peters *et al.* (2008b)	• See Table 2.3 for details	
5	Miceli *et al.* (2008)	• Sample: 50 healthy volunteers, 21 patients with untreated coeliac disease and 6 patients with refractory coeliac disease • Serum citrulline levels and duodenal lesions were evaluated at the time of diagnosis, and after at least 24 months of gluten-free diet • Serum citrulline concentrations were determined by ion exchange chromatography.	• In comparison to healthy volunteers, serum citrulline concentrations were significantly lower in untreated and refractory coeliac disease patients • No significant difference was found between untreated and refractory coeliac disease patients and between patients with different patterns of clinical presentation or various degrees of duodenal lesions • After a gluten-free diet, mean serum citrulline concentration increased

Table 2.4 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
6	Crenn *et al.* (2009a); Crenn *et al.* (2009b)	• Sample: 6 groups of HIV patients (n = 115): 1) undetectable viral load without chronic diarrhoea (a; n = 40) and with protease inhibitor-associated toxic chronic diarrhoea (b; n = 26), 2) detectable viral load and CD4 > 200/mm^3 without (a; n = 6) and with (b; n = 11) chronic diarrhoea, and 3) detectable viral load and CD4 <200/mm^3 without chronic diarrhoea (a; n = 7) and with opportunistic intestinal infections or HIV enteropathy (b; n = 25) • The influence of diarrhoea on citrulline was assessed by comparing subgroups a and b with healthy control subjects (n = 100).	• Citrulline was slightly decreased (22-30 μmol/L) in groups 1b and 2b • Citrulline levels in control subjects vs patients without chronic diarrhoea (groups 1a, 2a, and 3a): 38 ± 8 vs 36 ± 6 μmol/L • In group 3b, a citrulline concentration <10 μmol/L was associated with a clinical indication for parenteral nutrition ($p < 0.05$). • Citrulline correlated positively with albumin ($p < 0.01$) and BMI ($p < 0.05$) and negatively with C-reactive protein ($p < 0.01$). • When anti-infectious and nutritional therapies were successful, citrulline normalized in 2-12 weeks
7	Papadia *et al.* (2009a); Papadia *et al.* (2009b); Papadia *et al.* (2010)	• Post-absorptive fasting serum citrulline was measured in 150 tropical enteropathy patients (n = 44, HIV) with reverse phase, high performance liquid chromatography. • Absorptive capacity and permeability were measured after intrajejunal instillation of 4 sugars with assay by thin-layer chromatography. • Morphometric analysis was carried out on jejunal biopsies	• HIV positive vs HIV negative patients: median serum citrulline 19 (17-24) vs 27 (23-33) μmol/L; $p < 0.001$ • There were statistically significant correlations ($p < 0.005$) between citrulline and: crypt depth; villous height/crypt depth ratio; Shenk-Klipstein score; and xylose absorption, only in the HIV positive
8	Panetta *et al.* (2010); Diamanti *et al.* (2011a)	• Sample: 31 Crohn's disease patients and 44 controls (2008-2010) • Analysis: Differences between groups, at baseline, in plasma citrulline and glutamine and between their baseline and final values during the prospective survey, and correlation between baseline values of citrulline and duration of disease, C-reactive protein, and faecal calprotectin	• Mean citrulline value • Controls vs Crohn's disease: 33.0 ± 7.5 vs 23.5 ± 8.4 μmol/L ($p < 0.0001$) • Crohn's disease patients with small bowel disease vs ileo-colonic disease: 14.2 ± 5.5 vs 24.7 ± 8.0 μmol/L, $p = 0.0037$ • Citrulline ≤ 22 μmol/L had sensitivity of 100% and specificity of 98% for differentiating control subjects from Crohn's disease patients with small bowel disease
9	Bernini *et al.* (2011)	• Sample: 61 overt coeliac disease patients, 29 patients with potential coeliac disease, and 51 control subjects were examined by proton nuclear magnetic resonance of their serum and urine	• Potential coeliac disease largely shares the metabonomic signature of overt coeliac disease. Most metabolites found to be significantly different between control and coeliac disease subjects were also altered in potential coeliac disease
10	Blasco Alonso *et al.* (2011)	• Sample and design: Observational case-control study longitudinal in children 16 months to 14 years: 48 with untreated coeliac disease, 9 coeliac children under gluten free diet and 35 non-coeliac healthy children. • Plasma amino acids concentration was measured along with other clinical and analytical data	• Cases vs Controls: citrulline, arginine and glutamine 17.7 μmol/L, 38.7 μmol/L, 479.6 μmol/L respectively vs 28.9 μmol/L, 56.2 μmol/L, 563.7 μmol/L • Citrulline levels are significantly lower in the severe degrees of atrophy vs mild ones (13.8 μmol/L vs 19.7 μmol/L, $p < 0.05$)

Table 2.4 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
11	Ioannou *et al.* (2011)	• Sample: Fasting-plasma citrulline levels were determined by high-performance liquid chromatography in 23 patients with coeliac disease before gluten-free diet (ii) 20 patients with coeliac disease under treatment for more than 2 years responsive to gluten-free diet, (iii) 10 children with gastrointestinal symptoms and normal small bowel biopsy, and (iv) 20 healthy controls. • In group (i), citrulline levels were also measured after 1, 3, 6, and 12 months on a gluten-free diet	• Mean plasma citrulline levels: o Lower in untreated patients with coeliac disease 24.5 ± 4.9 μmol/L vs ▪ patients on a gluten-free diet: 31.2 ± 6.7 μmol/L, $p < 0.001$ ▪ patients with gastrointestinal symptoms and normal intestinal mucosa 30.3 ± 4.7 μmol/L, $p < 0.01$ ▪ and healthy controls: 32.4 ± 7.5 μmol/L, $p < 0.00$ • In untreated patients with coeliac disease, there was an inverse correlation between citrulline concentrations and severity of villous atrophy ($r = -0.67$, $p < 0.01$) • After 1 month on a gluten-free diet, patients had significantly higher levels than before diet ($p < 0.05$) and after 3 months on diet, levels were similar to those observed in the healthy controls
12	Elkhatib and Buchman (2012)	• Sample: 81 outpatients aged 18 to 65 years (mean, 40.6 ± 15.4 years) with a known history of Crohn's disease • Crohn's disease activity was measured by Harvey-Bradshaw Index and was correlated to the plasma citrulline concentration measured simultaneously (ion chromatography) • Spearman correlation coefficients were used to assess for an association between the 2 variables	• The mean plasma citrulline concentration was normal • It failed to distinguish between active and inactive patients based on the Harvey-Bradshaw Index (27.8 μmol/L, $p = 0.991$). • There was no significant linear association between the ranks of citrulline and ranks of Harvey-Bradshaw Index ($r = 0.012$, $p = 0.915$) • No association between plasma citrulline concentration and Harvey-Bradshaw Index ($p = 0.583$) • No difference in plasma citrulline concentrations among those with confirmed inflammation by imaging or endoscopy ($p = 0.583$)
13	Lee *et al.* (2013)	• Sample: 63 Crohn's Disease, 23 ulcerative colitis • Disease severity was assessed by paediatric Crohn's disease activity index, paediatric ulcerative colitis activity index, simplified endoscopic activity score for Crohn's disease, C-reactive protein, and erythrocyte sedimentation rate • Subgroup analysis whether correlations between plasma citrulline levels and disease activity depend on small bowel involvement in patients with Crohn's Disease.	• Plasma citrulline levels correlated negatively with C-reactive protein ($r = -0.332$, $p = 0.008$), erythrocyte sedimentation rate ($r = -0.290$, $p = 0.022$), and paediatric Crohn's disease activity index ($r = -0.424$, $p = 0.001$) in patients with Crohn's disease. • Plasma citrulline levels were lower in patients with jejunal involvement vs those without ($p = 0.027$) • In subgroup analysis, patients with Crohn's disease with jejunal involvement showed significantly negative correlations of plasma citrulline levels with CRP ($r = -0.628$, $p = 0.016$) and paediatric Crohn's disease activity index ($r = -0.632$, $p = 0.015$); no correlation was noted in patients without jejunal involvement and the simplified endoscopic activity score for Crohn's disease • No significant correlations of plasma citrulline levels with inflammatory parameters in ulcerative colitis
14	Basso *et al.* (2014)	• Design and Sample: Cross-sectional study of children and adolescent patients with coeliac disease (n = 48) and controls (n = 42) • Citrulline was measured with high performance liquid chromatography and correlated with disease severity	• Citrulline was significantly lower in coeliac disease patients compared to control subjects • Citrulline levels were negatively correlated with disease severity • A citrulline cut-off level of 27 μmol/L produced a sensitivity of 43% and specificity 90%

Table 2.4 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
15	Sevinc *et al.* (2015)	• Sample: 62 children with classic coeliac disease, 62 age and sex matched healthy control • Plasma amino acid levels measured with tandem mass spectrometry.	• Coeliac children had significant lower plasma levels of citrulline, glutamine and cystine than controls ($p < 0.05$) • Alanine, asparagine, glutamic acid, hydroxyproline, isoleucine, leucine, phenylalanine, proline, serine, threonine and valine were significantly higher in coeliac children than in controls ($p < 0.05$) • No significant difference in levels of arginine, argininosuccinate, aspartic acid, glycine, homocysteine, hydroxylysine, lysine, methionine, ornithine, tryptophan, tyrosine, histidine levels between celiac children and healthy controls ($p > 0.05$)

Table 2.5 Gastrointestinal toxicity from chemo-radiation therapy studies.

No	Authors	Settings (sample and design)	Main Results
1	Lutgens *et al.* (2002); Blijlevens *et al.* (2004)	• Sample: 32 haematopoietic stem cell transplant recipients following intensive myeloablative therapy during the first 3 weeks after transplantation when patients have oral mucositis	• Significant decline in serum concentrations of citrulline following intensive myeloablative therapy during the first 3 weeks after transplantation when patients have oral mucositis and a markedly disturbed gut integrity • Closer inspection of citrulline concentrations of 12 patients confirmed that decline corresponded to the onset of oral mucositis and altered gut integrity
2	Lutgens *et al.* (2004)	• Sample: 23 patients were studied weekly during treatment and at intervals of 2 weeks and 3 and 6 months after treatment by post-absorptive plasma citrulline concentration and clinical toxicity grading. • The interrelationship between these variables and the correlation with small-bowel dose and volume parameters were investigated.	• During fractionated radiotherapy, citrulline concentration significantly decreased as a function of the radiation dose ($p < 0.001$) and the volume of small bowel treated ($p = 0.001$) • Plasma citrulline concentration correlated with clinical toxicity during the last 3 weeks of treatment. As a whole, citrulline concentration correlated better with radiation dose and volume parameters than clinical toxicity grading.
3	Blijlevens *et al.* (2005a)	• Sample: 32 haematopoietic stem cell transplant recipients following intensive myeloablative therapy.	• Significant increase of interleukin-8, lipopolysaccharide-binding protein and C-reactive protein indicating mucosal barrier injury as measured by gut integrity, daily mucositis score and serum citrulline concentrations
4	Blijlevens *et al.* (2005b)	• Design: Prospective, randomised, double-blinded, placebo-controlled pilot study of parenteral nutrition supplemented with 0.57 g/kg glutamine-dipeptide in a homogeneous group of 32 allogeneic stem cell transplant recipients to determine its effect on mucosal barrier injury • Mucosal barrier injury measured by sugar permeability tests, daily mucositis score, daily gut score, and citrulline concentrations	• The daily gut score was significantly lower for the glutamine group on day 7 post-transplant ($p = 0.001$) whilst citrulline was lower ($p = 0.03$) for the placebo group on day 21 post-transplant • Albumin was significantly lower in the placebo group on day 21 post-transplant (32 ± 4 vs 37 ± 3, $p = 0.001$) whilst C-reactive protein was higher (74 ± 48 vs 34 ± 38, $p = 0.003$)
5	Lutgens *et al.* (2005)	• Design: Prospective study, 10 patients with haematological malignancies who were receiving myeloablative therapy had gut toxicity assessed with sugar permeability tests. • Serum citrulline concentrations also were determined using archival serum samples	• Sensitivity and specificity were better for the citrulline assay compared with sugar permeability tests • Maximum gut damage assessed with the citrulline assay was observed 1-2 weeks earlier compared with the sugar permeability test • Citrulline indicated recovery of gut damage at 3 weeks after transplantation, whereas most sugar permeability tests remained abnormal
6	Herbers *et al.* (2008)	• 29 patients with high-dose melphalan 200 mg/m^2 to prepare for an autologous Peripheral blood stem cell transplantation • Plasma samples from each patient starting before the myeloablative regimen and three times per week thereafter until discharge	• Baseline citrulline concentration was 27.6 ± 4.0 μmol/L, and citrulline concentrations declined rapidly thereafter reaching a nadir averaging 6.7 ± 2.7 μmol/L, 12 days after starting melphalan. • Citrulline concentrations, only increased gradually and were still low (12 ± 4 μmol/L) at discharge. • Their mean citrulline concentrations were lower at 5.5 ± 1.5 μmol/L than were those of patients without bacteraemia (10.2 ± 3.9 μmol/L)
7	Wedlake *et al.* (2008)	• Sample: 59 patients (30 males) with mixed pelvic malignancies, receiving 45-70 Gy were recruited • At baseline and weeks 4 or 5 of radiotherapy, blood samples for citrulline, C-reactive protein, eosinophil cationic protein and stool samples for faecal calprotectin were obtained	• Citrulline ($p = 0.02$) and faecal calprotectin ($p = 0.01$) values changed significantly between baseline and 4 or 5 weeks. • Inflammatory Bowel Disease Questionnaire - Bowel Subset fell significantly (mean fall = 10 points). Changes in markers did not correlate with symptoms.

Table 2.5 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
8	Derikx *et al.* (2009)	• Sample: 34 adult patients with haematological malignancy received allogeneic haematopoietic stem cell transplant 12 days after myeloablative conditioning with a regimen known to induce oral and intestinal mucosal barrier injury • Serum levels of citrulline, intestinal fatty acid binding protein and ileal bile acid-binding protein were measured on transplant days -12, -6, 0, +7, +14 and +21.	• Myeloablative conditioning resulted in a significant decrease in serum citrulline with the nadir on day 7 post-transplant; thereafter, levels rose gradually. • A significant decrease in intestinal fatty acid binding protein and ileal bile acid-binding protein levels occurred from the day of transplant until day +14.
9	van Vliet *et al.* (2009)	• Sample: Children with acute myeloid leukaemia • Investigations: various mucosal barrier injury-related clinical and laboratory tests, reflecting clinical severity (NCI symptomatic adverse events criteria), daily gut score, inflammation (plasma and faecal interleukin-8, faecal calprotectin), enterocytic loss (plasma citrulline, ratio faecal human DNA/total DNA) and intestinal permeability (sugar absorption tests)	• Intestinal mucosal barrier injury as detected by the NCI adverse events criteria was found in 55% of chemotherapy cycles, correlating well with the continuous daily gut score ($n = 55$, $r = 0.581$; $p < 0.001$) • Intestinal cell loss as measured by the ratio faecal human DNA/total DNA and plasma citrulline correlated well with both NCI criteria ($r = 0.357$, $p = 0.005$; $r = -0.482$, $p < 0.001$) and daily gut score ($r = 0.352$, $p = 0.009$; $r = -0.625$, $p < 0.001$) • Plasma interleukin-8 correlated strongly to plasma citrulline ($r = -0.627$; $p < 0.001$).
10	Herbers *et al.* (2010)	• Sample and design: Citrulline concentrations were determined at baseline and at least once weekly after the start of myeloablative chemotherapy until 30 days thereafter among 94 allogeneic or autologous haematopoietic stem-cell transplant recipients. • Intestinal mucosal damage was described either by level of citrulline on each day, on the basis of different thresholds of citrulline indicating the severity of villous atrophy, or by area under the curve using reciprocal value of 10/citrulline.	• Regimens that incorporated idarubicin induced the most severe intestinal toxicity. • Scores based on the level of citrulline, using severity thresholds, and on the area under the reciprocal curve are able to discriminate between the damage induced by different high-dose chemotherapy regimens.
11	Jakobsson *et al.* (2010)	• Sample: 29 women undergoing pelvic radiotherapy for anal or uterine cancer were prospectively followed • Fatigue and diarrhoea were assessed using patient self-reported questionnaires • Plasma citrulline concentration, as a sign of intestinal injury, and C-reactive protein, orosomucoid, albumin, alpha-1-antitrypsin, and haptoglobin, as signs of systemic inflammation, were analysed.	• Fatigue increased significantly ($p < 0.001$) and citrulline decreased significantly ($p < 0.001$) during treatment. • A significant negative correlation ($r = -0.40$; $p < 0.05$) was found between fatigue and epithelial atrophy in the intestine (as assessed by plasma citrulline) after 3 weeks of treatment and a significant positive correlation ($r = 0.75$; $p < 0.001$) was found between fatigue and diarrhoea.
12	van der Velden *et al.* (2010); van der Velden *et al.* (2013)	• Sample: Retrospective analysis in 163 stem-cell transplant recipients of which data had been collected prospectively on intestinal damage (citrulline), inflammation (C-reactive protein), and neutrophil count. • Six different conditioning regimens were studied; 5 myeloablative and 1 non-myeloablative • Linear mixed model multivariate and AUC analyses were used to define the role of intestinal damage in post-SCT inflammation.	• In the 5 myeloablative regimen there was a striking pattern of inflammatory response that coincided with the occurrence of severe intestinal damage • This contrasted with a modest inflammatory response seen in the non-myeloablative regimen in which intestinal damage was limited. • With linear mixed model analysis the degree of intestinal damage was shown the most important determinant of the inflammatory response, and both neutropenia and bacteraemia had only a minor impact. • AUC analysis revealed a strong correlation between citrulline and C-reactive protein ($r = 0.96$). Intestinal damage was associated with the occurrence of bacteraemia and acute lung injury, and influenced the kinetics of acute graft-versus-host disease

Table 2.5 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
13	Onal *et al.* (2011)	• Sample: 53 patients (36 prostate cancer, 17 endometrial cancer) who received 45 Gy pelvic radiotherapy using conventional fractionation • Patients with prostate cancer received an additional 25-30.6 Gy conformal boost. • Plasma citrulline levels were assessed on day 0, mid- (week 3) and post-radiotherapy (week 8), and four months post-radiotherapy. • Dose-volume histogram, citrulline concentration changes, and weekly intestinal toxicity scores were analysed.	• Citrulline concentrations were significantly reduced at week 3 (27.4 ± 5.9 μmol/L; $p < 0.0001$), treatment end (29.9 ± 8.8 μmol/L; $p < 0.0001$), and four months post-treatment (34.3 ± 12.1; $p = 0.01$). • The following factor pairs were significantly positively correlated: Citrulline concentration/mean bowel dose during, end of treatment, and four months post-radiotherapy; dose-volume parameters/citrulline change groups; cumulative mean radiation dose/intestinal toxicity at end and four months post-radiotherapy; citrulline changes/intestinal toxicity during and end of radiotherapy. • Citrulline concentration changes significantly differed during treatment according to radiotherapy oncology group intestinal toxicity grades ($p < 0.0001$)
14	Vokurka *et al.* (2013)	• Sample: prospective study in 11 adults (18 blood samples) with diarrhoea developed after allogeneic stem-cell transplant in between 2011-2012 compared to 20 healthy control samples	• Transplanted patients vs healthy controls: median (IQR) 9.3 (3.62-15.38) vs. 33.3 (26.82-36.23) μmol/L, $p<0.0001$ • Post-transplant toxic intestinal mucositis (n=8, days 1-22 post-transplant) vs. intestinal graft versus host disease (n=7, day 43-142) vs. other aetiology of diarrhoea (n=3, day 120-127): 9.55 (2.95-12.03) vs. 5 (3.85-9.05) vs. 15.6 (15.45-18.3) μmol/L ($p < 0.05$)
15	Gosselin *et al.* (2014)	• Sample: Multicentre, prospective cohort study of 26 children to define time-related changes in serum citrulline during the course of hematopoietic cell transplantation. Markers of gastrointestinal function including oral energy intake, emesis, stool volume, presence of graft-versus-host disease, oral mucositis severity, and cytokine and neurohormone levels were measured. • Weekly serum citrulline concentrations were obtained from 10 days prior until 30 days after hematopoietic cell transplantation.	• Mean baseline citrulline concentration was 22.7 μmol/L (95% CI 17.7-27.6) on day -10, which decreased to a nadir of 7.5 μmol/L (95% CI 3.1-18.0, $p = 0.017$) on day 8 following hematopoietic cell transplantation before returning to baseline by day 30. • After adjustment for interleukin-6 level (1.0% lower citrulline per 10% increase in interleukin-6, $p = 0.004$), presence of acute graft-versus-host disease (27% lower citrulline, $p = 0.025$), and oral energy intake (2.1% lower citrulline per 10% decrease in energy intake, $p = 0.018$), the nadir shifted to day 10, when mean citrulline concentration was lower in patients with severe oral mucositis (6.7 μmol/L, 95% CI 3.4-13.1) than in those without severe mucositis (11.9 μmol/L, 95% CI 5.8-24.4, $p = 0.003$). • Change in citrulline was not correlated with stool volume, C-reactive protein, tumour necrosis factor-alpha, leptin, or ghrelin.
16	Karlik *et al.* (2014)	• Aim: To determine whether citrulline levels correlate with clinical markers of intestinal injury in children undergoing a myeloablative allogeneic transplant regimen	• For every 1 μmol/L increase in citrulline, the odds of developing mucositis were 0.88 (95% CI 0.79-0.99, $p = 0.036$) • The odds of developing diarrhoea were 0.70 times less for every 1 μmol/L increase in citrulline (95% CI=0.59-0.84, $p < 0.0001$)
17	Brady *et al.* (2015)	• Sample: 15 patients treated with external beam radiation therapy to either prostate only (n=6) or prostate and pelvis (n=9). • Plasma citrulline levels were measured prior to radiotherapy and weekly during treatment and at 6 weeks, 3 months and 6 months post external beam radiation therapy • Bowel toxicity was assessed at the same time points using EPIC bowel summary scores.	• The strongest correlation between the fall in plasma citrulline levels from baseline and greatest bowel toxicity was observed after 3 weeks of radiotherapy ($p=0.03$). • A strong predictive trend was noted with positive correlations at 6 weeks post radiotherapy ($r = 0.594$, $p = 0.025$), 3 months post radiotherapy ($r = 0.534$, $p = 0.060$), 6 months post radiotherapy ($r = 0.606$, $p = 0.037$), 9 months post radiotherapy ($r = 0.618$, $p = 0.019$) and 1 year post radiotherapy ($r =0.358$, $p = 0.345$). • No significant correlation was found between changes in plasma citrulline levels or EPIC reported toxicity

Table 2.5 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
18	Kong *et al.* (2015)	• Sample: 42 patients with gastric or colorectal cancer underwent chemotherapy • Patients were asked to grade and record their symptoms of gastrointestinal toxicity daily • The urinary lactulose-mannitol ratio was measured to assess the intestinal permeability. • Plasma levels of citrulline, diamine oxidase, D-lactic acid, and endotoxin were also measured	• The urinary lactulose-mannitol ratio and plasma citrulline levels increased on the third and sixth post-chemotherapy days, respectively • There were no significant differences in the plasma levels of D-lactic acid, endotoxin or diamine oxidase activity compared to their levels before chemotherapy
19	Wang *et al.* (2015)	• Aim: To investigate the correlations between fatigue, diarrhoea, and alterations in gut microbiota induced by pelvic radiotherapy. •	• During the 5-week treatment of pelvic radiotherapy in 11 cancer patients, the general fatigue score significantly increased and was more prominent in the patients with diarrhoea. • The fatigue score was closely correlated with the decrease of serum citrulline and the increases of systemic inflammatory proteins, including haptoglobin, orosomucoid, alpha-1-antitrypsin and tumour necrosis factor-alpha.
20	Zezulová *et al.* (2016)	• Design: Plasma citrulline, serum neopterin and urinary neopterin were measured weekly in 49 patients with rectal carcinoma during chemoradiation	• Citrulline significantly ($p < 0.05$) decreased while serum and urinary neopterin concentrations increased during therapy. • Irradiated gut volume correlated significantly inversely with citrulline and positively with urinary neopterin. • Statistically significant inverse correlations were also observed between urinary neopterin and plasma citrulline concentrations during the treatment. • Urinary neopterin concentrations were significantly higher and citrulline concentrations were lower in patients who experienced grade ≥ 3 gastrointestinal toxicity

Table 2.6 Studies regarding citrulline levels in critical illness or other conditions.

No	Authors	Settings (sample and design)	Main Results
1	Backman *et al.* (1975)	• Design: The amino acid pattern in plasma was studied in a reference group (n=26) and in three groups of massive obese subjects (n=9, 8, and 9 respectively) before and at intervals after jejuno-ileostomy.	• The concentrations of lysine, tyrosine, cystine, and glutamic acids were higher, and aspargin, glutamine, serine, and glycine were lower than in the reference group. • During the post-operative period the amino acid pattern changed significantly with serine, glycine, and taurine increased and valine, lysine, leucine, tryptophan, thyrosine, cystine, and citrulline decreased. • The amino acid pattern in the obese group with the longest post-operative observation time and a stable body weight differed significantly from that in the reference group only with regard to a low valine concentration and high concentration of taurine and glutamic acid.
2	Müller *et al.* (1983)	• Sample: 6 patients - infusing 0.3 mg/24 h of exogenous glucagon	• In six normal subjects the same infusion reduced significantly ($p < 0.05$) plasma alanine, asparagine, glutamate, glutamine, glycine, proline, serine, threonine, arginine, ornithine, lysine and tyrosine • This particular glucagon sensitivity of duodenopancreatectomized patients suggests that glucagon deficiency is the cause of their hyperaminacidaemia.
3	Jeevanandam *et al.* (1991)	• Sample: 10 obese and 10 non-obese traumatized patients • Plasma levels of free amino acids in the early flow phase of injury when subjects were receiving maintenance fluids without calories or nitrogen	• Obese controls showed an increase in valine, leucine, isoleucine, and glutamic acid levels, and a decrease in glycine, tryptophan, threonine, histidine, taurine, citrulline, and cystine levels compared with lean controls. • Hypoaminoacidemia was equally seen in traumatized obese and non-obese patients, and it was mainly due to a 24% decrease in nonessential amino acids. • Essential amino acid levels were the same in all groups.
4	Sandstrom *et al.* (2003)	• Sample: Serum L-arginine and L-citrulline and urinary nitrite/nitrate concentrations 1 to 3 days after the onset of symptoms in 11 patients with gallstone pancreatitis, 10 patients with alcoholic pancreatitis, and 6 patients with idiopathic pancreatitis. 13 healthy control blood donors, 9 patients fasting before hernia operations, 8 patients with acute cholecystitis, and 9 alcoholic subjects but no pancreatitis. • Serum arginine and citrulline concentrations were measured with high performance liquid chromatography, and urinary nitrite/nitrate spectrophotometrically.	• Patients with acute pancreatitis had lower serum L-arginine and L-citrulline concentrations than controls
5	Sandstrom *et al.* (2008)	• Design: Serum amino acid spectrum was measured daily for five days and after recovery six weeks later in 19 patients admitted to the hospital for acute pancreatitis.	• These patients had abnormal levels of most amino acids including arginine, citrulline, glutamine and glutamate. • Phenylalanine and glutamate were increased, while arginine, citrulline, ornithine and glutamine were decreased compared to levels after recovery

Table 2.6 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
6	Thibault *et al.* (2008); Thibault *et al.* (2009)	• Sample: 20 morbidly obese patients operated by Roux-en-Y gastric bypass (17 women, 47 ± 12 years, BMI, 53.3±11.3 kg/m^2) • Body composition determined by single-frequency bioelectrical impedance analysis. Blood testing, • Plasma concentrations of 20 amino acids including citrulline were available for only 7 patients.	• Plasma citrulline (53.6 ± 16.0 μmol/L) and other amino acids, except cysteine, did not differ from normal values
7	Luiking *et al.* (2009)	• Aim: To compare arginine and citrulline metabolism in septic patients and nonseptic control patients in an intensive care unit and in healthy control subjects. • Sample: 10 patients with septic shock, 7 critically ill control patients, and 16 healthy elderly subjects	• Whole-body citrulline production was significantly lower in septic patients (4.5 ± 2.1 μmol/kg/h) than in intensive care control patients (10.1 ± 2.9 μmol/kg/h, $p < 0.01$) and in healthy control subjects (13.7 ± 4.1 μmol/kg/h, $p < 0.001$) • Citrulline production is severely low in patients with sepsis and is related to diminished de novo arginine and nitric oxide production
8	Peters *et al.* (2009)	• Aim: To assess citrulline generation test reference values in 14 stable intensive care patients with respiratory failure with normal renal function and able to tolerate enteral nutrition • Amino acid analysis was performed using reverse phase high performance liquid chromatography • 8 females, 6 males, mean age 60.2 years and BMI 27.2 kg/m^2	• The incremental area under the curve at 90 minutes during the test following enteral glutamine was 5,807,437 mmol/L.min for venous and 6,807,507 mmol/L.min for arterial citrulline sampling • Performing the test with intravenously administered glutamine resulted in an area of 7,707,235 mmol/L.min for venous and 9,297,223 mmol/L.min for arterial citrulline sampling • Positive correlation between venous and arterial citrulline sampling in enteral ($r = 0.96$, $p < 0.0001$) and intravenous glutamine ($r = 0.91$, $p < 0.0001$)
9	Crenn *et al.* (2010)	• Aim: To investigate in septic shock patients with multi-organ failure plasma citrulline pharmacokinetics, associated parameters and pro tumour necrosis factor alpha /anti–interleukin-10–inflammatory plasma cytokines • Two groups (n = 16 ,7 males, age 63 ± 12 years) were selected: survivors (n = 8), deceased patients (n = 8)	• Citrulline decreased during day 0 (29 ± 10 vs18 ± 6 μmol/L, $p < 0.05$) in most patients • Citrulline remained < 10 μmol/L in 2 patients of the deceased group whereas a transient citrulline <10 μmol/L was noted in 2 survivors. Citrulline normalised on day 7 in 5 survivors and 1 deceased patient ($p = 0.10$) • Citrulline was negatively correlated with C-Reactive protein ($r = 0.31$, $p< 0.01$) but positively with glutamine, arginine and creatinine ($r = 0.95, 0.92, 0.25$, $p< 0.05$) • No significant correlation was found between citrulline and albumin, tumour necrosis factor alpha, and interleukin-10
10	Pan *et al.* (2010)	• Sample: 32 patients with acute pancreatitis onset within 7 days • Severity of disease and gut dysfunction on admission, on day 7, and day 3 of enteral nutrition • Serum levels of intestinal fatty acid binding protein, citrulline, and C-reactive protein (CRP) and the lactulose and mannitol absorption ratio in urine were measured in parallel	• Intestinal fatty acid binding protein increased on admission and in severe attacks • All patients: ↑ gut dysfunction score, C-reactive protein, urine level of lactulose and mannitol absorption ratio; ↓ citrulline • Positive correlation noted between intestinal fatty acid binding protein and gut dysfunction score, Acute Physiology and Chronic Health Evaluation II score, C-reactive protein and intensive care stay • Negative correlation noted between intestinal fatty acid binding protein and citrulline
11	Piton *et al.* (2010)	• Design: Prospective observational pf 67 patient without small bowel disease and without chronic renal failure consecutively admitted to a single intensive care unit • Plasma citrulline concentrations were studied at admission, 12, 24, 48 hours, and the 7th day after admission	• 1st day: mean citrulline decreased from 18.8 to 13.5 μmol/L • Low plasma citrulline at 24 hours was associated with low plasma glutamine ($p = 0.01$) and arginine ($p = 0.04$), high plasma C-reactive protein ($p = 0.008$), nosocomial infection rate ($p = 0.03$), and 28-day mortality ($p = 0.02$) • Multivariate analysis: plasma citrulline ≤ 10 μmol/L at 24 hours and Sequential Organ Failure Assessment score ≥ 8 at 24 hours had higher 28-day mortality (odds ratio 8.7, 15.1, respectively)

Table 2.6 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
12	Través *et al.* (2010)	• Sample: 28 patients who underwent subtotal gastrectomy or hemicolectomy and were placed on short-term parenteral nutrition • Design: Assigned on a Parenteral-Oral (4-day parenteral nutrition and 4-day oral, n = 8) or a Parenteral-Only (7-day parenteral nutrition, n = 20ts) nutritional regime	• Pre-operative citrulline values were within range with those of a western population. • On day 4 in the Parenteral-Oral regime, citrulline levels were 60% lower than pre-operative levels • When enteral feeding was resumed, citrulline rose and was close to pre-operative values on day 8 • In the Parenteral-Only regime the parenteral nutrition solution composition had no influence on the citrulline
13	van Noord *et al.* (2011)	• Sample: Consecutive patients suspected of chronic gastrointestinal ischaemia (n = 40), healthy subjects (n = 9) • Blood samples for analysis of intestinal fatty acid-binding protein, D-dimer, lactate dehydrogenase, leucocyte counts, C-reactive protein, and L-lactate were drawn before and after a standard meal. • Intestinal mucosal injury was assessed with glutamine, citrulline and arginine in blood samples and compared to a sugar absorption test	• Ischaemia diagnosed in 32 patients • No difference noted in any parameter between patients with and without ischaemia • L-lactate was increased in ischaemia patients compared to non-ischaemia patients. • In ischaemia patients, D-dimer levels showed a significant elevation post-prandially compared to baseline.
14	Verdam *et al.* (2011)	• Aim: To investigate the relation between plasma markers of small intestinal function and chronic hyperglycaemia • Sample: Cross-sectional observational study of 40 severely obese subjects with chronic hyperglycaemia and 30 severely obese subjects without chronic hyperglycaemia who were indicated for bariatric surgery. • Measurement of plasma levels of citrulline, intestinal fatty acid binding protein, glucagon-like peptide-2, glycated haemoglobin HbA1c	• Plasma citrulline and intestinal fatty acid binding protein levels were significantly elevated in chronic hyperglycaemia compared to normal HbA1c (Citrulline: 35 ± 2.1 vs 26 ± 1.4 μmol/L, p = 0.001; intestinal fatty acid binding protein: 140 ± 22 vs 69 ± 14 pg/mL, p = 0.001 • Plasma citrulline and intestinal fatty acid binding protein correlated with HbA1c (r = 0.30, 0.33, p < 0.05, respectively). • Intestinal fatty acid binding protein to citrulline ratio was higher in subjects with elevated HbA1c (4.0 vs 3.1, p = 0.03) • Glucagon-like peptide-2 was not related to citrulline or intestinal fatty acid binding protein (p > 0.05)
15	Lundy *et al.* (2012)	• Design: Observations of serial plasma citrulline levels in a severely burned adult who ultimately died from non-occlusive mesenteric ischaemia leading to full-thickness small bowel necrosis	• Decrease of citrulline around settings of ischaemia and increase of lactate
16	Noordally *et al.* (2012)	• Aim: Prospective observational single-centre controlled study (n = 91, 31 females, mean 69.3 years) • Inclusion criteria: intensive care stay over 48 hours • Plasma citrulline: low (0-15 μmol/L), medium (16-35 μmol/L), and high (> 36 μmol/L)	• Mean citrulline: 21.7 ± 13.1 μmol/L • Patients with intestinal dysfunction had low plasma citrulline level < 15 μmol/L (p = 0.014) • No correlations noted between C-reactive protein, albumin, prealbumin, renal failure, inotrope use, Sequential Organ Failure Assessment score, Acute Physiology and Chronic Health Evaluation II score and citrulline
17	Grimaldi *et al.* (2013)	• Sample: 21 patients following resuscitation after cardiac arrest • Urinary intestinal fatty acid-binding protein, plasma citrulline, whole blood endotoxin were measured at admission, days 1-3 and 6 • Kinetics of release and the relationship between intestinal fatty acid-binding protein, citrulline and endotoxin values	• Lowest median of citrulline was attained at day 2 (11 vs 24 μmol/L at admission, p = 0.01) and normalised at day 6 (21 μmol /L) • Highest endotoxin level was negatively correlated lowest plasma citrulline levels (r^2 =0.55, p < 0.001)

Table 2.6 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
18	Kao *et al.* (2013)	• Aim: To investigate how sepsis affects glutamine metabolism, including its conversion to citrulline, by measuring glutamine and citrulline flux, fractional splanchnic extraction of glutamine and leucine, and the contribution of glutamine nitrogen to citrulline in septic patients and healthy controls • Sample: 8 patients with severe sepsis and 10 healthy controls were given primed, constant intravenous infusion of [$^{2}H_{2}$]citrulline and sequential administration of intravenous and enteral [α-^{15}N]glutamine and [^{13}C]leucine in the post-absorptive state	• Compared with healthy controls, septic patients had a significantly lower whole body citrulline flux and plasma concentration, higher endogenous leucine flux, and higher glutamine clearance • The majority of the ^{15}N label transferred from glutamine to citrulline was found at the α-position • Lower glutamine plasma concentrations in sepsis were a result of increased glutamine clearance • Despite adequate splanchnic uptake of glutamine, there is decreased production of citrulline, suggesting a defect in the metabolic conversion of glutamine to citrulline, decreased uptake of glutamine by the enterocyte but increased uptake by the liver, and/or shunting of glutamine to other metabolic pathways
19	Piton *et al.* (2013)	• Design and Sample: 103 intensive care patients, prospective observational study • Inclusion criteria: 18 years old or older; expected intensive care stay over 48 hours, without pregnancy, chronic small bowel disease, or chronic renal failure • Plasma intestinal fatty acid-binding protein, citrulline concentrations, and variables relating to prognosis and treatment, were measured on admission	• Intestinal fatty acid-binding protein elevation on admission was associated with catecholamine support, higher lactate concentration, higher Sequential Organ Failure Assessment score, and higher international normalized ratio ($p < 0.001$) • Plasma citrulline concentration ≤ 10 μmol/L on admission was associated with higher intra-abdominal pressure, higher plasma C-reactive protein concentration, and more frequent antibiotic use ($p < 0.005$) • No correlation between plasma levels of intestinal fatty acid-binding protein and citrulline • On admission, Sequential Organ Failure Assessment score ≥ 12, plasma citrulline ≤ 12.2 μmol /L, and plasma intestinal fatty acid-binding protein concentration ≥ 355 pg/mL were associated with higher 28-day mortality (odds ratio 4.39, 5.17, 4.46, respectively)
20	Ware *et al.* (2013)	• Sample: Plasma citrulline, arginine and ornithine levels and nitrate/nitrite were measured at baseline in 135 patients with severe sepsis • Acute respiratory distress syndrome was diagnosed by a consensus definition	• Plasma citrulline levels: • Below normal in all patients: median 9.2 (5.2-14.4) μmol/L • Acute respiratory distress syndrome vs non-acute respiratory distress syndrome: 6.0 (3.3-10.4) vs 10.1 (6.2-16.6) μmol/L, $p = 0.002$ • The rate of acute respiratory distress syndrome was 50% in the lowest citrulline quartile compared to 15% in the highest citrulline quartile ($p = 0.002$) • In multivariable analyses, citrulline levels were associated with acute respiratory distress syndrome after adjustment for covariates including illness severity
21	Alekseeva and Sal'nikov (2014)	• Sample: 27 critical condition patients (15 females, age 70 ± 14 years • On admission to intensive care, plasma glutamine, citrulline, glutamic acid (liquid chromatography), relative duodenal and jejunum electrical activity were measured	• No increase noted in plasma glutamine, citrulline or glutamic acid • Worst prognosis was observed when citrulline was ≤ 10 μmol/L and signified decrease in proximal small intestine relative electrical activity
22	Carswell *et al.* (2014)	• Sample: obese controls (BMI > 30 kg/m^{2}, n = 7), adjustable gastric banding (n = 6), Roux-en-Y gastric bypass (n = 7), biliopancreatic diversion with duodenal switch (n = 5). • Measurements: oro-caecal transit time, fasting plasma citrulline, 3 days of faecal elastase 1, calprotectin, fatty acids	• No difference in oro-caecal transit time ($p = 0.935$) or citrulline levels ($p = 0.819$) • Faecal calprotectin was elevated post- Roux-en-Y gastric bypass vs obese ($p = 0.016$) and faecal elastase 1 was decreased post- Roux-en-Y gastric bypass vs obese ($p = 0.002$)

Table 2.6 *(Continued)*

No	Authors	Settings (sample and design)	Main Results
23	Piton *et al.* (2015a)	• Sample: 69 patients with cardiac arrest of both cardiac and hypoxic origin admitted to intensive care • Design: Prospective, observational, single-centre study, evaluating plasma citrulline and intestinal fatty acid-binding protein concentrations on admission and after 24 hours • Comparison of the variables according to 28-day Cerebral Performance Category score of 1-2 (good neurological outcome) vs 3-5 (poor neurological outcome)	• On admission, citrulline was low in 65 % and plasma intestinal fatty acid-binding protein was high in 82 % • At 24 hours, citrulline was low in 82 % and intestinal fatty acid-binding protein was normal in 60 % • Patients with a poor neurological outcome had a lower plasma citrulline concentration and a higher intestinal fatty acid-binding protein on admission • Multivariate analysis: plasma citrulline levels ≤ 13.1 μmol/L and intestinal fatty acid-binding protein > 260 pg/mL were independently associated with a poor neurological outcome (odds ratio 21.9, 13.6, respectively)
24	Piton *et al.* (2015b)	• Aim: To examine whether catecholamines in critically ill patients may be associated with enterocyte damage • Design: Prospective observational study. • Sample: Critically ill patients requiring epinephrine and/or norepinephrine on admission to intensive care (n = 60). Controls not receiving catecholamines (n = 27) • Measurement on admission: plasma intestinal fatty acid-binding protein, plasma citrulline, abdominal perfusion pressure, and variables relating to prognosis and treatment	• Plasma intestinal fatty acid-binding protein was higher among patients receiving catecholamine vs controls • In patients receiving catecholamines, a dose of 0.48 γ/kg/min or more on admission was associated with a higher intestinal fatty acid-binding protein concentration • Sepsis-related Organ Failure Assessment score > 11 and plasma intestinal fatty acid-binding protein more than 524 pg/mL on admission were independently associated with 28-day mortality • Citrulline was not associated with catecholamine dose but was generally low: median 14.7 (8.8-27.9).
25	Poole *et al.* (2015)	• Sample: Prospective observational study, 15 healthy, 20 critically ill subjects • Fasting plasma citrulline concentrations were assayed in blood samples immediately prior to the administration of a liquid test meal (1 kcal/ml; containing 3 g of 3-O-methylglucose) that was infused directly into the small intestine • Serum 3-O-methylglucose concentrations were measured over the following 4 hours, with the area under the 3-O-methylglucose concentration curve calculated as an index of glucose absorption	• Healthy subjects vs critically ill patients: citrulline 26.5 vs 15.2 μmol/L, $p < 0.01$; glucose absorption 79.7 vs 61.0 mmol/L/240 min, $p = 0.05$ • No relationship between fasting citrulline concentration and subsequent glucose absorption was noted ($r = 0.28$; $p = 0.12$)

Table 2.7. Common causes of short bowel syndrome (Nightingale and Woodward, 2006; Pironi *et al.*, 2016; Pironi *et al.*, 2018).

Jejunum-colon	***Jejunostomy***
Crohn's disease	Crohn's disease
Mesenteric ischaemia	Ulcerative colitis
Irradiation	Irradiation
Small bowel volvulus	Mesenteric ischaemia
Adhesions	Desmoid

Table 2.8. Metaregression results of the correlation of citrulline with small bowel length with four potential sources of heterogeneity.

Variable	**Coefficient (95% CI)**	**SE**	***p*-value**
Male %	-0.0196 (-0.1155, 0.0763)	0.03	**0.562**
Age	0.0500 (-0.1267, 0.2268)	0.06	**0.434**
BMI	-0.0088 (-0.1593, 0.1416)	0.05	**0.864**
Mean citrulline concentration	-0.0779 (-0.3997, 0.2439)	0.10	**0.497**
Mean small bowel length	-0.0266 (-0.1043, 0.0510)	0.02	**0.355**
Constant	3.7196 (-5.4980, 12.9372)	2.90	**0.289**
Restricted maximum likelihood estimate of between-study variance: $\tau^2 = 0.8593$			
% residual variation due to heterogeneity: $I^2 = 98.9\%$			
Proportion of between-study variance explained: Adjusted $R^2 = 0\%$			
Joint test for all covariates: Model $F(5,3) = 0.34$			
With Knapp-Hartung modification: $p > F = 0.862$			

Table 2.9. Diagnostic accuracy data regarding studies investigating short bowel syndrome.

Study	TP	FP	FN	TN	Citrulline cut-off level (µmol/L)	AUC (ROC)	Sensitivity	Specificity	Groups under comparison
Crenn *et al.* (2000)	34	2	3	18	20		92 %	91 %	HPN dependency
Rhoads *et al.* (2005)	13	0	3	5	20	0.91 (0.79, 1.00)	81 %	100 %	HPN dependency
Peters *et al.* (2007c)	8	4	22	14		0.50 (0.30, 0.71)	25 %	77 %	SBS vs Controls
Papadia *et al.* (2007)	22	4	5	24	21	0.87	83 %	87 %	HPN dependency
Parekh *et al.* (2008)	30	21	0	28	20	0.82 (0.71, 0.93)	100 %	57 %	SBS vs Controls
Santarpia *et al.* (2008)	15	1	0	9	10	0.65 (0.43, 0.88)	100 %	9 %	HPN dependency
Fitzgibbons *et al.* (2009)	16	2	2	7	15	0.88 (0.75, 1.00)	89 %	78 %	HPN dependency
Bailly-Botuha *et al.* (2009)	20	9	1	3	20	0.46 (0.25, 0.67)	95 %	25 %	HPN dependency
Diamanti *et al.* (2010)	11	1	1	10	20		91 %	89 %	HPN dependency
Pironi *et al.* (2011)	25	25	2	20	20		50 %	91 %	HPN dependency
Raphael *et al.* (2011)	5	1	3	1	20		63 %	50 %	HPN dependency
Diamanti *et al.* (2011b)	9	1	5	13	10	0.90 (0.70, 1.00)	64 %	93 %	HPN dependency
Pironi *et al.* (2012)	33	0	34	26	14		49 %	100 %	HPN dependency
Suzuki *et al.* (2012)	2	1	0	3	15		100 %	75 %	HPN dependency
Amiot *et al.* (2013)	38	10	86	134	20	0.85 (0.78, 0.92)	31 %	93 %	HPN dependency
Pinto Costa *et al.* (2013)	6	4	5	20	25.5	0.67 (0.46, 0.88)	55 %	83 %	SBS vs Controls

Table 2.10. Results of diagnostic meta-analysis regarding sensitivity and specificity in patients with all conditions and only short bowel syndrome.

	All conditions (26 studies)				**Short bowel syndrome (16 studies)**			
	Coefficient (95% CI)	**SE**	***z***	***p* > *z***	**Coefficient (95% CI)**	**SE**	***z***	***p* > *z***
Bivariate Model								
Expected value of logit[Sensitivity]	1.37 (0.82, 1.92)	0.28			1.55 (0.70, 2.40)	0.43		
Expected value of logit[Specificity]	1.63 (1.19, 2.06)	0.22			1.52 (0.86, 2.17)	0.33		
Variance of logit[Sensitivity]	1.57 (0.77, 3.19)	0.57			2.22 (0.89, 5.57)	1.04		
Variance of logit[Specificity]	0.82 (0.37, 1.85)	0.34			1.13 (0.41, 3.12)	0.58		
Correlation between logits	-0.55 (-0.84, -0.02)	0.21			-0.64 (-0.92, 0.06)	0.25		
HSROC								
λ	3.08 (2.51, 3.64)	0.29			3.10 (2.31, 3.90)	0.41		
θ	-0.37 (-0.89, 0.14)	0.26			-0.24 (-0.96, 0.47)	0.36		
β	-0.32 (-0.82, 0.17)	0.25	-1.27	0.204	-0.34 (-0.94, 0.27)	0.31	-1.10	0.272
σ_α^2	1.03 (0.37, 2.82)	0.53			1.15 (0.26, 5.06)	0.87		
σ_θ^2	0.88 (0.45, 1.72)	0.30			1.30 (0.56, 2.98)	0.55		
Summary								
Sensitivity	**80 % (69%, 87%)**	**0.05**			**82 % (67%, 92%)**	**0.06**		
Specificity	**84 % (77%, 89%)**	**0.03**			**82 % (70%, 90 %)**	**0.05**		
Diagnostic odds ratio	**20.03 (11.55, 34.72)**	**5.62**			**21.43 (9.58, 47.90)**	**8.80**		
Positive likelihood ratio	**4.85 (3.47, 6.80)**	**0.84**			**4.58 (2.82, 7.44)**	**1.13**		
Negative likelihood ratio	**0.24 (0.16, 0.37)**	**0.05**			**0.21 (0.11, 0.41)**	**0.07**		
Inverse negative likelihood ratio	**4.13 (2.72, 6.25)**	**0.87**			**4.68 (2.43, 9.01)**	**1.56**		
Model characteristics	Covariance between estimates of Expected value of logit[Sensitivity] and Expected value of logit[Specificity]: -137.82452 Log likelihood = -0.0247892				Covariance between estimates of Expected value of logit[Sensitivity] and Expected value of logit[Specificity]: -0.06415 Log likelihood = -79.102622			

2.6 Figures

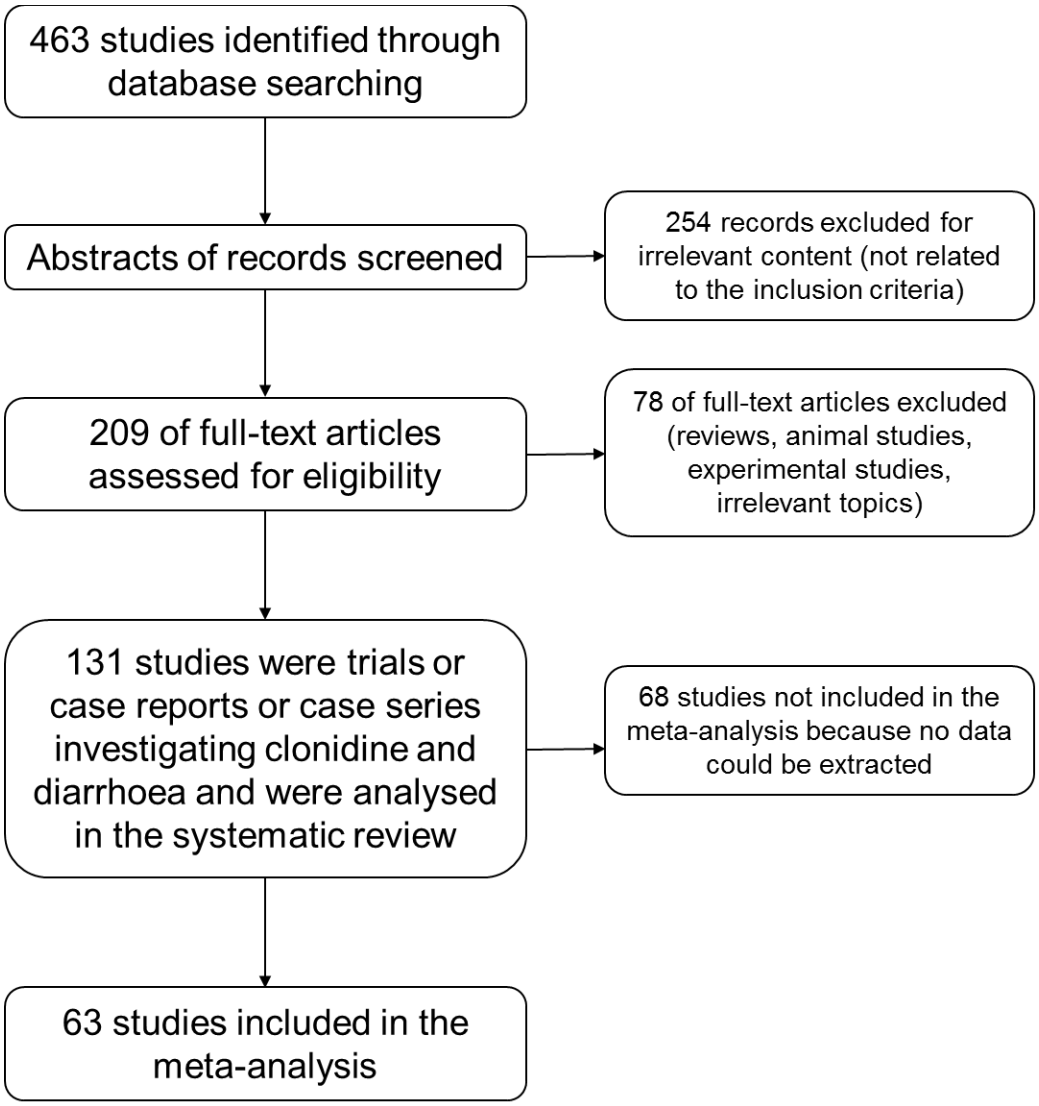


Figure 2.1. Flow chart for systematic review.

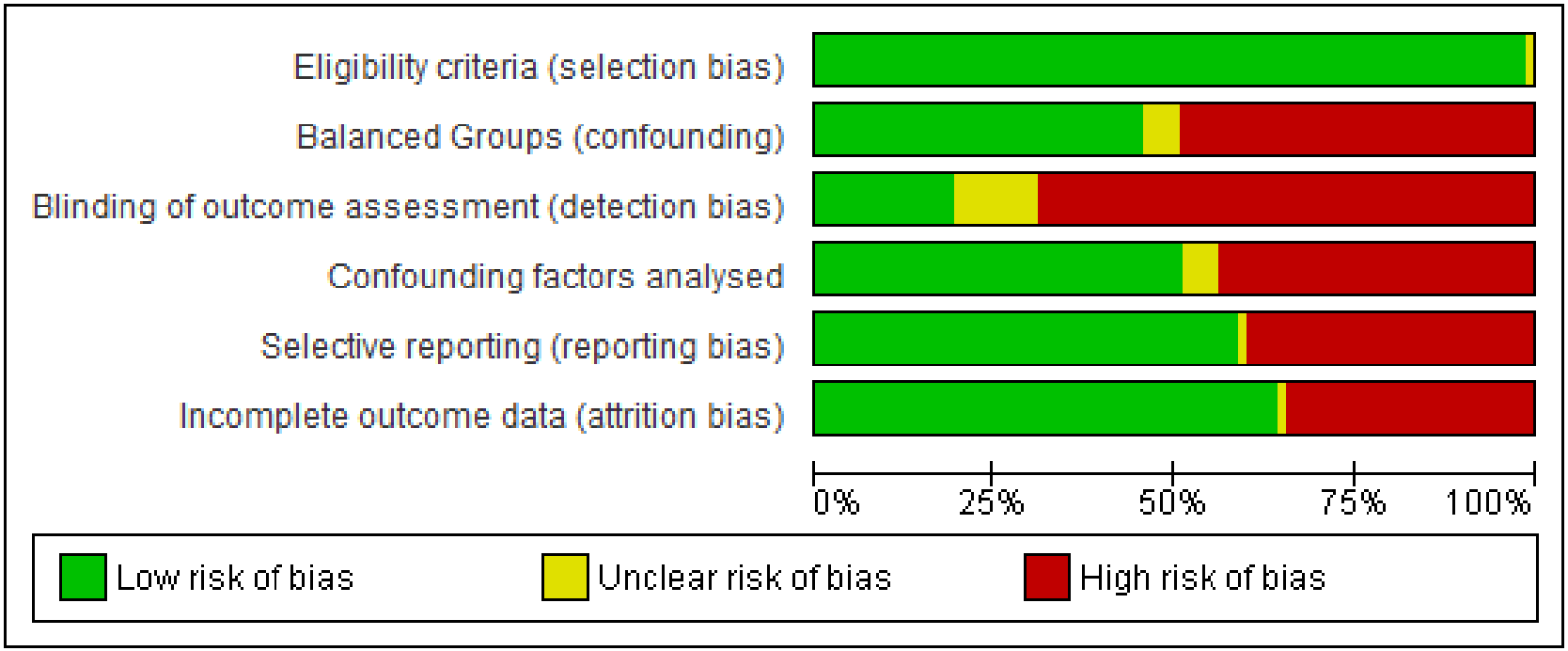


Figure 2.2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies (all studies).

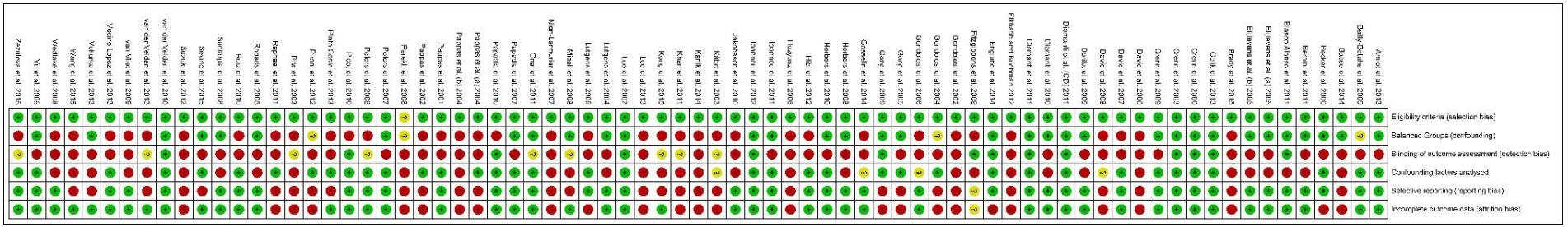


Figure 2.3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

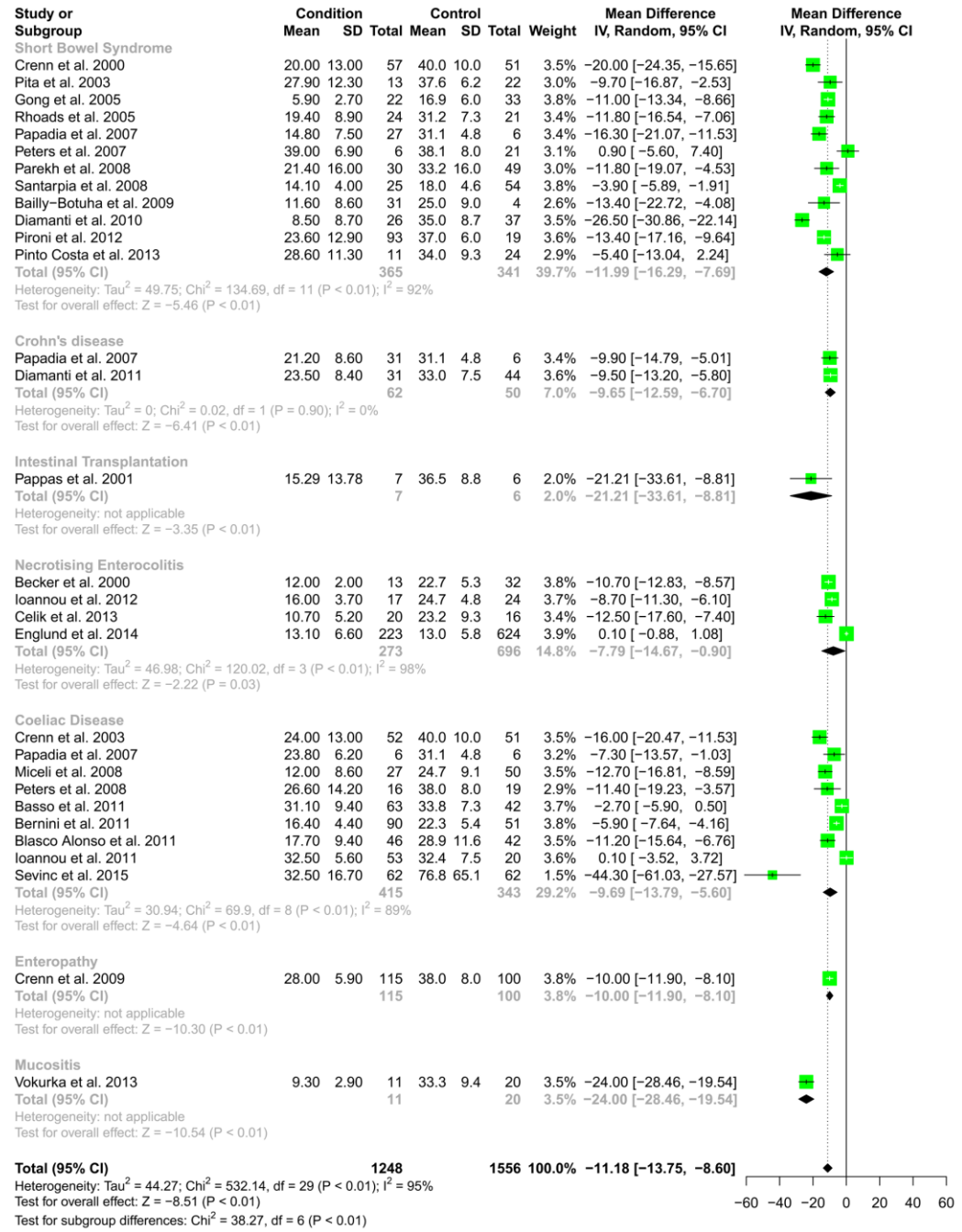


Figure 2.4. Forest plot with overall mean difference of patients with a condition against controls (30 studies) (overall and subgroups).

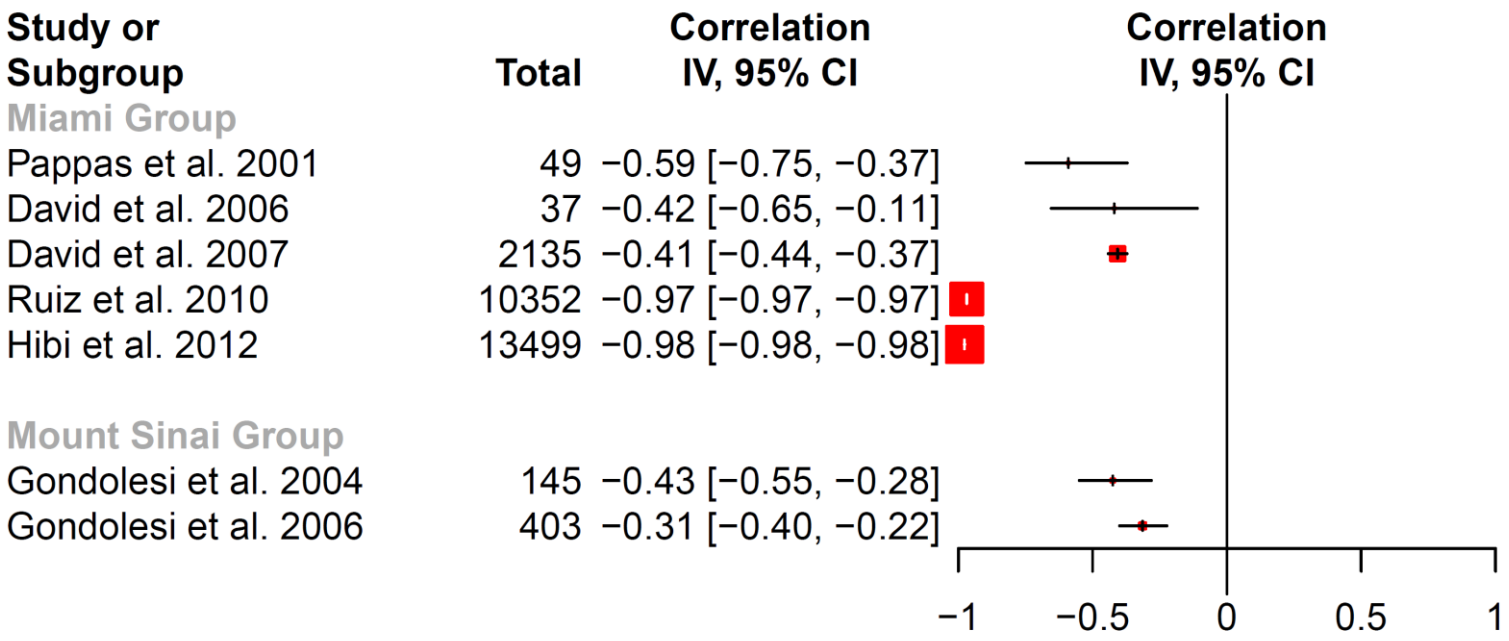


Figure 2.5. Forest plot of Intestinal Transplantation studies (Citrulline concentrations correlations with rejection) – without meta-analysis due to severe heterogeneity.

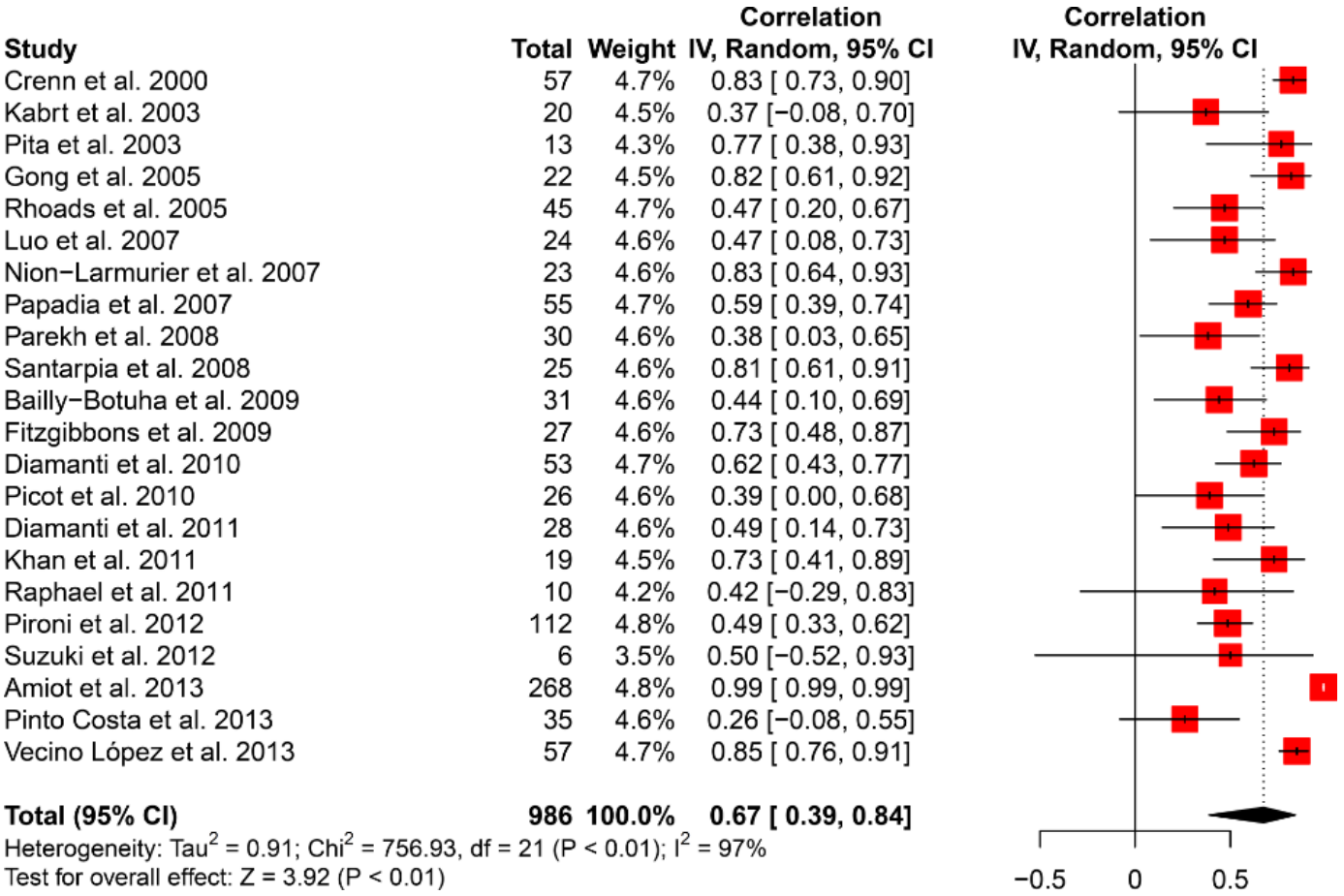


Figure 2.6. Forest plot of studies measuring plasma citrulline in short bowel syndrome. Correlation of citrulline with small bowel length.

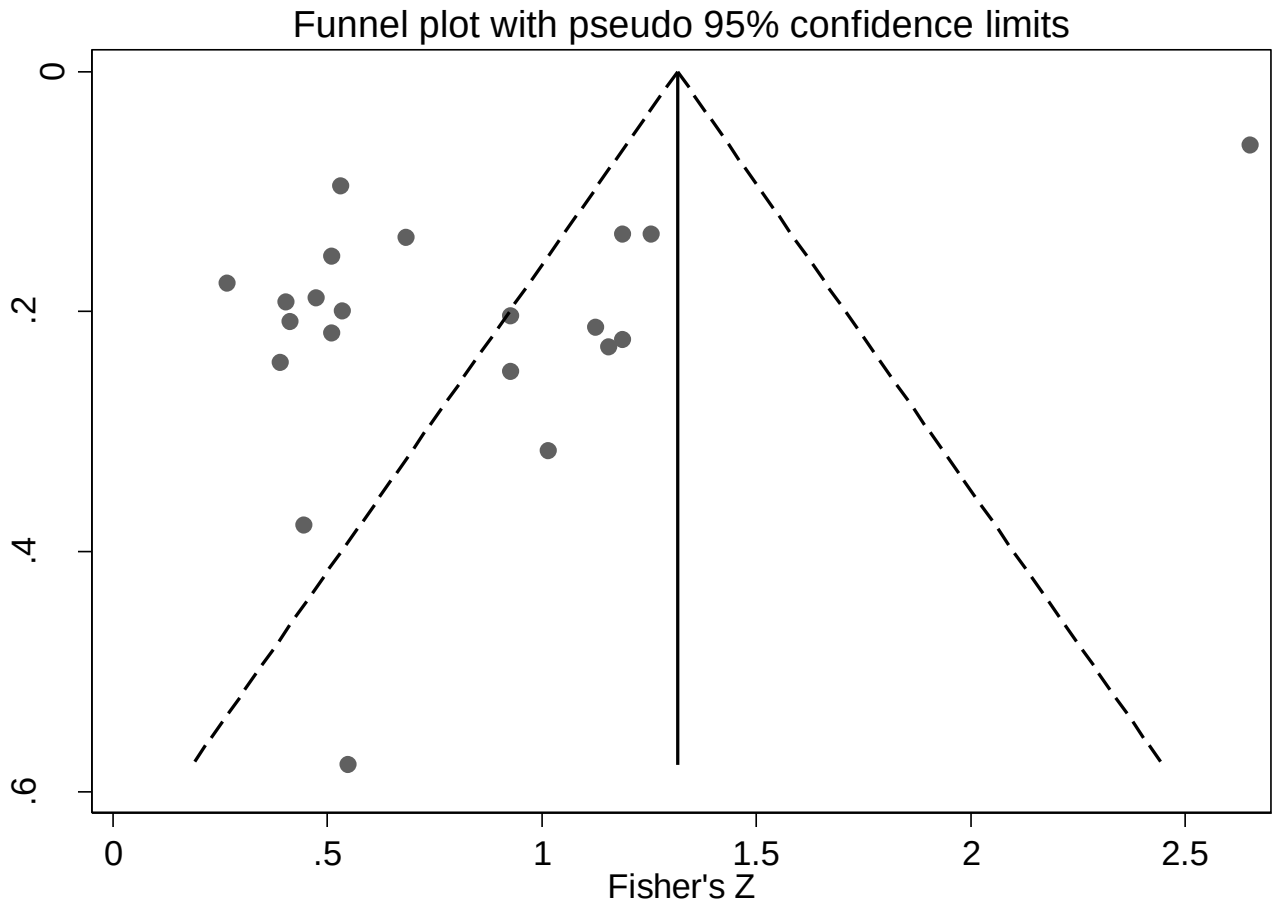


Figure 2.7. Funnel plot of short bowel syndrome studies. Correlation of citrulline with small bowel length – there is asymmetry in the plot indicating publication bias.

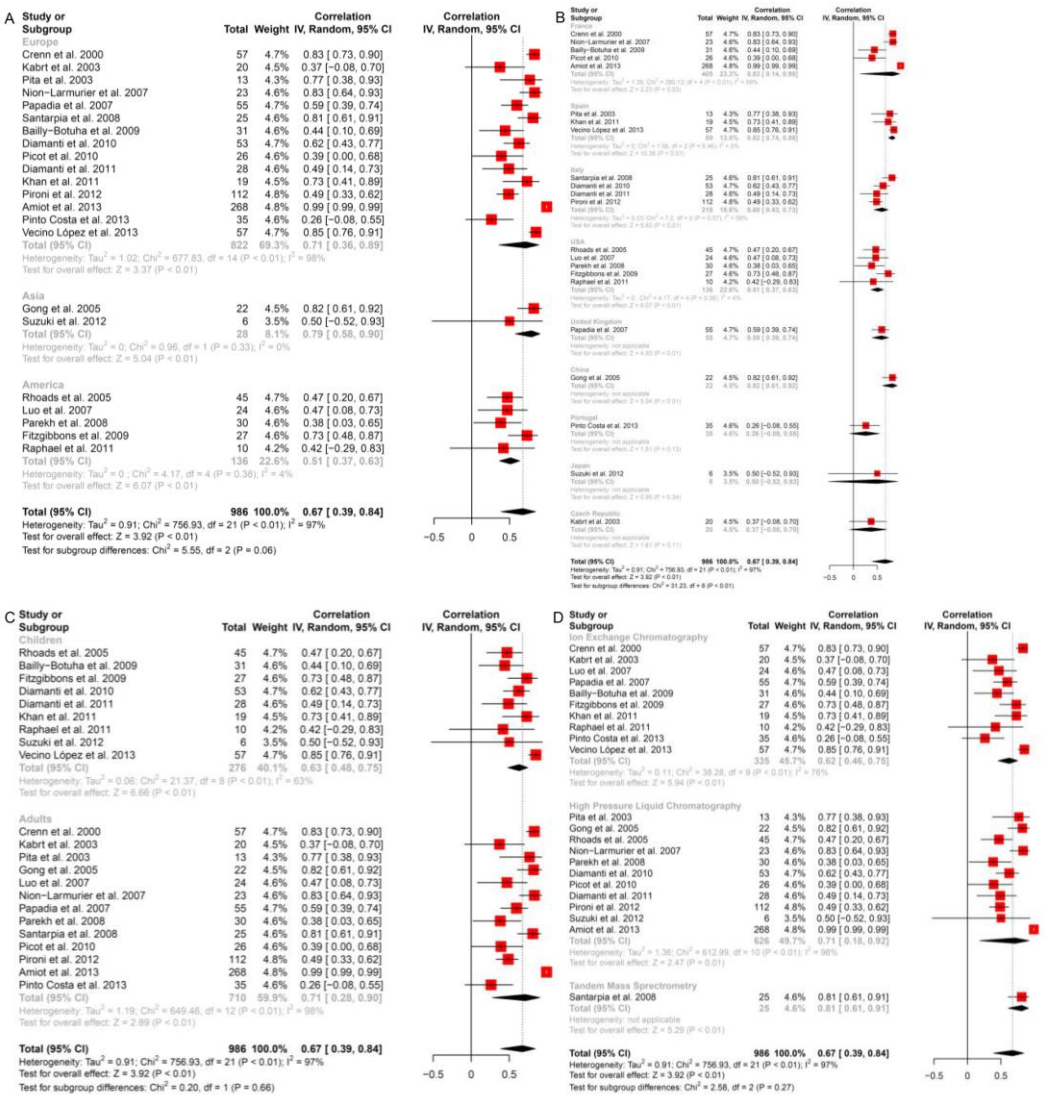


Figure 2.8. Forest plots of short bowel syndrome correlations with small bowel length – subgroup analyses by (A) continent, (B) country, (C) patient type, and (D) citrulline measurement method.

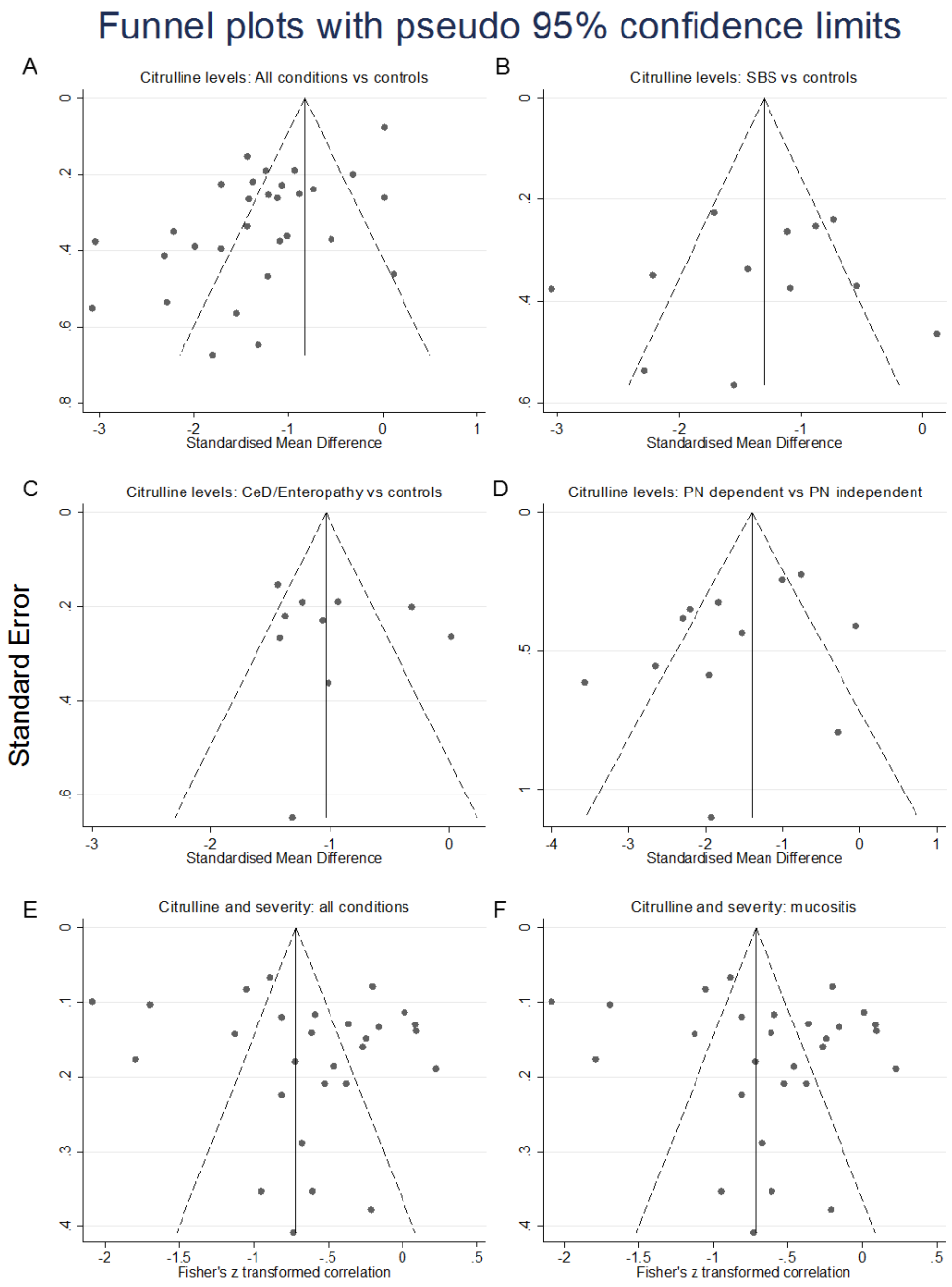


Figure 2.9. Funnel plots. A. Mean citrulline levels: All conditions vs controls (30 studies). No asymmetry seen. B. Mean citrulline levels: SBS vs controls. No asymmetry observed. C. Mean citrulline levels: CeD/Enteropathy vs controls. No asymmetry observed. D. Mean citrulline levels: PN dependent vs PN independent patients. No asymmetry observed. E. Citrulline levels with disease severity in all conditions (28 studies). No asymmetry seen. F. Citrulline levels with disease severity in mucositis after chemoradiation. No asymmetry observed.

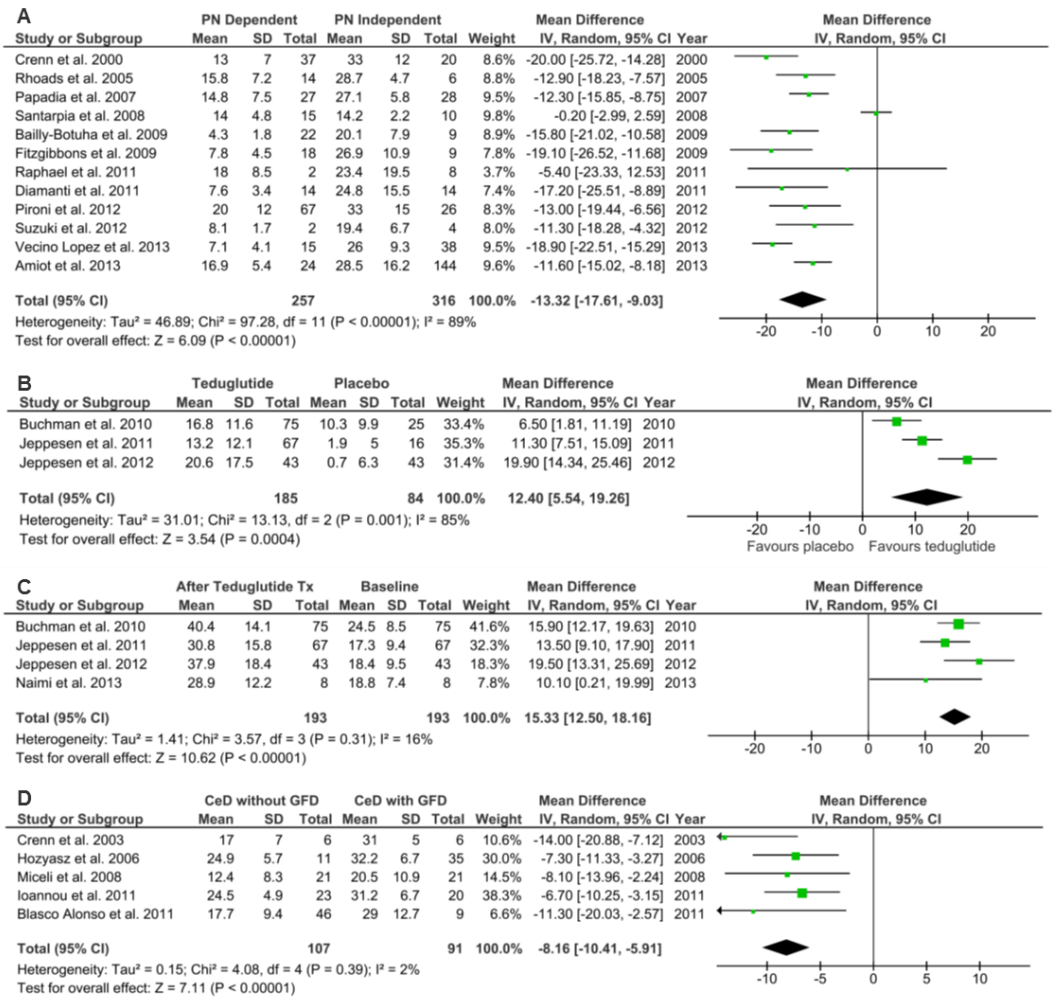


Figure 2.10. Forest plots. (A) Mean differences of citrulline levels between PN dependent and independent SBS patients. (B) Mean increase of citrulline levels after treatment with teduglutide vs placebo in SBS patients. (C) Mean increase of citrulline levels after treatment with teduglutide vs baseline in SBS patients. (D) Mean difference of citrulline levels in coeliac disease patients who had received GFD treatment vs those who had not.

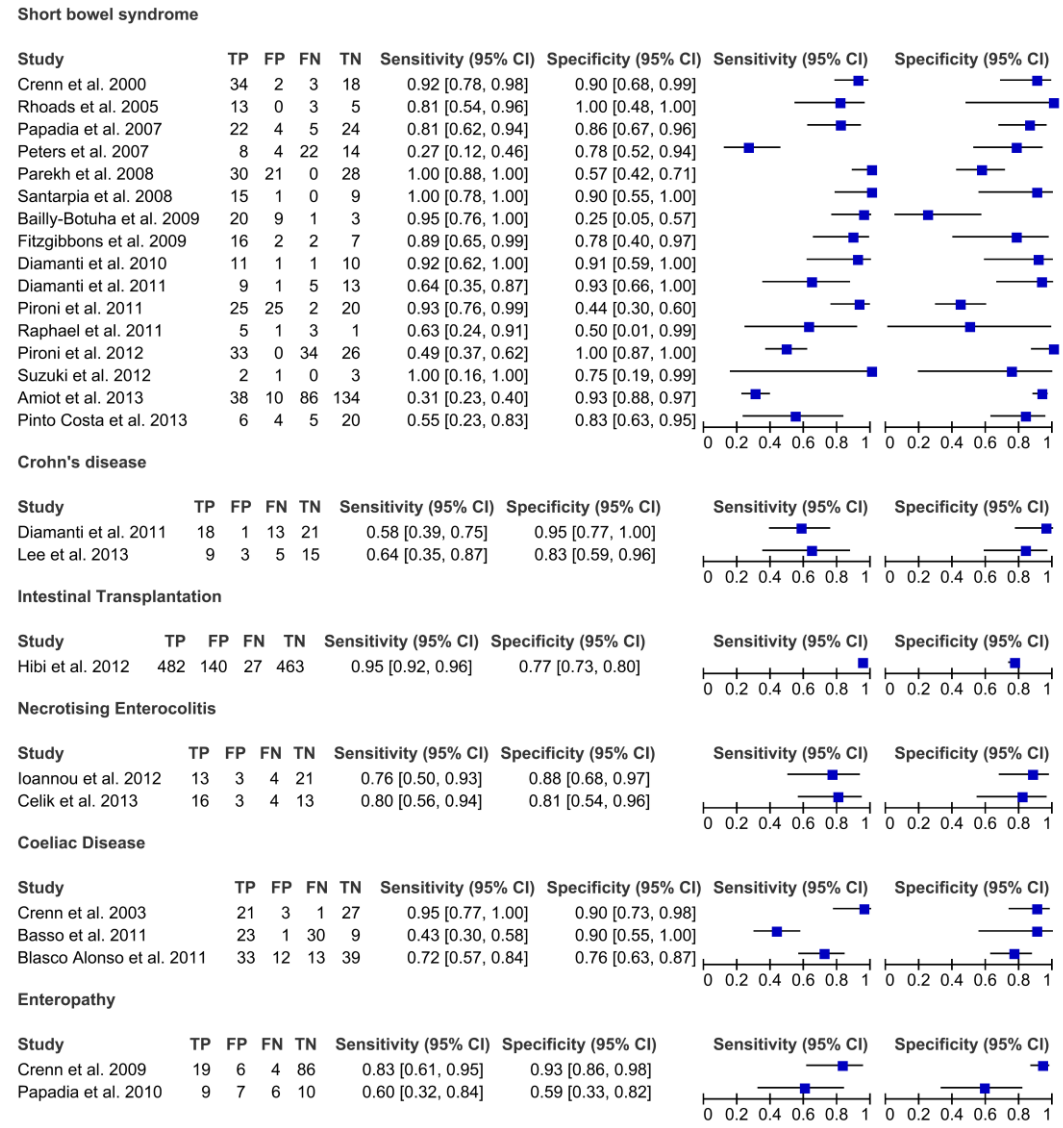


Figure 2.11. Forest plots of sensitivity and specificity in all patient conditions (26 studies).

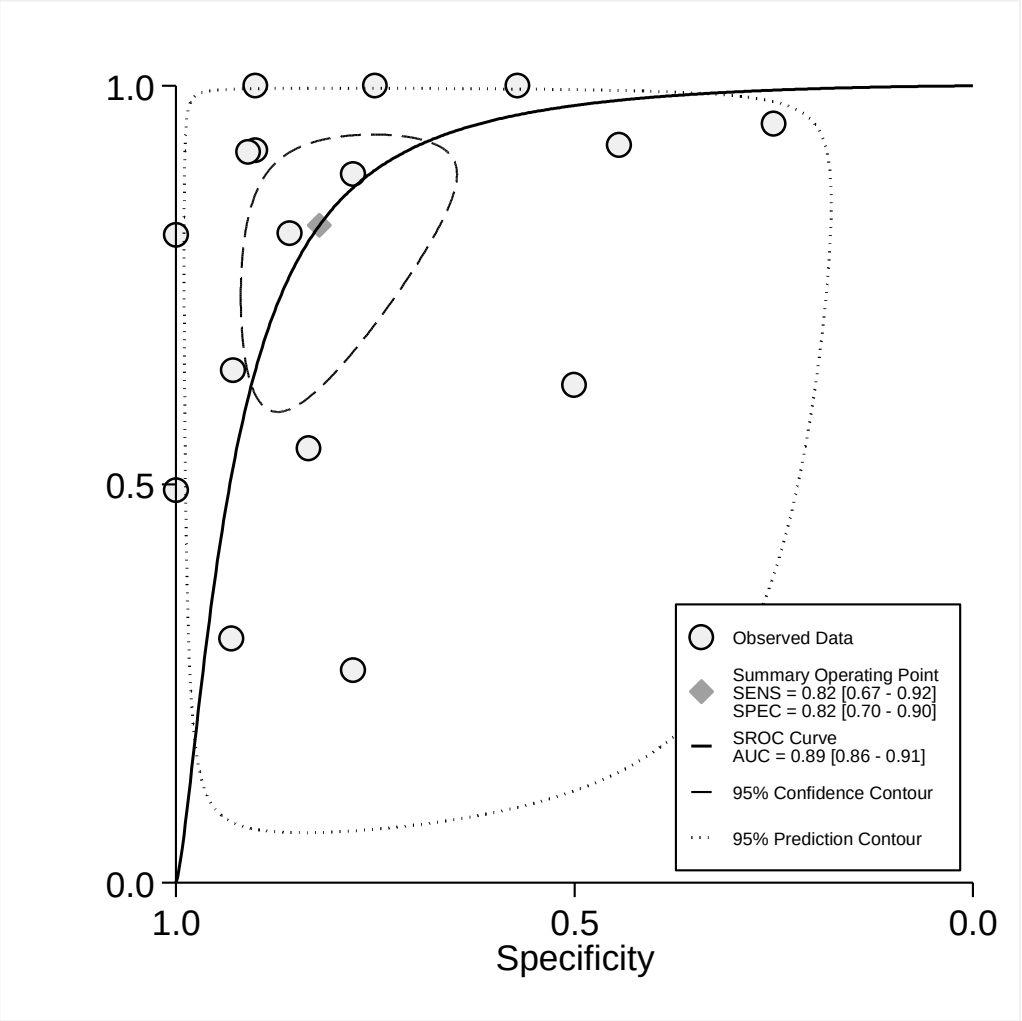


Figure 2.12. Summary ROC curve for diagnostic accuracy in SBS patients.

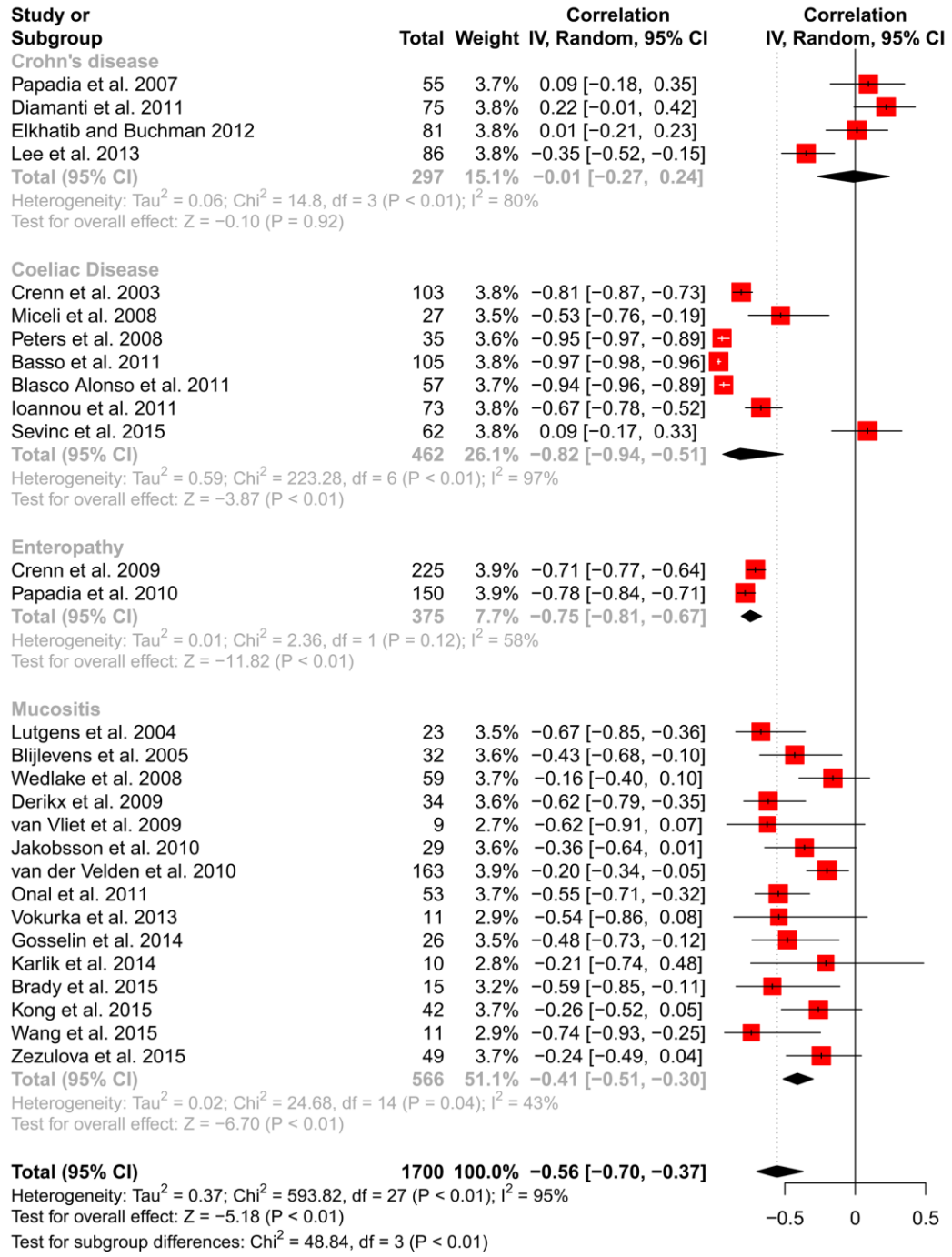


Figure 2.13. Forest plot with correlation coefficients with severity in all available studies (28 studies).

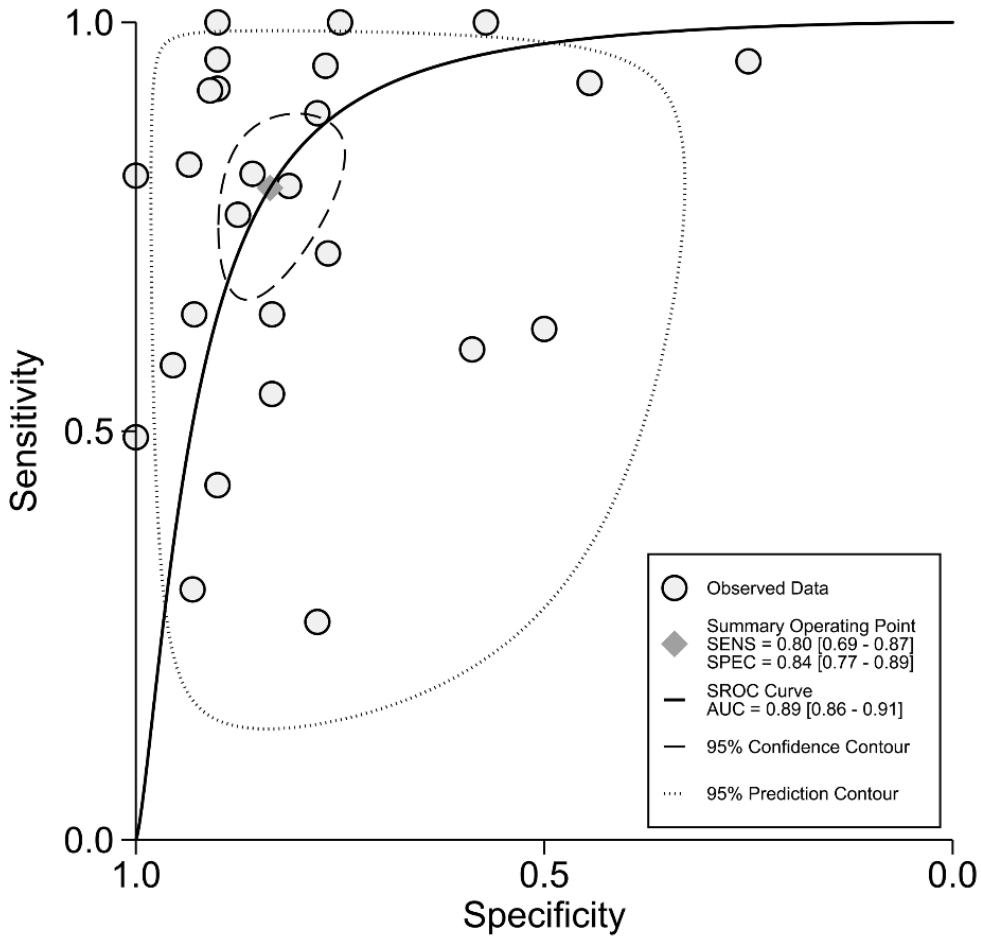


Figure 2.14. Summary ROC curve for all studies of diagnostic accuracy (26 studies).

Study	Total	Weight	Correlation IV, Random, 95% CI
Crenn et al. 2000	57	7.8%	0.48 [0.25, 0.66]
Gong et al. 2005	22	6.9%	0.56 [0.18, 0.79]
Rhoads et al. 2005	45	7.6%	0.85 [0.74, 0.92]
Blijlevens et al. 2005	32	7.4%	0.24 [−0.12, 0.54]
Lutgens et al. 2005	10	5.3%	0.00 [−0.63, 0.63]
Luo et al. 2007	24	7.0%	−0.05 [−0.44, 0.36]
Papadia et al. 2007	55	7.8%	0.42 [0.17, 0.61]
Peters et al. 2007	51	7.7%	0.20 [−0.08, 0.45]
Fitzgibbons et al. 2009	27	7.2%	0.63 [0.33, 0.81]
Gong et al. 2009	61	7.8%	0.94 [0.90, 0.96]
van Vliet et al. 2009	9	5.0%	0.00 [−0.66, 0.66]
Picot et al. 2010	26	7.1%	0.12 [−0.28, 0.49]
Papadia et al. 2010	150	8.2%	0.43 [0.29, 0.55]
Diamanti et al. 2011	28	7.2%	0.69 [0.43, 0.85]
Total (95% CI)	**597**	**100.0%**	**0.50 [0.26, 0.68]**

Heterogeneity: $Tau^2 = 0.24$; $Chi^2 = 130.94$, df = 13 ($P < 0.01$); $I^2 = 90\%$

Test for overall effect: $Z = 3.81$ ($P < 0.01$)

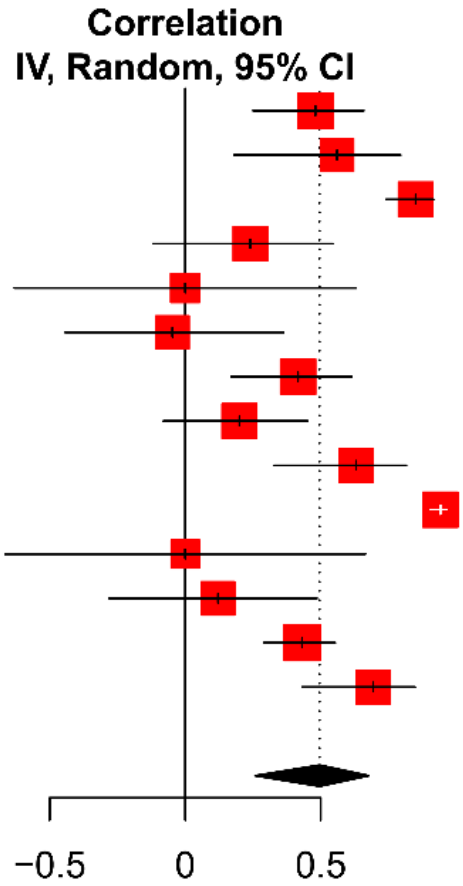


Figure 2.15. Forest plot with correlation coefficients of citrulline levels with enteral absorption (14 studies).

..

Chapter 3

Pharmacodynamic Study of Post-absorptive Plasma Citrulline after Oral Amino Acid Challenges

3.1 Introduction

Until now, it has been shown that citrulline is a significant diagnostic marker in intestinal failure patients. However, it is important for physicians to have better diagnostic tools at their disposal in order to tackle this disease. The studies mentioned in Chapter 2 take static measurements of citrulline in certain clinical conditions. These are mostly observational and show a static correlation of citrulline and intestinal function. There are no studies examining how amino acid challenges could reflect enterocyte function.

There is a central problem with the past data that although predictive for populations, the predictive value of a given citrulline level for an individual patient is poor i.e. not a sufficiently bimodal distribution. Pharmacodynamic challenge tests are an important step in diagnostic test development. Following paradigms from endocrinology and psychopharmacology, where challenge tests are integral to research and clinical practice (e.g. short synacthen test, glucose challenge test etc.), the Guideline for Good Clinical Practice of the International Conference on Harmonization may possibly be of great help in standardizing the development of challenge tests (EMEA, 1997; Gijsman *et al.*, 2004). Challenge tests are particularly useful in situations where measured markers are part of a physiological axis or pathway and challenging a member of this pathway is useful to clarify where the pathway might be failing.

This gap in nutrition literature is what this chapter addresses. I give the outline of a certain study design executed in order to explore the pharmacodynamic diagnostic properties of citrulline.

Study: "Randomised cross-over, single-site study on the bioavailability of Citrulline after an oral bolus of Citrulline or Glutamine or Arginine or 3-Methylhistidine or placebo in healthy subjects".

The present chapter gives a description of the actual design of this pharmacodynamics study with sections dedicated to trial design, sample size, and

laboratory analysis of samples. The sections have theoretical descriptions in between, where topics on sample size, crossover designs, randomisation, carryover and washout effects are discussed. First, I begin with a theoretical discussion on the design of experiments.

3.2 Design of Experiments: A (Theoretical) Primer

Before a detailed description of the clinical study is given, basic concepts regarding design of experiments are outlined. Design of experiments is a special chapter in statistics, which one can easily access by reading the books by Cox and Reid (2000), Giesbrecht and Gumpertz (2004) or Cochran and Cox (1957) among others. In this section, I intend to give some basics in the design of experiments and to elaborate deeper in crossover designs.

Thus, to begin with it is necessary to mention that statistics can have the greatest impact on the quality of an experimental study while in the planning stage. The feasibility of any experiment is constrained by limitations on time, equipment, funds, and ethical considerations. Ethical constraints range from sample size calculations which is related to use of resources (e.g. large experiments use more resources than needed but small experiments waste resources because they fail to give answers) to serious considerations, such as causing pain and suffering to living organisms or even to fellow human beings, or harming the environment. The goals of statistical experimental design are to provide tools that help to ensure that the experiment will be capable of providing answers to the research questions posed and that it will provide the information as efficiently as possible using the available resources and respecting all ethical constraints. An experimental plan that does not respect ethical constraints is not a good plan.

The statistician is rarely the lead investigator in scientific investigations, but statistics has some valuable insights and tools to offer to the process of designing experiments. Questions that come up when an experiment is designed involve:

1. How many participants should be examined? How much replication should there be?
2. How does one construct the experiment to provide as broad a basis for inference as possible?

3. How does one take into account the natural variability among experimental units?
4. How should treatments be allocated to the entities being studied?
5. How is the statistical analysis to be performed?

For these questions, there are four key principles of design of experiments that need to be emphasized:

(1) representativeness, (2) randomisation, (3) replication, and (4) error control or blocking. These ideas are fundamental to making an experiment useful for providing answers to the research questions. Representativeness, proper randomisation, and sufficient replication and error control are the grounds upon inferences can be made without bias and with enough precision to be meaningful. Randomisation provides insurance that unanticipated factors do not accidentally introduce unwanted biases into experimental results. Replication and error control are necessary to ensure that results are generally true and not just the product of the special circumstances of a particular run (Cochran and Cox, 1957; Giesbrecht and Gumpertz, 2004).

The process of designing an experiment has three parts. First, it is necessary to understand the objectives and constraints clearly, state what quantities are to be estimated, and identify what hypotheses are to be tested. The comment that a problem is too complex, or involves too many ethical or financial constraints for a properly designed experiment, reflects poor understanding of the experimental process. The second task is to consider possible designs that are tailored to the aims of the experiment and yet satisfy all ethical, physical, and financial constraints. The final part of the process is to evaluate the proposed design to establish that the objectives can be achieved, that the estimates are likely to have sufficient precision, and that any proposed tests of hypotheses have adequate power. Outlining the statistical analysis before the experiment is actually conducted is always a good idea. It allows the investigator to see, clearly, what can be estimated and what hypotheses can be tested before any data are collected. It is not uncommon to find an experiment that appears reasonable and yet on closer examination fails to provide estimates of important quantities or does not allow testing the hypotheses of interest because of insufficient replication or flawed allocation of treatments.

Outlining the statistical analysis during the planning stage helps prevent such mistakes (Cochran and Cox, 1957; Giesbrecht and Gumpertz, 2004).

3.2.1 Replication and Handling Unexplained Variability

The precision (or inversely, the variability) of estimates and the power of hypothesis tests depend on the amount of replication and the magnitude of the unexplained variation among experimental units. In general, the more replication, the more precise the estimates (Cox and Reid, 2000). The amount of replication and the pattern of how many units are assigned to each treatment are things that the investigator can control. If an experiment is not replicated properly, it may not be possible to estimate the precision at all. It is important to know how precise estimates are in order to have some idea of the likelihood or assurance that an estimate falls within reasonable distance of the true (but unknown) value.

In some cases it is possible to reduce unexplained variation by using the technique of blocking (Bailey, 2008). This does not reduce the unexplained variation per se, but it assigns treatments to experimental units in such a way that the unexplained variation is screened out before making comparisons among the treatments (Gates, 1995).

Finally, the unexplained variation can often be reduced by improving the model. This can be done by building in additional explanatory variables, also called covariates, or by changing the form of the model. The decision to measure additional variables should be made in the planning stages before the experiment is executed (Montgomery, 2009).

Of the three methods described: (1) improving experimental technique, (2) blocking, and (3) improving the model, the first is usually up to the subject-matter scientist, and the latter two are statistical devices. The goal of all three is to reduce the unexplained variation so that the effects of treatments stand out from the background noise and can be identified more easily (Giesbrecht and Gumpertz, 2004).

3.2.2 Randomisation: Why and How

The original notion of randomization is usually credited to Fisher (1935) and is accepted by most experimenters. Unfortunately, not all practice it. Too often,

haphazard assignment of treatments to experimental units is the practice used instead of true randomization. The two are not the same. Another common error is to assume that any randomization scheme that assigns each treatment to every experimental unit equally frequently is adequate. This is not true. For proper randomization, all possible assignments of treatments to experimental units must be equally likely to appear. An alternative but fully equivalent requirement, which is often easier to verify, is that each pair of treatments (control can be thought of as a treatment) has exactly the same chance of being assigned to every pair of experimental units (Hader, 1973).

Why randomize? Cox (1958, p. 85) states the positive advantages of randomization as assurances that:

(a) "In a large experiment it is very unlikely that the estimated treatment effects will be appreciably in error."

(b) "The random error of the estimated treatment effects can be measured and their level of statistical significance examined, taking into account all possible forms of uncontrolled variation."

3.2.3 Ethical Considerations

The ethical considerations are complicated and difficult in studies involving humans. In the clinical trials setting, and in fact in any experimental situation that deals with human subjects, the problem of whether to randomize treatment allocation tends to be confounded with the question of ethics. Is it ethical for a physician to allow some random device decide whether a patient is to receive a specific treatment?

3.2.4 Study Design

Steps to design an experiment include the design itself, calculating the sample size and performing randomisation. Important goals and purposes include:

1. To understand the objectives and constraints clearly, state what quantities are to be estimated, and identify what hypotheses are to be tested.
2. To consider possible designs that are tailored to the aims of the experiment and yet satisfy all ethical, physical, and financial constraints.

3. To evaluate the proposed design to establish that the objectives can be achieved, that the estimates are likely to have sufficient precision, and that any proposed tests of hypotheses have adequate power.

3.3 Study Design and Background of Study: Citrulline Bioavailability in Healthy Subjects

Despite many positive reports correlating fasting plasma citrulline concentrations to the degree of decreased enterocyte mass in various small intestinal disorders (Crenn *et al.*, 2000; Crenn *et al.*, 2003; Papadia *et al.*, 2007; Papadia *et al.*, 2010), Luo *et al.* (2007) reported no relation between fasting plasma citrulline concentrations and intestinal absorptive capacity in patients with short bowel syndrome. This latter finding was in line with data in patients with villous atrophy caused by coeliac disease and in patients with short bowel syndrome (Peters *et al.*, 2007c; Peters *et al.*, 2008b). Besides, Papadia *et al.* (2007) reported a quadratic (and not a linear) correlation between fasting plasma citrulline concentration and remnant small bowel length, indicating that decreased plasma citrulline concentrations predominantly reflect the extremes of disease spectrum.

Most of the circulating plasma citrulline is derived from glutamine conversion through the glutamate to ornithine pathway in the enterocyte (Curis *et al.*, 2005). Other amino acids such as proline, glutamate and arginine may also serve as precursors for citrulline. Supply of glutamine to the enterocyte occurs from both the arterial blood as well as from the intestinal lumen at the rate of 25%-33%, and 66%, respectively. Van De Poll *et al.* (2007a); Van De Poll *et al.* (2007b) recently reported glutamine consumption by the intestine to be dependent on glutamine supply and observed that 13% of intravenously administered glutamine taken up by the intestine was converted into citrulline. Citrulline appears to be an important source of nitric oxide, increasing even more the availability of it through arginine (Osowska *et al.*, 2008). 3-Methylhistidine is another important amino acid which has been tested as an absorption marker for the intestine and could function effectively as a way to detect a signal to noise ratio. As glutamine is the natural precursor of citrulline while arginine has been mentioned to produce citrulline, we hypothesized that loading the enterocyte with orally administered citrulline or glutamine or arginine or 3-methylhistidine might increase citrulline output to an extent reflecting enterocyte functional capacity. The rational for choosing each

amino acid will be described with details and references in section 3.3.2. Each amino acid will be given separately from the others in a cross-over design explained in section 3.3.4.

A similar experiment has been executed by Peters *et al.* (2007c); Peters *et al.* (2008b), commonly referred as *citrulline generation test*, but the authors did not include control challenges in their study, such as arginine, citrulline or 3-methylhistidine. This study did not include a comparison with placebo as well and open questions worth examining remained. Their results indicated that following the oral bolus of glutamine, plasma citrulline concentrations showed a time dependent rise in healthy subjects of 44% with higher rates of increase in healthy subjects compare to coeliac disease patients. Additionally, in patients with short bowel syndrome, the citrulline generation test indicated a higher incremental area under the curve till 90 minutes in adapted (defined as over 24 months since their final digestive circuit modification) compare to non-adapted cases with borderline significance but overall fasting plasma citrulline concentrations had poor test characteristics for detection of decreased intestinal energy absorption capacity in patients with enterocyte damage. Hence, their challenge test indicated an interesting potential but left many unanswered questions due to the fact that there weren't control challenges, design biases with unbalanced groups and possible confounding bias (see Figure 2.2 from the previous chapter).

3.3.1 Hypotheses

My primary hypothesis is that a single oral bolus of glutamine or citrulline or arginine or 3-methylhistidine or placebo results in a time-dependent increase in plasma citrulline concentrations in healthy subjects in order to establish a normal range before repeating the doing the same procedure later on in intestinal failure patients. I hypothesize that this loading to reflect enterocyte mass, thus showing a delayed or attenuated citrulline response in patients with intestinal failure. 3-Methylhistidine and placebo will serve as controls allowing to test with rigour whether citrulline, arginine or glutamine have an effect.

Secondary Hypotheses:

1. Are there differences in the bioavailability of citrulline after giving the five treatments (3-methylhistidine, arginine, glutamine, citrulline or placebo)?

2. Is there a significant difference in the period which each is given? Each amino acid will be given in a difference sequence and this could produce a period bias. This is discussed below in study design.
3. Is there a significant difference between the sequences of treatment?
4. I will investigate as well the bioavailability of the amino acids glutamine, arginine, ornithine and 3-methyl-histidine. These are related to the boluses given because they are connected through the biochemical pathways discussed in Chapter 1. For these amino acids, I postulate as well the same questions:
    a. Are there differences in the bioavailability of these amino acids after giving the five treatments (3-methylhistidine, arginine, glutamine, citrulline or placebo)?
    b. Is there a significant difference in the period which each is given for these amino acids?
    c. Is there a significant difference between the sequences of treatment for these amino acids?

3.3.2 Rationale for Using these Particular Amino Acids

Citrulline has shown a high diagnostic sensitivity in previous studies with intestinal clinical conditions, as discussed in Chapter 2. Arginine and glutamine are precursors of citrulline. For 3-methylhistidine, I believe the following reasons make it important for using it as a challenge:

- The absorption of the free 3-methylhistidine is rapid.
- The serum levels are proportional to the amount of ingested muscular proteins; thus, it may be a useful tool for studying the pathophysiology of protein absorption in man.
- It is intended to be used as a marker that will standardize the other measurements, giving greater discrimination (tolerance test).

3.3.3 Pharmacokinetics

Arginine and the related amino acids glutamine and citrulline are widely used at pharmacological dosages for various purposes in a number of situations, e.g., athletes, bodybuilders, elderly and immunocompromised patients. When studied in

healthy subjects, the main aim of amino acid supplementation is to elicit growth hormone secretion or to sustain nitric oxide production.

Arginine. In a study by Tangphao *et al.* (1999), 10 healthy volunteers (6 males, 4 females) aged 23-52 years received 10 g of L-arginine in 100 mL water. Fürst (2001) mentions that we can reach even to 20 g of oral amino acid; hence, according to this data, arginine was administered orally in 20 g for one single dose, followed by a washout period of one week.

Glutamine. L-glutamine is a protein amino acid found in proteins of all life forms. It is classified as a semi-essential or conditionally essential amino acid. This means that under normal circumstances the body can synthesize sufficient L-glutamine to meet physiological demands. However, there are conditions where the body cannot do so. Recently, L-glutamine has come to be regarded as one of the most important amino acids when the body is subjected to such metabolic stress situations as trauma (including surgical trauma), cancer, sepsis and burns. Under such conditions, L-glutamine becomes an essential amino acid, and it is therefore very important to ensure adequate intakes of the amino acid in order to meet the increased physiological demands created by these situations.

L-glutamine is the most abundant amino acid in the body, and plasma glutamine levels are the highest of any amino acid. L-glutamine is predominantly synthesized and stored in skeletal muscle. The amino acid L-glutamate is metabolized to L-glutamine in a reaction catalysed by the enzyme glutamine synthase. The typical dietary intake of L-glutamine is 5 to 10 g daily. The dose can reach to 20 g (Fürst, 2001), and Ziegler *et al.* (1990) reached the dose to 0.3 g/kg (around 21 g in a 70 kg person). In the present study, L-glutamine was administered orally in one single dose of 20g, followed by a washout period of one week.

Citrulline. Citrulline is a non-protein amino acid that is present in substantial amounts in watermelon (*Citrullus vulgaris*), with a mean content of 2.1 mg/g fresh weight, ranging from 0.5 to 3.6 mg/g according to variety (Curis *et al.*, 2005). A pharmacokinetic study was performed in six healthy adults receiving 3.3 kg wet weight of the red fruit of a ripe watermelon (Mandel *et al.*, 2005); the study is limited by uncertainty as to the actual amount of citrulline ingested, but the average citrulline intake was probably around 7 g. Moinard *et al.* (2008) studied eight young male healthy adults (age: 27.6 ± 1.5 years; BMI = 22.3 ± 0.5 kg/m^2) who received

2 g, 5 g, 10 g, or 15 g of citrulline in random order on separate occasions, with a 15 day washout between doses. The dose can reach to 20 g (Fürst, 2001), which was administered orally in the present study in one single dose, followed by a washout period of one week.

3-Methylhistidine. This amino acid is produced by methylation of the actin and myosin peptide chains in the muscle. Metabolism after intravenous administration of L-3-methylhistidine involves excretion in the urine of 75% of the administered dose in 24 hours and 95% in 48 hours. The plasma disappearance curves of [^{14}C]3-methylhistidine suggested a half-life of approximately 130 minutes (Long *et al.*, 1975). Bachmann *et al.* (1984) used 3-methylhistidine for the assessment of intestinal protein absorption by studying its plasma kinetics for potential use as a marker of protein degradation. Oral 3-methylhistidine was given in doses 20 g, 60 g or 120 mg. The absorption of the free 3-methylhistidine is rapid and reaches a peak after 75 minutes. The peak is delayed after meat ingestion and reaches a maximum after 180 minutes. Peak height and area under the plasma concentration versus time curve were proportional to the dose received, indicating first order kinetics for 3-methylhistidine. It has a half-life of 12.2 hours. Hence, 3-methylhistidine was administered orally as 120 mg for one single dose, followed by a washout period of one week.

Placebo. Dextrose (glucose) was used in a dose of 20 g in this study. This is line with the study of Bachmann *et al.* (1984), who used glucose as placebo against 3-methylhistidine, and Figueroa *et al.* (2009), who used maltodextrine against oral citrulline.

NOW Foods (Bloomingdale, IL, R&D Scientist Dr L. Ber) kindly provided glutamine, citrulline, arginine and placebo for the study; 3-methyl-histidine was used from Sigma-Aldrich.

3.3.4 Trial Design

For the detection of difference between these four loadings and placebo, a crossover design was chosen. It is a randomised cross-over single-site study on the bioavailability of predominantly citrulline after an oral bolus of citrulline, glutamine, arginine, 3-methylhistidine or placebo in healthy subjects. Secondary measurements included measurements of the amino acids glutamine, arginine,

ornithine and 3-methylhistidine as well. In order to have a balanced design, free of carryover effects, each participant was allocated in a random manner to one of the ten sequences of the Williams balanced cross-over design, the optimum design for reducing carryover effect in crossover designs. After screening, the participants began receiving the first oral load of the sequence they were randomised to and subsequently had the appropriate blood samples taken. The first load and measurements lasted six hours. After this, the participant had a washout period of one week and returned for their second loading and measurements. This was repeated five times for each participant and then the study ended. The detailed diagram for each participant is shown in Figure 3.1. In case participants dropped out, the sequence was allocated again to a new participant.

3.3.4.1 Statistical Theory behind Crossover Designs

A crossover design is a modified, randomized block design in which each block receives more than one formulation of a drug at different periods.[1] A block may be a subject or a group of subjects. Subjects in each block receive a different sequence of formulations. A crossover design is called a complete crossover design if each sequence contains each of the formulations (Chow and Liu, 2008). For a crossover design, it is not necessary that the number of formulations in each sequence be greater than or equal to the number of formulations to be compared. We shall refer to a crossover design as a $g \times p$ crossover design if there are g sequences of formulations administered at p different periods. For bioavailability and bioequivalence studies, the crossover design is viewed favourably by the US Food and Drug Administration (FDA) and other regulatory agencies in the world, such as the European Medicines Agency (EMA), because of the following advantages:

1. Each subject serves as their own control. It allows a within-subject comparison between formulations.
2. It removes the inter-subject variability from the comparison between formulations.

[1]A crossover design is an experimental design in which each *experimental unit (subject)* receives a series of experimental treatments over time. The order in which an experimental unit receives the treatments is called a sequence (example, ABC). The time interval during which a treatment is administered to a subject is called a *period.* A period could range from a few minutes to several months depending on the study. Sequences usually involve subjects receiving a different treatment in each successive period. However, one or more treatments may occur more than once in any sequence (example, AABB). Treatments and periods are compared 'within subject', i.e. each subject acts as its own control. Therefore any component that is related to subject differences is removed from treatment and period comparisons. Carryover effects are residual effects from a previous treatment manifesting themselves during subsequent periods (Shanga, 2003).

3. With a proper randomisation of subjects to the sequence of formulation administrations, it provides the best unbiased estimates for the differences (or ratios) between formulations.
4. This design has the need of fewer subjects than a parallel design, with an important decrease on costs of the study.

The use of crossover designs for clinical trials has been extensively discussed in the literature. See, for example Senn (2002) and Jones and Kenward (2003).

Washout and Carryover Effects

It is helpful to introduce the concepts of washout and carryover effects (or residual effects) in a crossover design because the presence of carryover effects usually has an influence on statistical inference of bioavailability between formulations.

The washout period is defined as the rest period between two treatment periods for which the effect of one formulation administered at one treatment period does not carry over to the next. In a crossover design, the washout period should be long enough for the formulation effects to wear off so that there is no carryover effect from one treatment period to the next. The washout period depends on the nature of the drug. A suitable washout period should be long enough to return any relevant changes that influence bioavailability to baseline (usually, at least five times the blood-plasma elimination half-life of the active ingredient, therapeutic moiety or its metabolite, or the decay of the immediate pharmacological effect since the last sampling time point of the previous period).

If a drug has a long half-life or if the washout period between treatment periods is too short, the effect of the drug might persist after the end of dosing period. In this case, it is necessary to distinguish the difference between the direct drug effect and the carryover effects. The direct drug effect is the effect that a drug product has during the period in which the drug is administered, whereas the carryover effect is the drug effect that persists after the end of the dosing period. Carryover effects that last only one treatment period are called first-order carryover effects. A drug is said to have *c*-order carryover effects if the carryover effects last up to *c* treatment periods. In bioavailability and bioequivalence studies, however, it is unlikely that a drug effect will carry over more than one treatment period because a sufficient length of washout is usually considered.

Crossover Designs for Three or More Formulations

The crossover designs for comparing three or more formulations are much more complicated than those for comparing two formulations. For comparing three formulations of a drug, there are a total of three possible pairwise comparisons between formulations: formulation 1 versus formulation 2, formulation 1 versus formulation 3, and formulation 2 versus formulation 3. It is desirable to estimate these pairwise differences in average bioavailability between formulations with the same degree of precision. In other words, it is desirable to have equal variances for each pairwise differences in average bioavailability between formulations (i.e. $V(\widehat{F}_i - \widehat{F}_j) = \nu\sigma_e^2$) where ν is a constant and σ_e^2 is the intra-subject variability. Designs with this property are known as variance-balanced designs. It should be noted that, in practice, *n* may vary from design to design. Thus, an ideal design is one with the smallest *n*, such that all pairwise differences between formulations can be estimated with the same and possibly best precision. However, to achieve this goal, the design must be balanced. A design is said to be balanced if it satisfies the following conditions (Jones and Kenward, 2003):

1. Each formulation occurs only once with each subject.
2. Each formulation occurs the same number of times in each period.
3. The number of subjects who receive formulation *i* in some period followed by formulation *j* in the next period is the same for all $i \neq j$.

Under the constraint of the number of periods (p) being equal to the number of formulations (t), balance can be achieved by using a complete set of orthogonal Latin squares (Jones and Kenward, 2003). However, if $p = t$, a complete set of orthogonal Latin squares consists of $t \times (t\text{-}1)$ sequences except for $t = 6$. As a result, when the number of formulations to be compared is large, more sequences and consequently more subjects are required. This, however, may not be of practical use.

A more practical design has been proposed by Williams (1949). This shall be referred to as a Williams design. A Williams design possesses balance property and requires fewer sequences and periods. The algorithm for constructing a Williams design with t periods and t formulations is summarized in the numerical steps mentioned in the next section (Jones and Kenward, 2003).

Williams Designs: Construction and Randomization

The Williams design is a special case of the cross-over and Latin square designs. A Latin square, in which every treatment is represented once, and once only, in each column and in each row, yields uniform cross-over designs; it represents uniformity both within periods and within sequences (Wang *et al.*, 2009). To achieve the highest possible efficiency, the design must be balanced. In a balanced design, each of the treatments occurs the same number of times in each period and the number of subjects who receive treatment *i* in one period and treatment *j* in the next period is the same for all $i \neq j$. Fisher and Yates (1963) give examples of Latin square designs. Some of them have additional properties that other Latin squares do not. For example, in the design listed in Table 3.1, every treatment follows every other treatment the same number of times. Such a cross-over design, which was introduced by Williams (1949), is said to be balanced with respect to first-order carry-over effects. Williams's designs require fewer subjects than those based on complete sets of orthogonal Latin squares, which is another way to produce balanced cross-over designs. If the number of treatments to be tested is even, the design needs only one Latin square, called the Williams single; otherwise it consists of two Latin squares (except in a few special cases), called the Williams pair (Bate and Jones, 2006).

Practical Construction of a Williams Design

Although Williams (1949) describes the steps needed to construct one of his designs, a more easily remembered algorithm is given by Sheehe and Bross (1961). The details of the construction methods of a Williams design are found in Jones and Kenward (2003). An encyclopaedia review article on the related subject is found in Bate and Jones (2006).

A Williams design can be generated by re-arranging a 'standard' Latin square design, in which the first row and column consists of the letters written in alphabetical order. The practical steps in Sheehe and Bross (1961) algorithm are as follows Jones and Kenward (2003):

1. Number the treatments from 1 to *t* (*t* = the number of treatments to compare).
2. Start with a cyclic $t \times t$ Latin square. In this square the treatments in the *i*th row are *i*, $i + 1$, , … , *t*, 1, 2, … *i*-1.

3. Interlace each row of the cyclic Latin square with its own mirror image (i.e. its reverse order). For example, if $t = 4$, the first row of the cyclic square is 1, 2, 3, 4. Its mirror image is 4, 3, 2, 1. When the two sequences are interlaced, the result is 1, 4, 2, 3, 3, 2, 4, 1.

4. Slice the resulting $t \times 2t$ array down the middle to yield two $t \times t$ arrays. The columns of each $t \times t$ array correspond to the periods, the rows are the treatment sequences, and the numbers within the square are the treatments.

5. If the number of treatments is even, any one of the two $t \times t$ arrays are chosen. If the number of treatments is odd, both arrays are used (e.g. for $t = 5$, both Latin squares are used producing 10 sequences).

The design for $t = 4$ obtained by using this algorithm and choosing the left-hand square is shown in Table 3.1.

The Statistical Model of a Crossover Design (Matthews, 1988)

In a crossover design, because the direct drug effect may be confounded with any carryover effects, it is important to remove the carryover effects from the comparison if possible. To account for these effects, the following statistical model is usually considered. Let Y_{ijk} be the response (e.g., area under the curve) of the ith subject in the kth sequence at the jth period. Then:

$$Y_{ijk} = \mu + S_{ik} + P_j + F_{(j,k)} + C_{(j-1,k)} + \varepsilon_{ijk}$$

where

- μ is the overall mean
- S_{ik} is the random effect of the ith subject in the kth sequence, where $i = 1, 2, \dots, g$
- P_j is the fixed effect of the jth period, where $j = 1, \dots, p$ and $\Sigma_j P_j = 0$.
- $F_{(j,k)}$ is the direct fixed effect of the formulation in the kth sequence which is administered at the jth period, and $\sum F_{(j,k)} = 0$.
- $C_{(j\text{-}1,k)}$ is the fixed first-order carryover effect of the formulation in the kth sequence which is administered at the $(j\text{-}1)$th period, where $C_{(0,k)} = 0$; and $\sum C_{(j-1,k)} = 0$.

- ε_{ijk} is the (within-subject) random error in observing Y_{ijk}.

It is assumed that S_{ik} are independently and identically distributed with mean 0 and variance σ_S^2, and ε_{ijk} are independently distributed with mean 0 and variance σ_S^2, where t =1, 2, ..., L (the number of formulations to be compared); and ε_{ijk} are assumed mutually independent. The estimate of σ_t^2 is usually used to explain the inter-subject variability, and the estimates of $\sigma_t^2$are used to assess the intra-subject variabilities for the *t*th formulation (Chow and Liu, 2008).

A term for the carryover treatment effect is usually introduced into the model to allow for the absence or inadequacy of washout periods. If the effect of treatment does persist into the period following the period of administration, but a carryover term is not included in the model, then the estimates of the direct treatment effect will be biased. For a more detailed discussion of the issues involved, see Abeyasekera and Curnow (1984). If there is a carryover effect of the treatment, then it must be decided whether it is the direct or carryover effect that is of interest. The relevance of the 'total effect' $F_{(j,k)} + C_{(j-1,k)}$ and the possibility of modelling carryover effect using fewer parameters, perhaps by assuming proportionality between direct and carryover effects, could be considered (Patterson and Lucas, 1962).

The aim is to devise designs that allow the treatment effect in the equation to be estimated within-unit and within-period. If the S_{ik} term is taken to be fixed, then any estimator of treatment effect must be within-unit. Some writers assume that the unit effects are random: this means that one can also use between-unit information in one's analysis. For quantities that can be estimated within-unit, it is seldom profitable to use this less precise source of information.

Between-unit information is sometimes used when a treatment × period interaction is being considered. Good discussions of the problems of treatment ×x period interactions in the context of the simple two-treatment, two-period crossover design are given by Hills and Armitage (1979) and Cox and Reid (2000). Little has been written of such interactions in more complicated designs. In particular the relationship between treatment × period interactions and the set of carryover effects, of all orders, terms having the same number of degrees of freedom, does not seem to have been investigated.

Consideration of unit × treatment interaction does not seem to have received any attention in the literature. Such a term could be of interest; in a medical context it may be of importance to know which patients react favourably to the treatments. If there is a measurable feature of the units that could influence treatment effects, then it may be instructive to consider the interaction between this quantity and treatment effects**.** However, if one could not characterise the patients through such a measurement, then consideration of unit × treatment interaction might be less valuable.

A final remark on the model concerns the issue of baseline measurements: these are measurements taken on the response variable (or, occasionally, on some closely related variable) at the start of the experiment, and perhaps also at the start of each treatment period. Baseline measurements taken only at the beginning of the experiment are of no relevance to within-unit comparisons. It is less well understood how baseline readings taken at the start of each treatment period, perhaps after a washout period, should be used. Kershner and Federer (1981), Laska *et al.* (1983) and Laska and Meisner (1985) assume that the baseline readings follow model but with the direct treatment effect omitted, and with an error term that is independent of that for the response variables. These authors use this approach in their comparisons of various two-treatment designs. Identifying the parameters in the models for the baseline and response variables in this way is open to a number of practical criticisms.

Many of the present design issues will be discussed with respect to present design in further sections of the present chapter.

3.3.5 Selection of Subjects

3.3.5.1 Inclusion Criteria

1. Healthy volunteers fasted over the previous night.
2. Age equal or over 18.
3. No significant past illness, subject to investigator’s judgement.
4. All volunteers provided written informed consent before study initiation.

3.3.5.2 Exclusion Criteria

1. Having drunk coffee or alcohol during the previous night.
2. Person on regular medication or with a significant past medical illness(e.g. cardiovascular, hepatic, renal, psychiatric, neurologic, hematologic, or metabolic disease; drug or alcohol abuse within 2 years before the start of the study; smoking; HIV, hepatitis B virus, or hepatitis C virus infection)
3. Consumption of any prescribed or over-the-counter drug within 2 weeks before the start of the study;
4. Participation in a similar study within the past 6 months.
5. Female subjects were not pregnant, planning to become pregnant, or breastfeeding at the time of the study

3.3.6 Study Procedures and Schedule of Assessments

3.3.6.1 Randomisation Procedures

Each participant was randomized to one of the 10 sequences of the Williams design discussed above. This randomisation list was produced with a SAS algorithm which is discussed in the statistical section of this protocol.

3.3.6.2 Screening Assessments

Screening assessments involved determining whether potential participants fulfil the inclusion and exclusion criteria. This was done:

1. with a thorough medical history of all previous diseases and symptoms that might or might not be affecting the participant. The participant was asked if he/she had drunk coffee or alcohol during the previous night.
2. with a detailed history of regular medication.
3. through a physical examination of approximately 10 minutes on all systems of the body (gastrointestinal, cardiovascular, respiratory, general appearance).
4. with a urine pregnancy test.

3.3.6.3 Schedule of Evaluations and Procedures

All clinical study evaluations prior to screening, during screening and after screening were performed according to the schedule shown in Table 3.2. Visits were scheduled at as consistent a time as possible that were routinely available for the subject and investigator, preferably in the morning. There was a visit window from Visit 1 (V1) to Visit 5 (V5) of ± 1 day. All visits were calculated with references to V1, not with reference to the previous visit. When 20 subjects had completed V5 no further screening was performed.

This randomised-sequence, open-label, 5-period crossover study was conducted from August 2011 to May 2012. After an overnight fast, subjects received a single oral dose of either one of the amino acids or placebo with 200 mL of water. There was a 7-day washout between periods, after which subjects received the other formulation. Randomization was performed using a random number table and the treatment sequences were balanced with a Williams design. Written informed consent was obtained from all subjects after the nature and purpose of the study had been explained and before clinical screening, which included physical examination and laboratory tests. After signing informed consent, patients underwent clinical examination. Laboratory analyses consisted of determination of blood haemoglobin levels, haematocrit, and total white blood cell counts. Biochemistry and haematology safety were performed only at screening.

Subjects were confined to the centre during each visit for the assessments and blood sampling (6 hours). They received a payment for travel expenses regardless of whether they completed the study. To avoid repeated venepuncture, a peripheral venous catheter was placed during each visit in an antecubital vein and flushed with 0.5 mL of a 1:20 dilution of sodium heparin in saline after each sampling. Before collection of the next sample, 5 mL of blood was discarded to ensure that the sample was free of the flushing solution. The peripheral venous catheter was removed at the end of each visit and a new one was inserted at the beginning of the next visit.

The study protocol and informed consent forms were approved by the East London Research Ethics Committee (Ref. No 11/H0703/4) and the R&D Department of UCL/UCLH (Ref. No 10/0458). The study was performed in accordance with the Declaration of Helsinki (revised, Tokyo 2004) (World-Medical-Association, 2013) and the Good Clinical Practice guidelines (EMEA, 1997).

3.3.7 Laboratory Procedures

3.3.7.1 Plasma Amino Acid Measurement Method

For determination of plasma amino acid levels (citrulline, arginine, glutamine, ornithine, 3-methyl-histidine), blood samples (8 mL) were collected in potassium EDTA with 0.1 mL 1 sodium metabisulfite solution at the following times: predose and 15, 30, 45, 60, 90, 120, 180, and 240 minutes. Samples were centrifuged immediately at approximately 3000 g for 10 minutes at 4°C. The resulting plasma was separated, and aliquots were stored at -80°C until required for analysis. As stated by (Neveux *et al.*, 2003), the deproteinated samples can be stored at -70°C until analysis with no loss of amino acids, including glutamine, for at least several months to a year. Amino acids were separated and quantified by mass spectrometry. All samples were analysed with mass spectrometry at UCL Cancer Centre under the supervision of Dr Cali Hyde. The kit EZ:Faast was used which included internal standards and solvents for the study. Product information: http://www.phenomenex.com/Products/AminoAcidDetail/EZfaast.

The EZ:faast amino acid analysis procedure consists of a solid phase extraction step followed by a derivatization and a liquid/liquid extraction; derivatized samples are quickly analysed by liquid chromatography-mass spectrometry. The solid phase extraction is performed via a sorbent packed tip that binds amino acids while allowing interfering compounds to flow through. Amino acids on sorbent are then extruded into the sample vial and quickly derivatized with reagent at room temperature in aqueous solution. Derivatized amino acids concomitantly migrate to the organic layer for additional separation from interfering compounds. Organic layer is then removed, evaporated, and re-dissolved in aqueous mobile phase and analysed on a LC/MS system. Total sample preparation time takes around 8 minutes and analysis is performed in around 12 minutes for a total start to finish time of around 20 minutes.

3.3.7.2 Summary of Procedure

1. For each sample line up one glass sample preparation vial in the vial rack.
2. Pipette 100 µL sample and 100 µL Reagent 1 into each sample preparation vial.

3. Attach a sorbent tip to a 1.5 mL syringe; pass the solution in the sample preparation vial through the sorbent tip by slowly pulling back the syringe piston.
4. Pipette 200 µL Reagent 2 (Washing Solution) into the sample preparation vial.
5. Slowly pass the solution through the sorbent tip and into the syringe barrel.
6. Detach the sorbent tip, and discard the liquid accumulated in the syringe.
7. Pipette 200 µL Eluting Medium (prepared fresh each day) into the sample preparation vial.
8. Pull back the piston of a 0.6 mL syringe halfway up the barrel and attach the sorbent tip.
9. Wet the sorbent with Eluting Medium; stop when the liquid reaches the filter plug in the sorbent tip.
10. Eject the liquid and sorbent out of the tip and into the sample preparation vial. Repeat, until all sorbent particles in the tip are expelled into the sample preparation vial. Discard the empty tip.
11. Using the Drummond Dialamatic Microdispenser, transfer 50 µL Reagent 4.
12. Emulsify by repeatedly vortexing the solution for about 5 seconds. Allow reaction to proceed for about 1 minute.
13. Vortex the solution again for a few seconds to re-emulsify the content of the vial. Allow the reaction to proceed for at least one additional minute.
14. Using the Microdispenser, transfer 100 µL Reagent 5, and re-emulsify by vortexing for about 5 seconds. Let the reaction proceed for 1 minute.
15. Transfer part of the (upper) organic layer (50-100 µL) with a Pasteur pipette into an autosampler vial. Avoid transferring aqueous layer along with the organic layer. Evaporate the solvent slowly to dry under a gentle stream of nitrogen (maximum 10 minutes). Re-dissolve amino acid derivatives in 100 µL (or less) of a mixture of LC mobile phase components A:B 1:2 (v/v). Transfer the reconstituted sample into an insert, and place the insert in the same autosampler vial. The reconstituted sample is ready for LC/MS analysis.

3.3.7.3 LC-MS Analysis

LC Settings

Mobile phase:

A: 10 mM Ammonium formate in water

B: 10 mM Ammonium formate in methanol

Gradient: 0.00min 68%B 13.00 83%B 13.01 68%B 17.00 68%B

Re-equilibrate column for 4-6 min before next injection depending on HPLC system used.

Flow rate: 0.50 mL/min. for 3.0 mm ID column 0.25 mL/min. for 2.0 mm ID column

Column temperature: 35 °C

Injection volume: 1 µL

MS Settings

Either ESI or APCI was used

Mode: Positive Ion

Scan range: 100-650 m/z

ESI ion source temperature: 365 °C (Bruker); 425 ˚C (AB API3000)

APCI ionization chamber temperature: 450 °C

3.3.8 Statistical Considerations

3.3.8.1 Primary Endpoints

The primary end point for this study was the Area under the Curve (AUC) for citrulline after each oral load. This is the fraction of the administered dose that reaches the systemic circulation. Bioavailability is 100% for intravenous injection. It varies for other routes depending on incomplete absorption, first pass hepatic metabolism etc. Thus one plots plasma concentration against time, and the bioavailability is the AUC.

3.3.8.2 Secondary Endpoints

- AUC of all other amino acids after oral load.
- Peak concentration (C_{max}, µmol/L) of all amino acids after oral load.
- Increment (%) of all amino acids after oral bolus.

- Time to peak (T_{max}, min) of all amino acids after oral load.
- Time of maximum concentration (TOMC)
- Slope of line between Concentration at Baseline and C_{max} (μmol/L/min) for all amino acids.

3.3.8.3 Sample Size Calculation

Since this was an exploratory study with no similar in the literature, there were no sources for prior variability to allow subsequent sample size computation. However, the design of a balanced crossover study created a few conditions which allowed for sample size estimation. Thus, the restraint of the Williams design was that the number of subjects must be a multiple of 10. Thus, our chosen sample size is 20, in order to avoid possible underpowered study on 10 healthy subjects (this was reviewed by the Ethics Committee which agreed that a sample size of 20 participants was likely an appropriately powered study whilst 10 participants would be an underpowered study). Approximately 2 patients were recruited per week, based on the advertisements placed.

Theory for Sample Size Calculation

The sample is the group of subjects on which the study is performed. The choice of the sample requires qualitative and quantitative considerations. Among the qualitative aspects of the sample selection, crucial is the need to ensure that the sample is representative of the population to which one wants to extend the conclusions of the study. Among the quantitative ones, crucial is the need to quantify the concept of ‘sufficiently large sample’, i.e. of a sample large enough to allow the detection of the treatment effect, separating it from the variability of the phenomenon, with an acceptable degree of certainty. This is achieved through statistical methods. The size of the sample required for a given study depends on the magnitude of the signal, the risks we are willing to accept of making type I and type II errors, the type and variability of the end-point(s), the design of the study, and the number of treatments and primary end-points. Once the information on the size of the sample required for a given study is obtained, it is essential to evaluate the feasibility of the study, based on practical considerations, such as the number of patients we are likely to obtain from the participating centres, the study duration

considered acceptable and the projected costs. If the study is not feasible, sometimes changes in the design will allow the researcher to conform to the limits set by the abovementioned practical considerations. However, in other cases, it is best to give up. This is much less frustrating than proceeding with a study which is bound to fail (Bacchieri and Della Cioppa, 2007). In exploratory studies it might prove the case to base sample size on statistical estimations that will likely provide the necessary power for the study.

3.3.8.4 Statistical Analysis Plan

Primary Endpoint Analysis

The AUC of citrulline for the time 0-6 hours (AUC_{0-6}) was calculated by the trapezoidal rule. The AUC from the last experimental time to infinity ($AUC_{6-\infty}$) was calculated by extrapolation, dividing the last measured plasma concentration value by the apparent elimination rate constant (k_e). The $AUC_{0-\infty}$was calculated by adding AUC_{0-6} and $AUC_{6-\infty}$. All AUCs corrected for baseline concentration, which were taken as the concentration at $t = 0$ hours, are termed ΔAUC.

Three factors were considered for the analysis: treatment, patient and period of administration. The experiment followed a double Latin square design assuming an additive model and no interaction between the two squares. Three-factor ANOVA followed by Tukey's honestly significant differences post hoc test was used to estimate effects of each of the three factors on all the above computed plasma pharmacokinetic parameters. Tests were applied to the natural logarithm-transformed values of ΔAUC in order to homogenise variances, if their distribution was found to be non-parametric.

Secondary Endpoint Analysis

AUC_{0-6} for all other amino acids were smoothed by cubic spline interpolation and the interpolated curve was derived numerically in order to obtain a smoothed C(t) curve. C_{max} and T_{max} were deduced from this smoothed C(t) curve. All C_{max} corrected for baseline concentration were termed ΔC_{max}.

Clearance (Cl) was evaluated as Cl = dose/$\Delta AUC_{0-\infty}$. Apparent distribution volume (V_d) was computed as Cl/k_e. The apparent half-life of elimination ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2/k_e$.

Pharmacokinetic and Statistical Analyses

Amino acid plasma concentrations were analysed as a function of time. The following pharmacokinetic parameters were obtained for each formulation: C_{max}, Time of maximum concentration (TOMC), AUC_{0-t} (where *t* is the last time point with a measurable concentration), and $AUC_{0-\infty}$. C_{max} and TOMC were obtained directly from the original data set, and AUC_{0-t} was calculated using the linear trapezoidal rule. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/k_e$, where C_t was the last measured concentration and k_e, the elimination rate constant, was calculated using linear regression of the log-linear portion of the plasma concentration–time curve. The half-life was calculated as $\ln 2/k_e$. Mean values for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were adjusted for gender and body mass index using a multivariate analysis-of-variance model. Comparisons were performed with and without adjustment.

Applying a noncompartmental model to the concentrations of all amino acids, the pharmacokinetic parameters (C_{max} and AUC_{0-t}) were compared by analysis of variance for a crossover design, taking into account the effects of formulation, period, sequence, and subject (Chow and Liu, 2008).

The Schuirmann and Anderson-Hauck tests were used to examine bioequivalence. The ratios and 90% CIs of C_{max} and AUC_{0-t} were calculated for both formulations, and 2 one-sided *t*-tests were employed to evaluate whether the 90% CIs of the geometric mean ratios (test:reference) for these parameters met the criteria for bioequivalence (i.e. if they were within the range from 80% to 120%) (Schuirmann, 1987; Anderson and Hauck, 2007; Chow and Liu, 2008).

Unless otherwise stated, data are presented as the mean (SD). Differences were considered significant at $p < 0.05$. Stata 12.0 and SPSS 22.0 were used for analyses. Missing values were replaced by the mean values of their categories.

Randomisation

To generate a proper Williams design, based the Sheehe-Bross algorithm, a simple Latin square is constructed at random, and then re-arranged (Sheehe and Bross, 1961). In a crossover trial, subjects are not randomized to treatment in the same sense as they are in a parallel-group design. In cross-over studies, only the treatment sequences are randomized (Jones and Kenward, 2003). Consequently, after the Williams design is constructed at random, the appropriate sequences are then randomly assigned to the subjects. In addition, equal numbers of subjects may be

allocated to all the sequences to ensure balance. Thus, the number of subjects required in the trial is usually a multiple of the number of sequences. In practice, there are usually several patients assigned to each sequence. The appropriate statistical software to design a Williams design study is the statistical package SAS. Hence, the SAS code for a Williams design and randomization for a 5 × 5crossover trial and sample size = 20 was (Wang *et al.*, 2009):

```
%WILLIAMS(
  TmtsNum=5,
  TmtNames= Cit Arg Gln 3-MH Placebo,
  Samplesize=20,
  OutRtf=D:\WilliamsN24.rtf,
SeedNum=);
```

Below is a balanced set of sequences for five treatments. In this case 10 sequences are needed to keep the balance (and each treatment is followed by every other treatment twice), so multiples of 10 subjects are needed in the study:

1: A B C D E	6: E D C B A
2: B D A E C	7: C E A D B
3: D E B C A	8: A C B E D
4: E C D A B	9: B A D C E
5: C A E B D	10: D B E A C

Williams Design and Randomisation List for the Current Study

This was produced with SAS code (SAS 9.2) and is shown in Table 3.3. Table 3.4 shows each sequence per subject.

Outliers' Analysis

Outliers were analysed to investigate for influential or outlier observations. Heteroscedasticity was tested with the Breusch-Pagan / Cook-Weisberg test (Breusch and Pagan, 1979; Cook and Weisberg, 1983) and influential observations were identified per treatment with Cook's distance (Cook, 1977, 1979) while DFBETAs were graphed per subject per treatment (Dehon *et al.*, 2009).

A collection of variables is heteroscedastic if there are sub-populations that have different variabilities from others. The Breusch-Pagan / Cook-Weisberg test tests whether the estimated variance of the residuals from a regression or ANOVA are dependent on the values of the independent variables. In that case, heteroscedasticity is present. ANOVA is quite frequently robust, in a manner that results are resistant to departion form usual pre-requisites for performing ANOVA; nevertheless, influential points provide insight into the presence and sources of variability.

Cook's distance (Cook's D) is a commonly used estimate of the influence of a data point when performing regression analysis and ANOVA. There are different opinions regarding what cut-off values to use for spotting highly influential points: a simple guideline of $D > 1$ has been suggested while others have suggested $D > 4/n$, where n is the number of observations; the latter is used in the present study. DFBETAs are another metric used to estimate and visualize influential observations. The DFBETA for a particular observation is the difference between the regression coefficient for an included variable calculated for all of the data and the regression coefficient calculated with the observation deleted, scaled by the standard error calculated with the observation deleted. The cut-off value for DFBETAs is $2/\sqrt{n}$, where again n is the number of observations (Dehon *et al.*, 2009).

3.4 Results

3.4.1 Patients

Twenty Subjects were enrolled: 4 females and 16 males, mean age 22.3 years (SD = 4.7), mean height 1.75 m (SD = 0.06), mean weight 74.8 kg (SD = 11.9) and mean BMI 24.3 kg/m^2 (SD = 3.5).

3.4.2 Method Validation

Extraction recovery of amino acids measured in plasma samples was adequate. Means, standard deviations and other statistical measures for retention time, area height, concentration (μmol/L), and internal standard (ISTD) area are shown in Table 3.5 and Table C.1, Appendix C. Dates of analysis are shown in Table C.2 and correspond to the different standard curves used for computing the concentration of plasma amino acids. The retention times for arginine, citrulline, glutamine,

ornithine and 3-methylhistidine were 3.06, 3.64, 3.73, 6.56, and 4.49 minutes, respectively. For each internal standard, retention times were similar. Linearity was achieved over a concentration range of 50 μmol/L to 400 μmol/L (Figures C.1-C.5, Appendix C), with the typical equation for the calibration curve reaching R^2 over 0.968, with most of them being approximately 0.999.

3.4.3 Bioavailability Analysis

3.4.3.1 Spaghetti Plots, Individual Subject Plots and Means Plots

Spaghetti plots, individual subject plots and means plots are first presented for each amino acid concentration (citrulline, arginine, glutamine, ornithine and 3-methyl-histidine) and per treatment (citrulline, arginine, glutamine, placebo and 3-methyl-histidine) (Figures 3.2-3.26). Time-dependent increases in citrulline, arginine, glutamine and ornithine levels post-ingestion of arginine, citrulline or glutamine are observed. Placebo and 3-methylhistidine ingestion do not seem to produce an effect. No inferences can be made though; this will be performed with statistical testing in the next section.

3.4.3.2 Crossover ANOVA for Means of Concentrations before Transforming Into Area under the Curve

Initially, an analysis of variance (ANOVA) for a crossover study with respect to each amino acid concentration under each treatment was performed. This analysis does not take into account the different time points, but it will generate initial proxy results when the area under the concentration-time curve (AUC) ANOVA is performed, which is the main result from a crossover study. The results are shown in the Table C.3 and in summary they are:

1. All amino acids appear to have been affected by the treatments significantly (ANOVA $p < 0.001$).
2. There isn't a carryover effect with respect to citrulline concentrations abut there is one with respect to arginine, glutamine, ornithine and 3-methylhistidine (ANOVA $p < 0.001$).
3. These results are only proxies to the final results when analysis is done with an ANOVA for the AUC. They are to be treated with caution but if similar patterns are observed with next results, this strengthens the results.

3.4.3.3 AUC Analysis

Means of AUC, C_{max} and TOMC

This information is shown in Table 3.6 and in Figure 3.27. This table is quite important because it depicts means and standard deviations (SDs) for the AUC, C_{max} and TOMC for each plasma amino acid measured according to treatment. The results reveal the following:

1. When the subject is loaded with citrulline there are increases in subsequent plasma levels of glutamine, citrulline and arginine (as compared with placebo) (greater AUC) (Table 3.6, Figure 3.27). When loaded with glutamine or arginine there are also increases in subsequent levels of glutamine, citrulline and arginine. The magnitude of these increases is greatest when the loading dose was of citrulline.
2. The effect of citrulline comes earlier in time compared to glutamine (greater TOMC).
3. There is a spread of effects – mostly with citrulline and least with glutamine – for the doses used in these experiments. Citrulline, arginine, glutamine and ornithine levels appear to be affected more when given citrulline and arginine than any other treatment.
4. The distribution of AUC and C_{max} is not normal as shown by the histograms (Figures C.6 and C.7) and Shapiro Wilk test ($p < 0.05$).

Statistical inference testing of these figures is performed next to investigate any potential significant differences.

Crossover ANOVA for AUC and C_{max}

The AUC for each amino acid concentration didn't follow a normal distribution (Shapiro Wilk's test p value < 0.05 for all AUCs; see histograms Figures C.6 and C.7). Initial Kruskal-Wallis testing for non-parametric data revealed similar p-values with ANOVA, because ANOVA is a robust technique which is able to perform satisfactorily even when data are not parametric (Schmider *et al.*, 2010). The results are shown in Tables 3.7 to 3.9, Table C.4 (Appendix C) and Figures C.8-C.40 (Appendix C). Overall:

1. There was no effect of age, gender and BMI on the AUC, C_{max} and TOMC (Figures C.8-C.22 for age, Figures C.23-C.37 for BMI and Figures C.38-C.40 for gender and MANOVA Table C.4; $p < 0.05$). This essentially indicates that

basic anthropometric characteristics and age have no effect on pharmacological post-ingestion measures.

2. Citrulline, arginine and ornithine concentrations were more affected by the treatment overall (crossover ANOVA $p < 0.05$, Tables 3.7 and 3.8) in comparison with glutamine and 3-methylhistidine which were not affected ($p > 0.05$). Both the AUC and the C_{max} were affected indicating that overall post-absorptive concentrations and the maximum concentration were influenced by the type of treatment.
3. There was no carryover and period effect for all treatments, except for 3-methylhistidine which had a significant period effect (possibly due to small concentrations, $p < 0.05$; Tables 3.7 and 3.8). This confirms that the study design maintained low likelihood for carryover or period effects.
4. Citrulline administration increased arginine concentrations significantly compared to placebo and 3-methylhistidine. Citrulline loading also had a stronger effect than glutamine but not arginine ($p < 0.05$ from Tukey multiple comparisons Table 3.9; see also Table 3.6, Figure 3.27).
5. Arginine administration increased arginine concentrations significantly compared to placebo and 3-methylhistidine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9). Additionally, arginine loading increased arginine concentrations with a stronger effect than glutamine but not compared to citrulline (although the increase was higher after arginine loading than after citrulline loading) (Table 3.9; see also Table 3.6, Figure 3.27).
6. Citrulline administration increased citrulline concentrations significantly compared to placebo and 3-methyl-histidine (controls) as well as arginine and glutamine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9). Increase of citrulline concentrations was larger with arginine loading compare to glutamine loading ($p < 0.05$ from Tukey multiple comparisons, Table 3.9).
7. Citrulline administration increased ornithine levels significantly compared to placebo and 3-methylhisitidine (controls) as well as arginine and glutamine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9).
8. Arginine administration increased ornithine levels compared to placebo and 3-methylhistidine (controls) as well as glutamine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9); but less prominently than citrulline loading ($p < 0.05$ from Tukey multiple comparisons, Table 3.9).

3.4.3.4 Bioequivalence Testing

The bioequivalence of formulations was examined and results are presented in Table 3.10. Interestingly no treatment was equivalent to treatment with citrulline for producing equivalent post-absorptive concentrations ($p > 0.05$). With respect to all other amino acid concentrations, equivalence was reached with all treatments (Anderson-Hauck test $p < 0.05$). Hence, it is noted that an effect has not been demonstrated but the absence of an effect cannot be inferred from this calculation.

3.4.3.5 Analysis of Outliers

There was heteroscedasticity in the plasma levels of citrulline, arginine, placebo and 3-methylhistidine but not glutamine (as measured by AUC) (Table 3.11). The percentage of influential observations in each plasma group was between 6% and 12%. Figure 3.28 is a graph of the DFBETAs per plasma amino acid levels (calculated in AUC) per treatment. Increased variability and more influential points were noted in the treatment groups for the plasma amino acid measured. For example, with respect to plasma citrulline AUC, citrulline treatment group had more influential points compare to the others. Variability in the placebo group was markedly less compared to the other treatments. This was similarly observed in the other sub-graphs of Figure 3.28. The placebo treatment group had increased variability with respect to measurement of plasma glutamine AUC, almost comparably to glutamine and arginine, indicating a need for caution when interpreting the ANOVA results for plasma glutamine AUC. Overall, although there are influential points in the placebo treatment groups across all plasma amino acids measured, their influential potential is much less prominent and less variable compared to the other treatment groups, except from the case where plasma glutamine was measured. This also suggests that the variability in the spaghetti plots for the placebo groups is comparably acceptable.

3.5 Summary of Findings and Discussion

In the present chapter post-absorptive patterns of five plasma amino acids after five challenges (citrulline, arginine, glutamine, placebo and 3-methylhistidine) were investigated. All results were presented in tables and figures. Results were satisfactory from robustness, despite an apparent variability. In other words, the

results are true and can be believed to then interpret them. Elements that provide robustness are:

1. Powerful enough design with no carryover effects for main outcomes (Tables 3.7 and 3.8).
2. Acceptable accurate standard curves when analysing data in mass spectrometer (Figures C.1-C.5).
3. Crossover ANOVA is reportedly robust (Driscoll, 1996).
4. Outlier analysis depicted markedly less influential points in the placebo treatment group in all plasma amino acids measured, except for plasma glutamine. In this case results should be treated with caution.

Returning to the original hypotheses formulated:

1. Citrulline can function successfully as an oral challenge test. This oral challenge test is time dependent with citrulline decreasing after the oral challenge is performed. Interestingly, citrulline was the most potent stimulator for all other amino acids, despite glutamine challenges being readily considered the most potent stimulators of citrulline production (Peters *et al.*, 2007c; Peters *et al.*, 2008b). It could be that this demonstrates more efficient amino acid absorption and that this could be an important step to countering disease-related catabolism or assist in protein anabolism. Hence, citrulline challenges could be useful in intestinal failure but also in liver failure where urea cycle pathways including glutamine, arginine and ornithine are implicated.
2. There were differences in the bioavailability of all five amino acids as shown with statistical testing (ANOVA $p < 0.05$ for citrulline, arginine and ornithine).
3. There was no period, carryover or sequence effect except for 3-methylhistidine which had a significant period effect (possibly due to small concentrations).
4. No treatment was equivalent to treatment with citrulline for producing equivalent post-absorptive concentrations ($p > 0.05$).

Citrulline challenges could be useful in intestinal failure but also in liver failure where urea cycle pathways including glutamine, arginine and ornithine are implicated. Limitations exist and include open-label design and short duration of follow-up. It was conducted in a small sample of healthy volunteers, who have different bioavailability than do patients when taken orally. Thus, the clinical significance of the findings – namely, challenges in patients for functional reason – must be clarified in additional studies involving patients with intestinal or liver

failure (Ouelaa *et al.*, 2017; Sellmann *et al.*, 2017). Furthermore, gastric emptying and intestinal transit factors were not measured in the present study and these could have been linked to citrulline challenges (Straathof *et al.*, 2000; Bruins *et al.*, 2004). Previous studies demonstrated that under stress during critical illness bowel motility was affected which was restored by administering L-arginine (Bruins *et al.*, 2004), which however didn't affect the motility of the lower oesophageal sphincter in healthy volunteers (Straathof *et al.*, 2000).

The present study creates the potential for future studies in the field. These could involve:

1. A dynamic study with oral citrulline challenges in states of compromised intestine, such as extensive enteropathies (e.g. coeliac disease, mucositis, radiation, Crohn's disease) or short bowel syndrome or intestinal failure.
2. Citrulline challenges in liver failure, since citrulline is part of the urea cycle.
3. Oral challenges in cases of compromised gut during critical illness. This is similar to extensive enteropathy but it is broader since multiple factors can contribute to critical illness enteropathy: endotoxemia, sepsis, catabolism, nitric oxide changes, arginine fluxes, presence of excessive lactate, etc. In Chapter 2 it was shown that citrulline was measured in critical illness but mainly as a static measure, with all the relevant shortcomings. A study in this setting would examine the potential of an oral challenge against single or serial measurements of citrulline.

3.6 Tables

Table 3.1. An example of the Williams design for a four-treatment, four-period cross-over trial.

Subject no	Sequence no	Actual sequence	Period			
			1	2	3	4
1	1	A-B-C-D	A	B	C	D
2	2	B-D-A-C	B	D	A	C
3	3	C-A-D-B	C	A	D	B
4	4	D-C-B-A	D	C	B	A

Table 3.2. Schedule of evaluations and procedures.

Procedures **Visit Number:**	**Prior to screening**	**Screening** **SV**	**Week 1** **V1**	**Week 2** **V2**	**Week 3** **V3**	**Week 4** **V4**	**Week 5 End of study** **V5**
Study Day		0	0	7	14	21	28
Visit Window (days)				±1	±1	±1	±1
Informed consent	X	X[a]					
Eligibility criteria		X	X[d]	X	X	X	X
Medical History, Demographics		X	X[d]	X	X	X	X
Physical Examination[b]		X	X[d]	X	X	X	X
Concomitant medication[c]		X	X[d]	X	X	X	X
Urine pregnancy test		X	X[d]	X	X	X	X
Vital Signs			X	X	X	X	X
Height			X				
Body weight			X	X	X	X	X
Inserting intravenous cannula			X	X	X	X	X
Blood sample before 1st loading (considered as time 0)			X	X	X	X	X
Oral loading			X	X	X	X	X
Blood samples at 0.25, 0.5, 0.75, 1, 1.5, 2,3, 6h after the load			X	X	X	X	X
Urine samples			X	X	X	X	X
Brief Medical History at end of visit and removal of cannula			X	X	X	X	X
Urine samples of 16 and 24h post-administration			X	X	X	X	X
Brief Medical History via phone after end of study							X

[a] Informed Consent must be signed before any screening visit assessments will be done.
[b] Full physical examination (gastrointestinal, cardiovascular, respiratory, general appearance).
[c] At screening, information on all medications taken in the previous 30 days will be collected.
[d] This information will be identical with the screening visit, if the participant passes screening.

Table 3.3. A Williams Design for the Cross-over Trial. The number of treatments in this trial = 5.

Sequence No	Period1	Period2	Period3	Period4	Period5
1	Placebo	3-MH	Gln	Arg	Cit
2	Gln	Placebo	Cit	3-MH	Arg
3	Cit	Gln	Arg	Placebo	3-MH
4	Arg	Cit	3-MH	Gln	Placebo
5	3-MH	Arg	Placebo	Cit	Gln
6	Cit	Arg	Gln	3-MH	Placebo
7	Arg	3-MH	Cit	Placebo	Gln
8	3-MH	Placebo	Arg	Gln	Cit
9	Placebo	Gln	3-MH	Cit	Arg
10	Gln	Cit	Placebo	Arg	3-MH

Table 3.4. The Randomisation schedule for the trial. The number of treatments in this trial = 5.

Subject ID	Sequence
001	Arg--3-MH--Cit--Placebo--Gln
002	3-MH--Arg--Placebo--Cit--Gln
003	Cit--Gln--Arg--Placebo--3-MH
004	Cit--Arg--Gln--3-MH--Placebo
005	Placebo--3-MH--Gln--Arg--Cit
006	Gln--Placebo--Cit--3-MH--Arg
007	Arg--Cit--3-MH--Gln--Placebo
008	Gln--Cit--Placebo--Arg--3-MH
009	3-MH--Placebo--Arg--Gln--Cit
010	Placebo--Gln--3-MH--Cit--Arg
011	3-MH--Placebo--Arg--Gln--Cit
012	Arg--3-MH--Cit--Placebo--Gln
013	Gln--Cit--Placebo--Arg--3-MH
014	3-MH--Arg--Placebo--Cit--Gln
015	Cit--Gln--Arg--Placebo--3-MH
016	Cit--Arg--Gln--3-MH--Placebo
017	Arg--Cit--3-MH--Gln--Placebo
018	Gln--Placebo--Cit--3-MH--Arg
019	Placebo--3-MH--Gln--Arg--Cit
020	Placebo--Gln--3-MH--Cit--Arg

Table 3.5. General descriptive statistics regarding arginine, citrulline, glutamine, ornithine, and 3-methylhistidine concentrations (μmol/L).

	N	Mean		SD	Range	Minimum	Maximum	Variance	Skewness		Kurtosis	
		Statistic	SE	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	SE	Statistic	SE
Arginine Concentration (μmol/L)	900	153.8219	4.02715	120.8146	706.94	17.26	724.2	14596.16	1.986	0.082	4.345	0.163
Citrulline Concentration (μmol/L)	900	115.49	5.15092	154.5275	835.63	4.67	840.3	23878.74	1.818	0.082	1.755	0.163
Glutamine Concentration (μmol/L)	900	534.1462	8.06871	242.0614	1023.72	43.15	1066.87	58593.7	0.126	0.082	-1.141	0.163
Ornithine Concentration (μmol/L)	900	119.6382	3.32826	99.8479	431.58	3.68	435.26	9969.603	1.738	0.082	2.363	0.163
3-MH Concentration (μmol/L)	900	4.4797	0.04579	1.37376	6.48	0.18	6.67	1.887	-0.756	0.082	0.161	0.163

Table 3.6. Means of AUC, C_{max} and TOMC per treatment for plasma amino acids arginine, citrulline, glutamine, ornithine, and 3-methylhistidine.

		Treatment																				
		Citrulline					Arginine				Glutamine				Placebo				3-Methyl-Histidine			
	Plasma Amino Acid measured	**N**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
AUC	Arginine	20	1607.51	865.71	721.59	3307.66	1339.23	605.6	335.65	2726.94	694.87	285.49	366.75	1538.1	503.68	258.17	205.73	1180.56	554.49	281.34	124.06	1125.67
	Citrulline	20	245.41	230.5	214.598	2866.79	258.27	76	175.55	485.22	380.63	113.8	248.57	624.67	245.04	60.55	103.89	371.4	247.03	56.43	145.19	365.53
	Glutamine	20	2819.73	777.83	1338.13	4608.58	3379.98	1284.56	1070.06	5347.03	3482.87	1273.98	1302.25	5159.22	2810.08	1421.87	611.1	5747.18	3287.2	1330.94	1346.92	5309.75
	Ornithine	20	1451.77	592.59	470.28	2370.12	1055.85	573.16	497.65	2432.07	454.41	246.29	91.12	1092.55	363.29	149.19	160.81	811.46	412.63	175.54	136.22	840.92
	3-Methyl-Histidine	20	27.69	7.77	8.36	38.92	26.21	7.48	10.38	39.05	27.17	9.3	8.37	38.89	27.57	7.82	14.54	38.88	26.69	7.19	12.16	39.07
C_{max}	Arginine	20	342.57	180.05	148.83	658.45	368.25	143.55	186.52	724.2	146.95	82.58	73.41	449.18	102.06	40.1	42.24	210.18	108.59	53.69	35.43	227.3
	Citrulline	20	505.82	96.77	440.43	840.3	588.6	194.3	336.8	1112	1242.5	342.4	838.5	1966.5	525	122.2	317.1	777.7	510.4	117.1	296.7	774.8
	Glutamine	20	706.3	203.91	318.55	1066.87	681.62	198.4	348.91	895.95	821.56	164.67	534.03	1053.19	664.17	234.79	221.19	1062.78	692.6	191.68	270.04	902.25
	Ornithine	20	314.77	106.62	133.18	435.26	265.6	102.76	129.47	431.57	98.59	61.3	24	280.42	90.04	67.1	36.2	315.84	101.17	54.2	37.91	227.12
	3-Methyl-Histidine	20	4.91	1.17	2	6.5	4.84	1.07	2.53	6.53	4.98	1.32	2.24	6.54	4.79	1.15	3.14	6.51	4.79	1.12	2.74	6.67
TOMC	Arginine	20	2.19	0.87	0.5	3	1	0.44	0.5	2	0.96	0.9	0	3	1.69	2.41	0	6	1.68	1.82	0	6
	Citrulline	20	1.04	0.63	0.25	3	1.39	0.75	0.25	3	1.78	0.62	0.25	3	1.11	1.68	0	6	1.4	1.7	0	6
	Glutamine	20	3.44	2.71	0	6	2.96	2.46	0	6	1.01	0.5	0.25	2	2.04	2.51	0	6	3.54	2.65	0	6
	Ornithine	20	1.85	1.15	0.5	6	1.81	1.52	0.5	6	1.56	1.4	0	6	0.9	1.41	0	6	0.81	1	0	3
	3-Methyl-Histidine	20	1.74	1.74	0.25	6	1.13	1.32	0	6	1.61	2.09	0	6	1.95	2.27	0	6	2.01	2.45	0	6

Table 3.7. Crossover ANOVA for AUC.

	CITRULLINE						ARGININE					
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**
Intersubjects												
Sequence effect	72446.37	9	8049.6	0.62	0.76		4.26E+06	9	473056.06	1.34	0.3252	
Residuals	130497.52	10	13049.75	0.75	0.6783		3.52E+06	10	352390.08	1.58	0.1304	
Intrasubjects												
Treatment effect	7.13E+07	4	1.78E+07	1020.43	**<0.001**	**<0.001**	2.01E+07	4	5.02E+06	22.55	**<0.001**	**<0.001**
Carryover effect	42336.77	4	10584.19	0.61	0.6599		643278.28	4	160819.57	0.72	0.5795	
Period effect	64058.35	4	16014.59	0.92	0.4596		2.00E+06	4	501133.66	2.25	0.0725	
Residuals	1.19E+06	68	17476.63				1.51E+07	68	222555.52			
Total	7.71E+07	99					4.56E+07	99				
	GLUTAMINE						**ORNITHINE**					
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**	**Partial SS**	**df**	**MS**	F	p	**p-value form Kruskal Wallis test**
Intersubjects												
Sequence effect	1.07E+07	9	1.18E+06	0.44	0.8827		1.51E+06	9	168237.3	1.54	0.255	
Residuals	2.68E+07	10	2.68E+06	1.98	0.0486		1.09E+06	10	109244.7	0.65	0.762	
Intrasubjects												
Treatment effect	5.42E+06	4	1.36E+06	1	**0.4118**	**0.324**	1.85E+07	4	4.62E+06	27.66	**<0.001**	**<0.001**
Carryover effect	6.94E+06	4	1.74E+06	1.29	0.2843		663833	4	165958.3	0.99	0.4169	
Period effect	7.88E+06	4	1.97E+06	1.46	0.2243		496669.4	4	124167.4	0.74	0.5656	
Residuals	9.18E+07	68	1.35E+06				1.14E+07	68	166958.8			
Total	1.54E+08	99					3.38E+07	99				
	3-METHYL-HISTIDINE											
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**						
Intersubjects												
Sequence effect	1221.07	9	135.67	0.89	0.5648							
Residuals	1524.77	10	152.48	3.99	0.0003							
Intrasubjects												
Treatment effect	31.63	4	7.91	0.21	**0.9337**	**0.916**						
Carryover effect	33.52	4	8.38	0.22	0.9268							
Period effect	521.38	4	130.35	3.41	0.0133							
Residuals	2598.37	68	38.21									
Total	6026.68	99										

Note for all models: Omnibus measure of separability of treatment and carryover = 77.6393%

Table 3.8. Crossover ANOVA for C_{max}.

	CITRULLINE						ARGININE					
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**
Intersubjects												
Sequence effect	5353.95	9	594.88	0.3	0.9564		243271.47	9	27030.16	1.79	0.1887	
Residuals	19584.58	10	1958.46	0.8	0.6305		151000.57	10	15100.06	1.42	0.1925	
Intrasubjects												
Treatment effect	2.94E+06	4	734320.3	299.35	**<0.001**	**<0.001**	1.35E+06	4	336784.4	31.57	**<0.001**	**<0.001**
Carryover effect	13212.66	4	3303.16	1.35	0.2618		36438.96	4	9109.74	0.85	0.4962	
Period effect	6668.03	4	1667.01	0.68	0.6085		60617.76	4	15154.44	1.42	0.2366	
Residuals	166804.75	68	2453.01				725518.99	68	10669.4			
Total	3.30E+06	99					2.59E+06	99				
	GLUTAMINE						**ORNITHINE**					
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**	**Partial SS**	**df**	**MS**	F	p	**p-value form Kruskal Wallis test**
Intersubjects												
Sequence effect	263896.04	9	29321.78	0.4	0.9102		61251.74	9	6805.75	1.11	0.435	
Residuals	739672.92	10	73967.29	2.05	0.0415		61490.01	10	6149	0.95	0.4973	
Intrasubjects												
Treatment effect	187888.36	4	46972.09	1.3	**0.279**	**0.112**	852429.69	4	213107.4	32.81	**<0.001**	**<0.001**
Carryover effect	248811.04	4	62202.76	1.72	0.1555		38430.72	4	9607.68	1.48	0.2182	
Period effect	38022.01	4	9505.5	0.26	0.9007		26520.23	4	6630.06	1.02	0.403	
Residuals	2.46E+06	68	36144.88				441721.8	68	6495.91			
Total	4.11E+06	99					1.55E+06	99				
	3-METHYL-HISTIDINE											
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**p-value from Kruskal Wallis test**						
Intersubjects												
Sequence effect	20.84	9	2.32	0.77	0.6505							
Residuals	30.23	10	3.02	3.06	0.0029							
Intrasubjects												
Treatment effect	0.31	4	0.08	0.08	**0.9884**	**0.842**						
Carryover effect	1.08	4	0.27	0.27	0.8947	1.08						
Period effect	8.85	4	2.21	2.24	0.0734	8.85						
Residuals	67.12	68	0.99			67.12						
Total	130.5	99										

Note for all models: Omnibus measure of separability of treatment and carryover = 77.6393%

Table 3.9. Multiple comparisons between mean AUC (Tukey). Differences express (I-J).* The mean difference is significant at the 0.05 level.

Dependent Variable	(J) Treatment	(I) Treatment			
		Citrulline	Arginine	Glutamine	Placebo
AUC_{0-6} for Arginine	Arginine	268.3			
	Glutamine	912.6*	644.4*		
	Placebo	1103.8*	835.6*	191.2	
	3-Methylhistidine	1053.0*	784.7*	140.4	-50.8
AUC_{0-6} for Citrulline	Arginine	2195.1*			
	Glutamine	2072.8*	-122.4*		
	Placebo	2208.4*	13.2	135.6*	
	3-Methylhistidine	2206.4*	11.2	133.6*	-2
AUC_{0-6} for Glutamine	Arginine	-560.3			
	Glutamine	-663.1	-102.9		
	Placebo	9.6	569.9	672.8	
	3-Methylhistidine	-467.5	92.8	195.7	-477.1
AUC_{0-6} for Ornithine	Arginine	395.9*			
	Glutamine	997.4*	601.4*		
	Placebo	1088.5*	692.6*	91.1	
	3-Methylhistidine	1039.1*	643.2*	41.8	-49.3
AUC_{0-6} for 3-Methylhistidine	Arginine	1.5			
	Glutamine	0.5	-1		
	Placebo	0.1	-1.4	-0.4	
	3-Methylhistidine	1.0	-0.5	0.5	0.9

Table 3.10. Test : reference values for AUC. Anderson Hauck test *p*-value < 0.05 indicates equivalence.

	Treatment			
Treatment	**Citrulline**	**Arginine**	**Glutamine**	**Placebo**
Citrulline concentration				
Arginine	70.31% to 71.19% ($p < 0.05$)			
	Anderson Hauck test *p*-value: < 0.05			
Glutamine	70.43% to 71.06% ($p < 0.05$)	58.47% to 58.83% ($p < 0.05$)		
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05		
Placebo	70.51% to 70.98% ($p < 0.05$)	57.95% to 59.36% ($p < 0.05$)	60.83% to 61.83% ($p < 0.05$)	
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	
3-Methylhistidine	70.54% to 70.96% ($p < 0.05$)	58.18% to 59.12% ($p < 0.05$)	61.04% to 61.62% ($p < 0.05$)	58.03% to 58.50% ($p < 0.05$)
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05
Arginine concentration				
Arginine	96.61% to 99.11% ($p = 0.752$)			
	Anderson Hauck test p-value: < 0.05			
Glutamine	96.73% to 98.99% ($p = 0.759$)	97.23% to 98.40% ($p = 0.799$)		
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05		
Placebo	96.83% to 98.882% ($p = 0.763$)	97.38% to 98.25%	97.38% to 97.82% ($p = 0.759$)	
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	
3-Methylhistidine	96.42% to 99.30%	97.56% to 98.07% ($p = 0.794$)	96.69% to 98.51% ($p = 0.777$)	96.65% to 98.26% ($p = 0.760$)
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05
Glutamine concentration				
Arginine	101.09% to 102.45%			
	Anderson Hauck test *p*-value: < 0.05			
Glutamine	100.70% to 102.85% ($p = 0.763$)	101.30% to 102.18% ($p = 0.775$)		
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05		
Placebo	101.50% to 102.05% ($p = 0.789$)	101.06% to 102.43%	100.62% to 102.85% ($p = 0.763$)	
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	
3-Methylhistidine	100.82% to 102.73% ($p = 0.747$)	101.03% to 102.45% ($p = 0.752$)	100.69% to 102.78% ($p = 0.762$)	101.05% to 102.55% ($p = 0.786$)
	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05	Anderson Hauck test *p*-value: < 0.05
Ornithine concentration				

	Treatment			
Treatment	**Citrulline**	**Arginine**	**Glutamine**	**Placebo**
Arginine	94.16% to 96.03% (p = 0.764)			
	Anderson Hauck test p-value: < 0.05			
Glutamine	94.2% to 95.99% (p = 0.737)	93.74% to 95.94% (p = 0.795)		
	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05		
Placebo	94.92% to 95.27% (p = 0.767)	93.70% to 95.98% (p = 0.771)	92.86% to 95.35%	
	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	
3-Methylhistidine	94.55% to 95.63% (p = 0.744)	93.63% to 96.06% (p = 0.764)	93.49% to 94.71% (p = 0.765)	93.17% to 94.71% (p = 0.785)
	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05
3-Methylhistidine concentration				
Arginine	96.68% to 100.36%			
	Anderson Hauck test p-value: < 0.05			
Glutamine	96.75% to 100.29% (p = 0.752)	96.20% to 100.80%		
	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05		
Placebo	97.21% to 99.84% (p = 0.740)	97.38% to 99.62%	97.32% to 99.69%	
	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	
3-Methylhistidine	95.38% to 101.67% (p = 0.772)	97.17% to 99.83% (p = 0.804)	95.30 to 101.70% (p = 0.780)	96.37% to 100.68% (p = 0.771)
	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05	Anderson Hauck test p-value: < 0.05

Table 3.11. Heteroscedasticity tests and influential observations according to Cook's distance.

Plasma Citrulline AUC			**Plasma Arginine AUC**			**Plasma Glutamine AUC**			**Plasma Ornithine AUC**			**Plasma 3-Methylhistidine AUC**		
Breusch-Pagan / Cook-Weisberg test for Heteroscedasticity														
χ^2	44.78		44.63			0.15			42.78			11.71		
p	< 0.001		< 0.001			0.6987			< 0.001			0.0006		
Cook's Influential Observations														
ID	*Treatment*	*Cook's D*	*ID*	*Treatment*	*Cook's D*	*ID*	*Treatment*	*Cook's D*	*ID*	*Treatment*	*Cook's D*	*ID*	*Treatment*	*Cook's D*
S09	Citrulline	0.044265	S15	Citrulline	0.16398	S21	Citrulline	0.044138	S02	Citrulline	0.061632	S10	Citrulline	0.048262
S10	Citrulline	0.087887	S16	Citrulline	0.11762	S10	Arginine	0.072873	S09	Citrulline	0.073003	S09	Glutamine	0.067065
S12	Citrulline	0.06152	S19	Citrulline	0.047331	S23	Arginine	0.040662	S12	Citrulline	0.043224	S04	3-Methyl-H	0.06143
S18	Citrulline	0.150955	S23	Citrulline	0.084224	S13	Glutamine	0.069266	S13	Citrulline	0.058716	S07	3-Methyl-H	0.068636
S20	Citrulline	0.041899	S15	Arginine	0.056279	S20	Glutamine	0.070475	S14	Citrulline	0.042913	S09	3-Methyl-H	0.135173
S21	Citrulline	0.070912	S23	Arginine	0.095587	S09	Placebo	0.058897	S17	Citrulline	0.0773	S10	3-Methyl-H	0.056019
S22	Citrulline	0.045516	S21	Glutamine	0.049899	S12	Placebo	0.072856	S18	Citrulline	0.051824			
S23	Citrulline	0.046197	S16	3-Methyl-H	0.04345	S20	Placebo	0.052024	S23	Citrulline	0.072304			
S10	Arginine	0.054543				S23	3-Methyl-H	0.078569	S02	Arginine	0.119699			
S12	Glutamine	0.074601							S15	Arginine	0.04179			
S14	Glutamine	0.044515							S18	Arginine	0.040249			
									S22	Arginine	0.135838			

ID: Subject ID; Treatment: Amino Acid ingested; Cook's D: Cook's Distance; 3-Methyl-H: 3-methylhistidine.

3.7 Figures

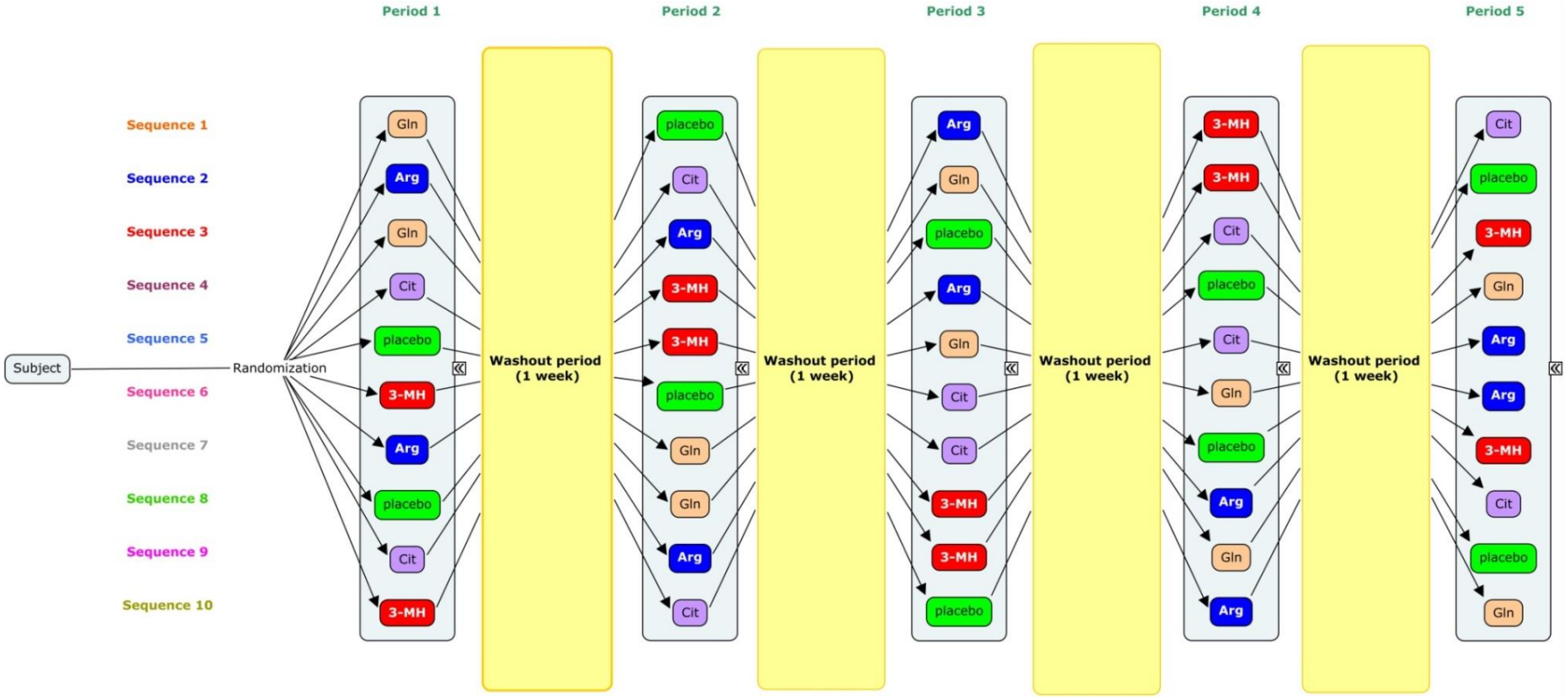


Figure 3.1. Study diagram. Example of the ten sequences of amino acids the subjects received.

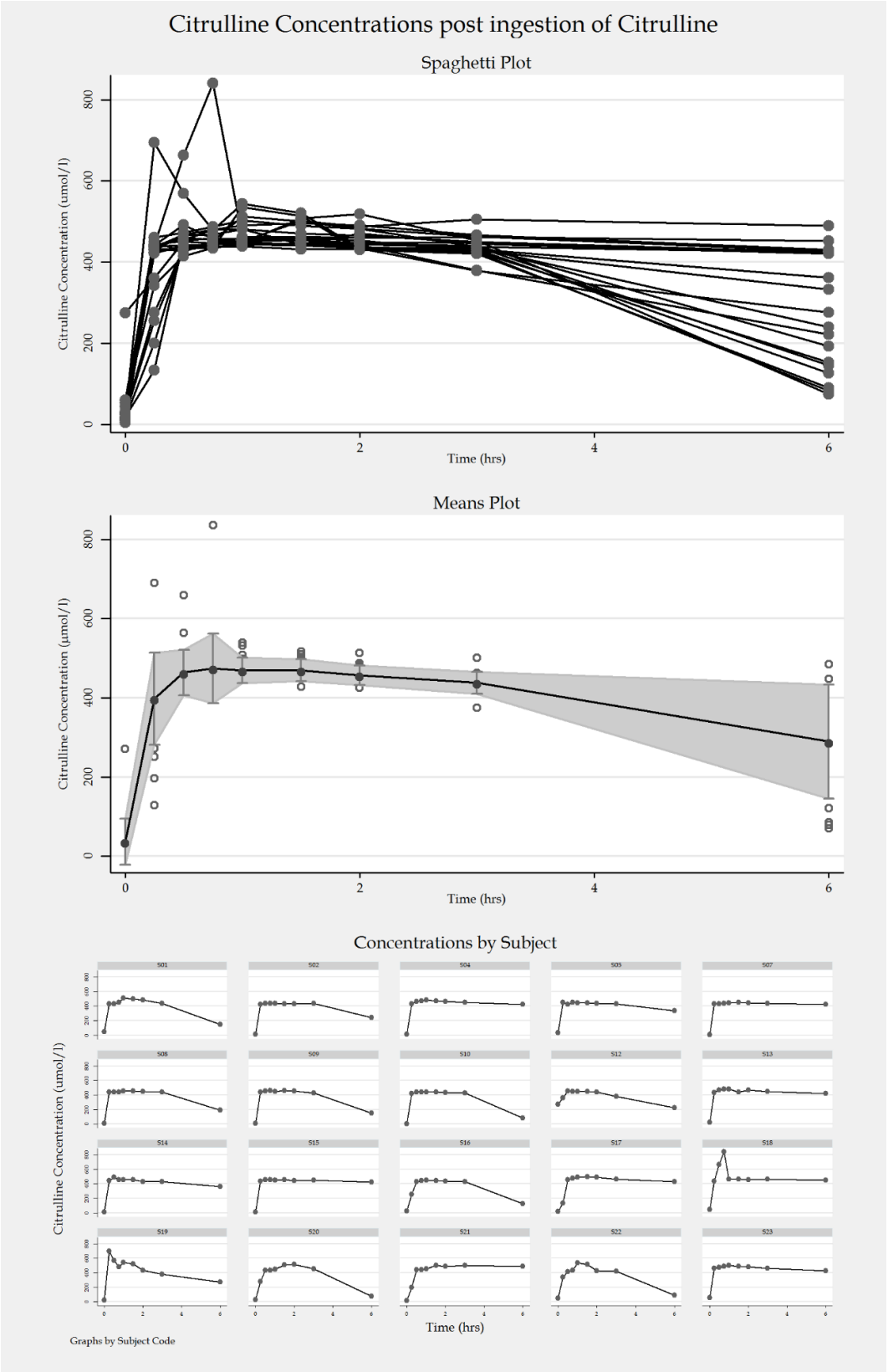


Figure 3.2. Citrulline concentrations post ingestion of citrulline.

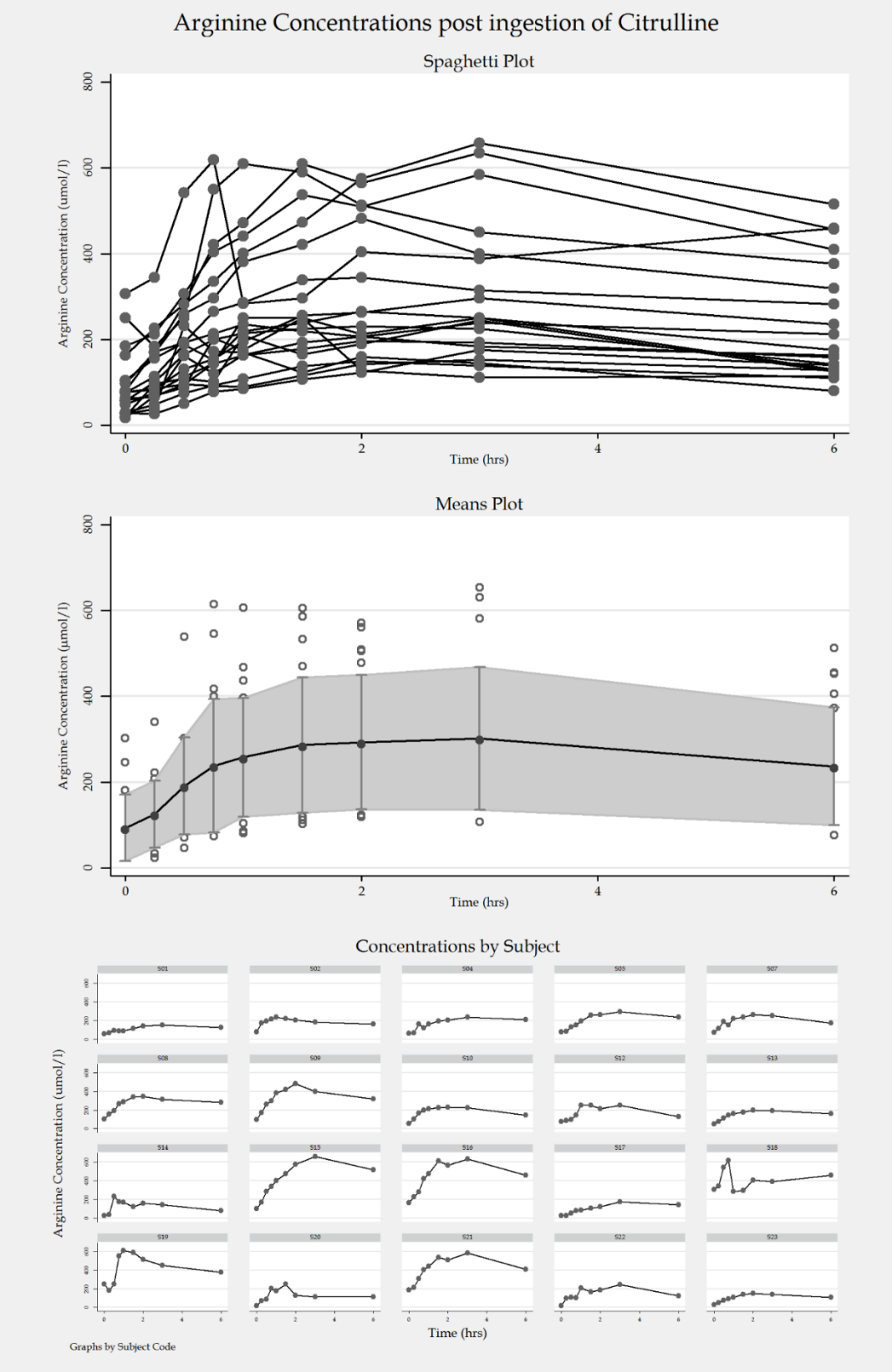


Figure 3.3. Arginine concentrations post ingestion of citrulline.

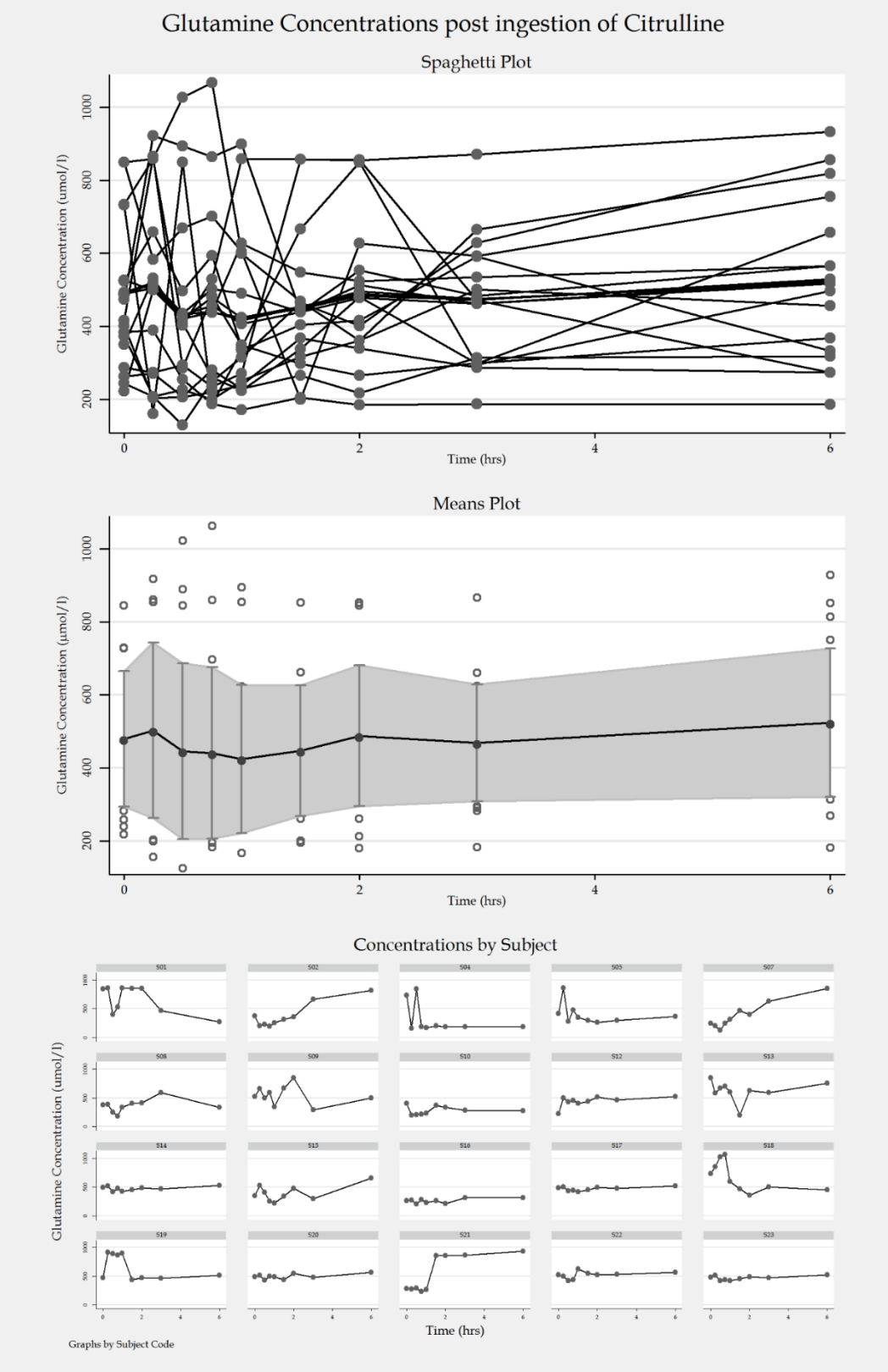


Figure 3.4. Glutamine concentrations post ingestion of citrulline.

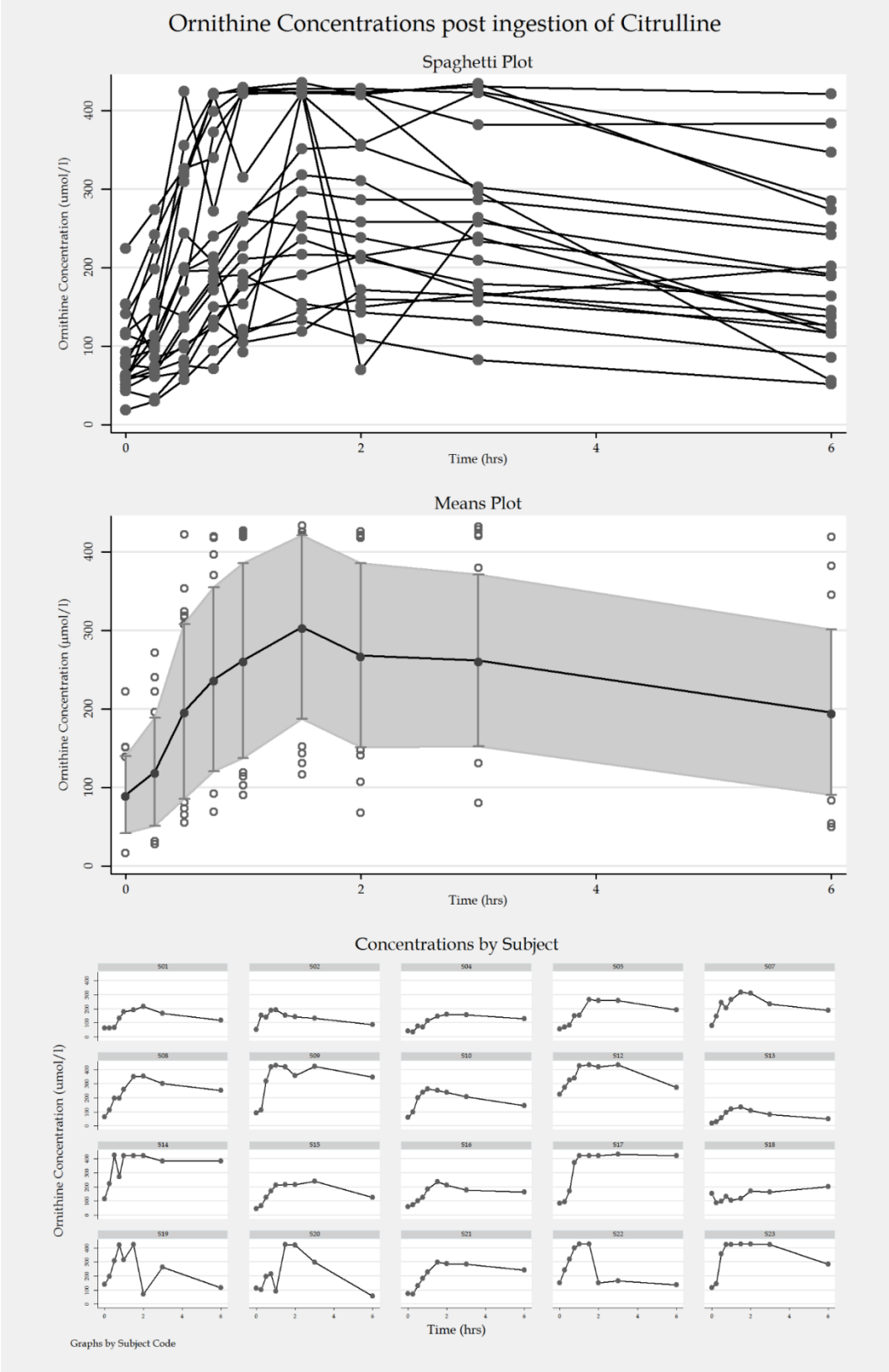


Figure 3.5. Ornithine concentrations post ingestion of citrulline.

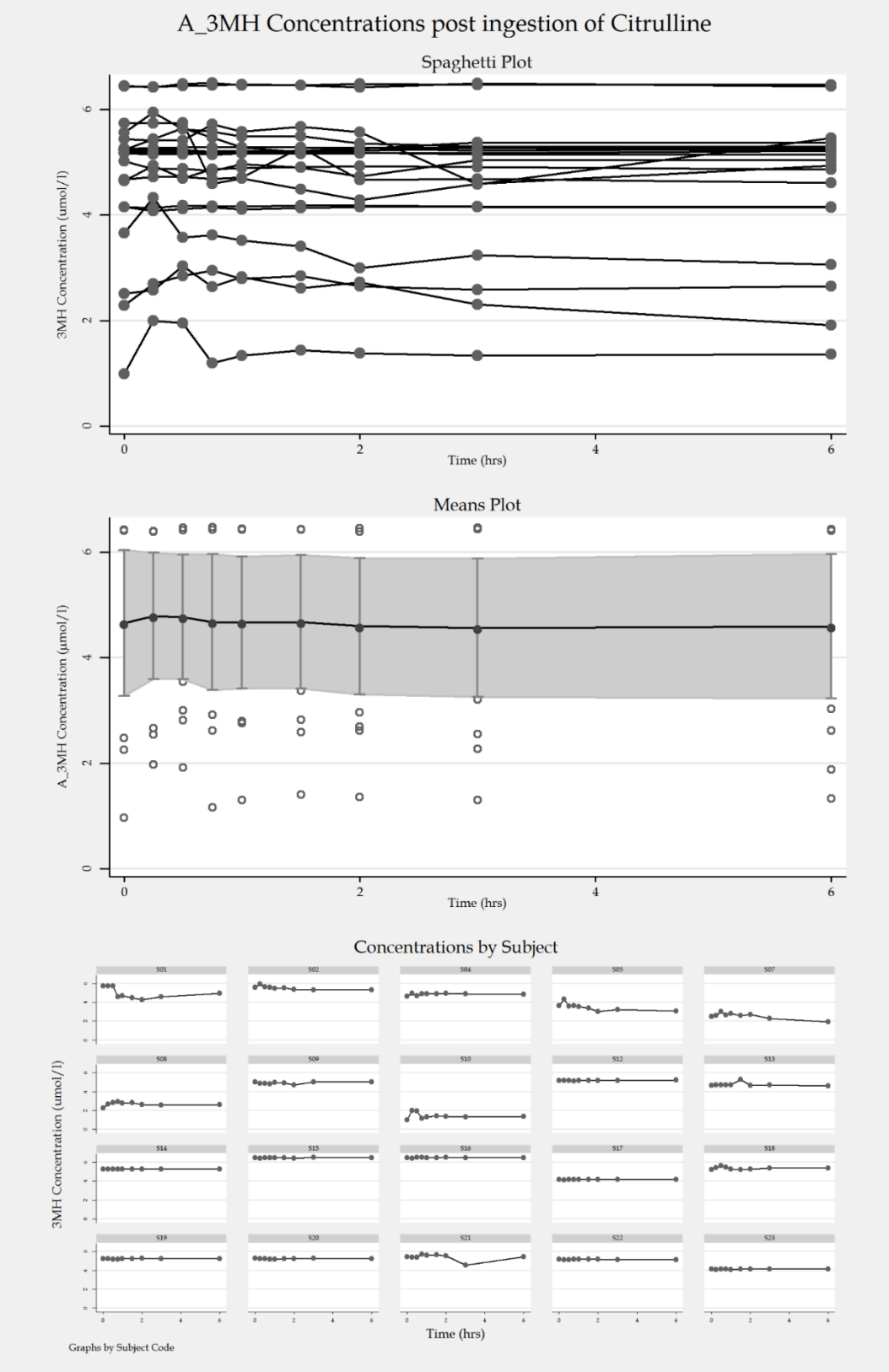


Figure 3.6. 3-Methylhistidine concentrations post ingestion of citrulline.

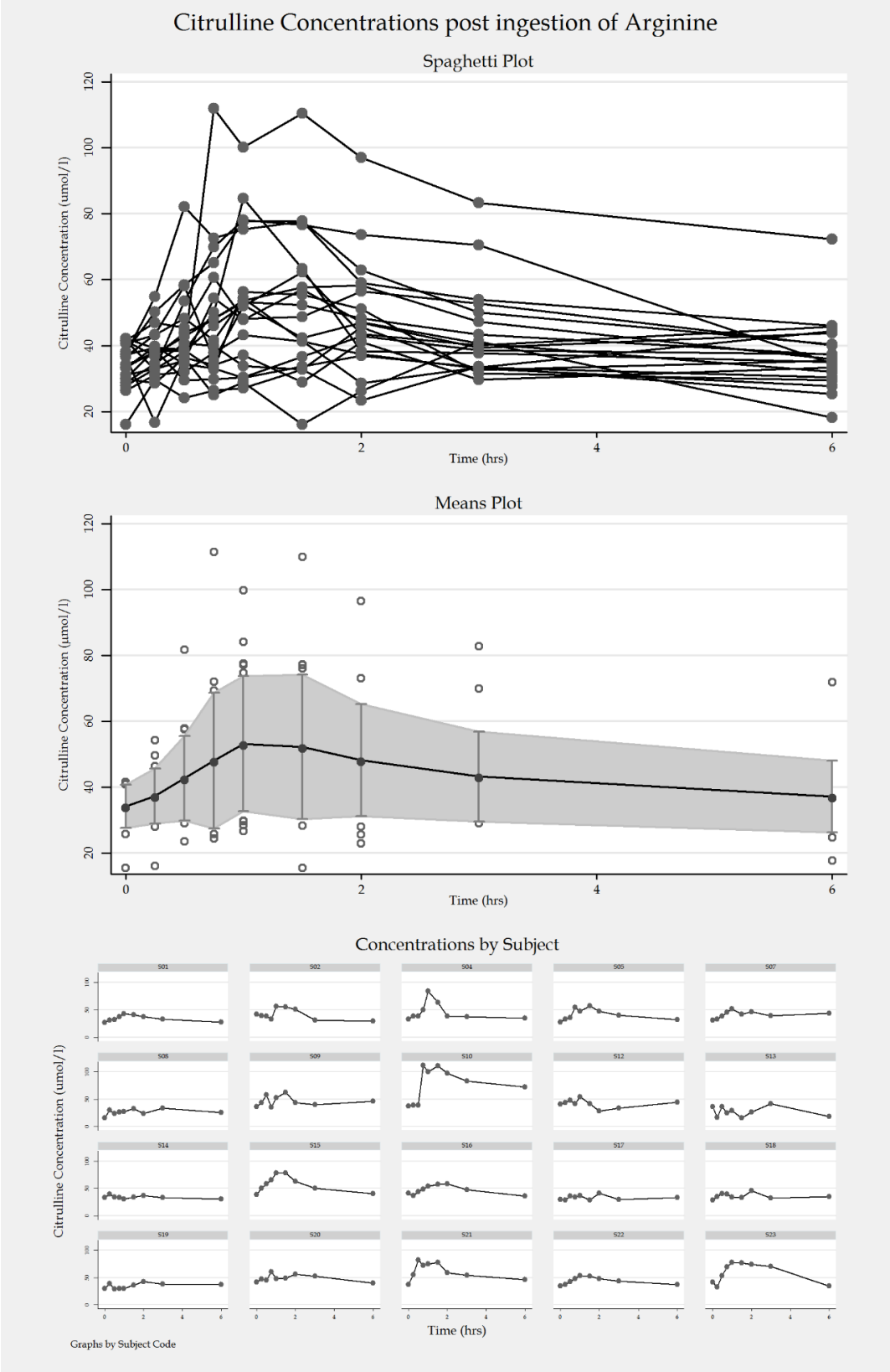


Figure 3.7. Citrulline concentrations post ingestion of arginine.

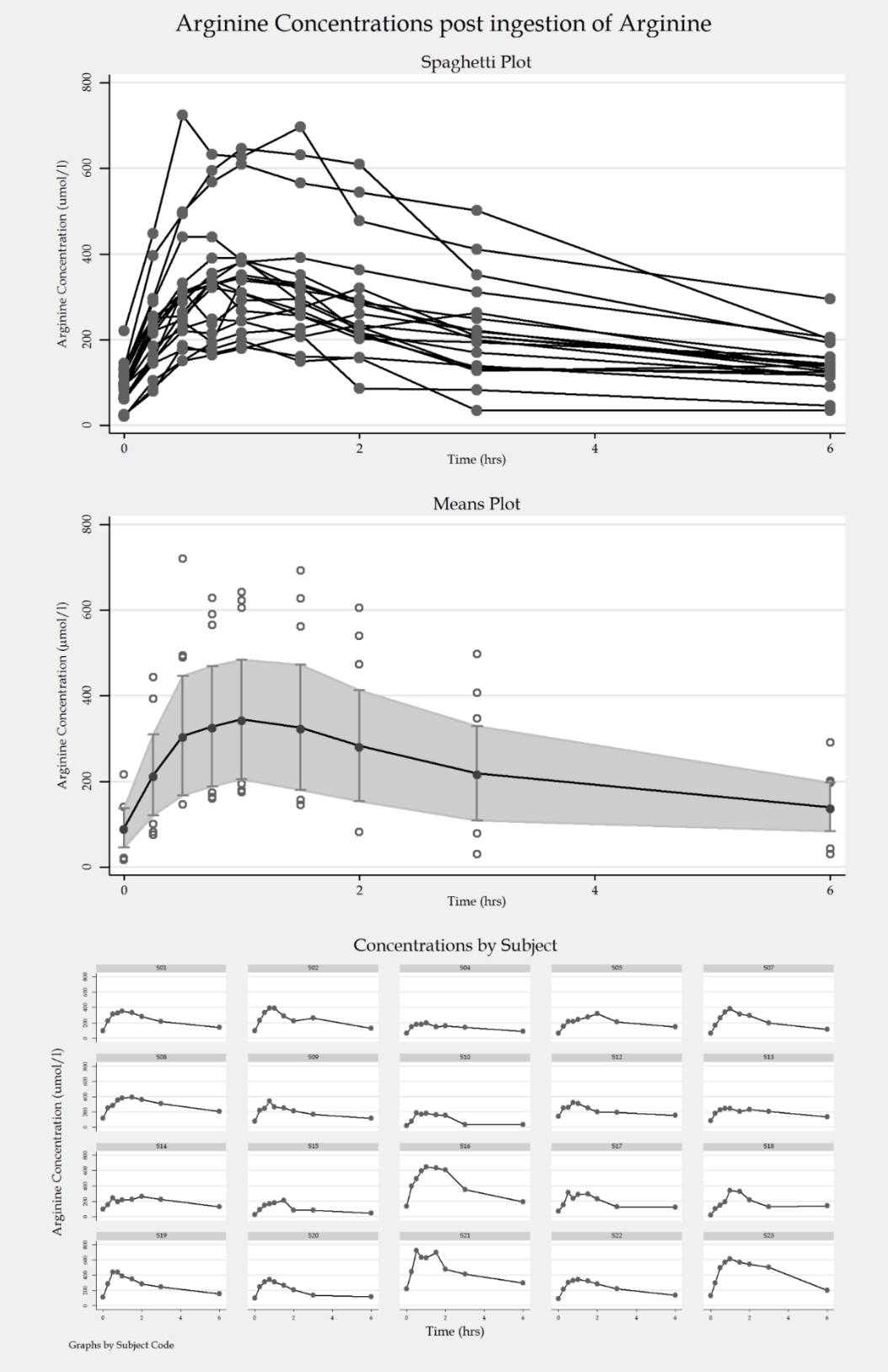


Figure 3.8. Arginine concentrations post ingestion of arginine.

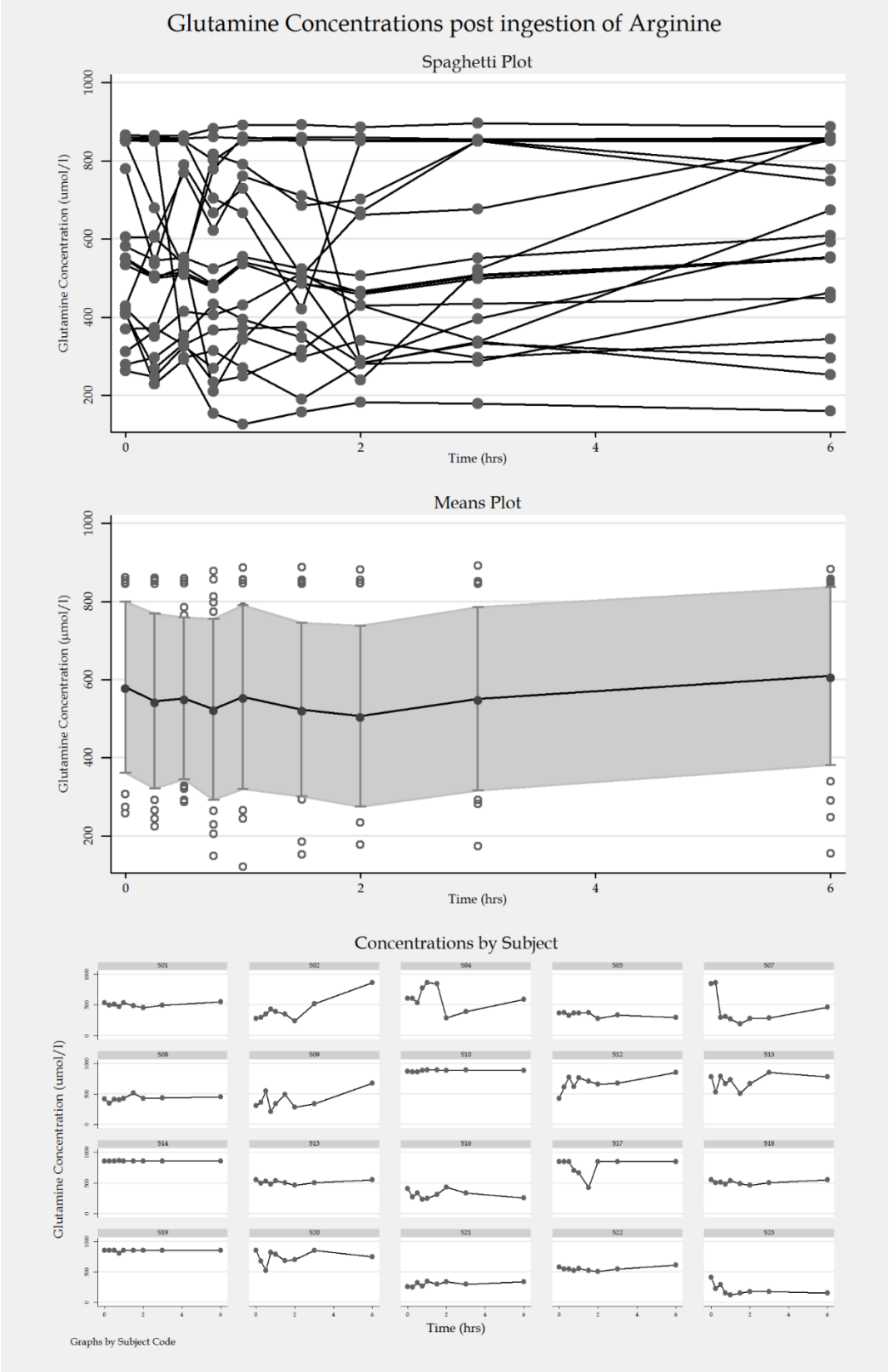


Figure 3.9. Glutamine concentrations post ingestion of arginine.

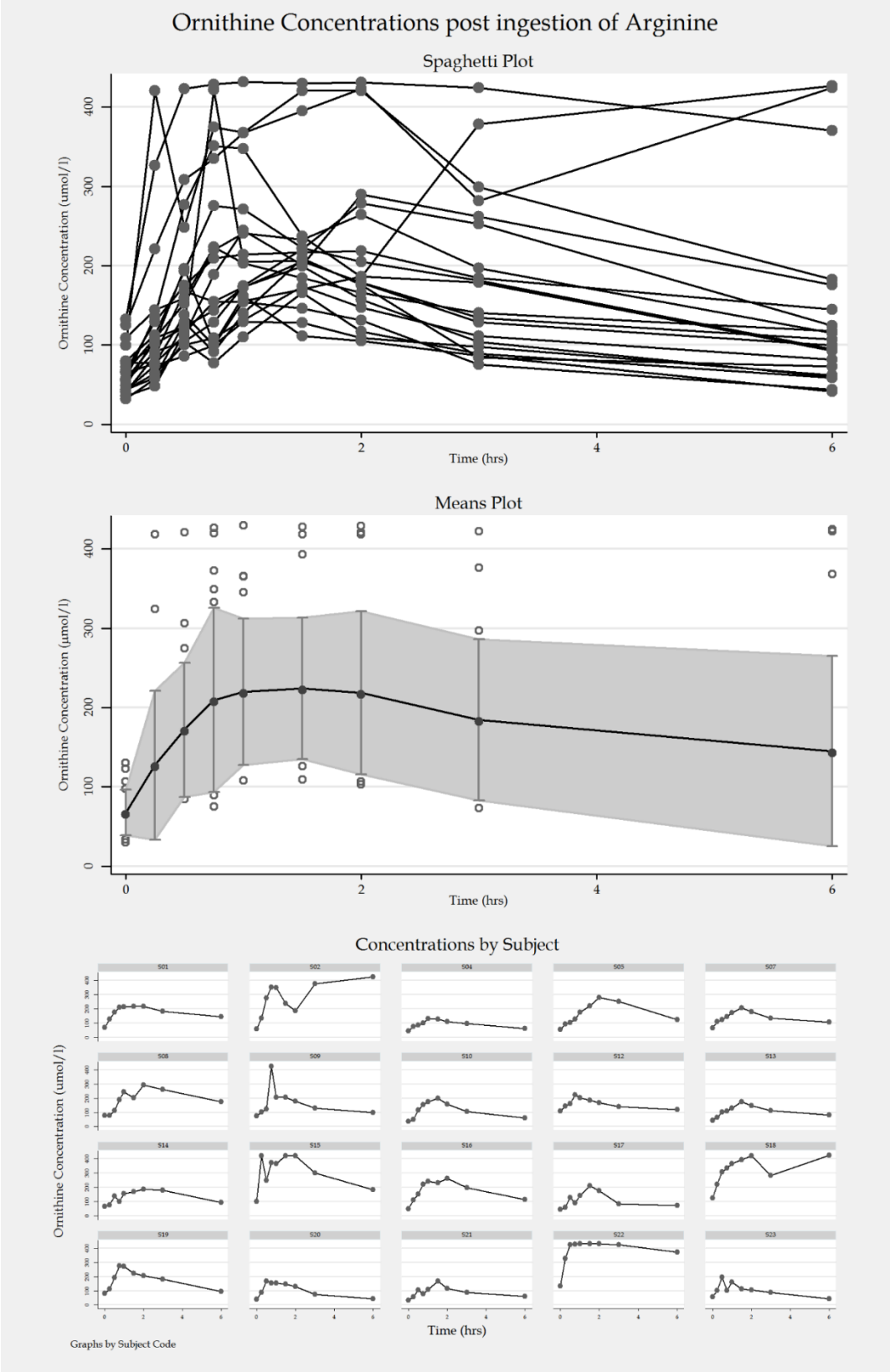


Figure 3.10. Ornithine concentrations post ingestion of arginine.

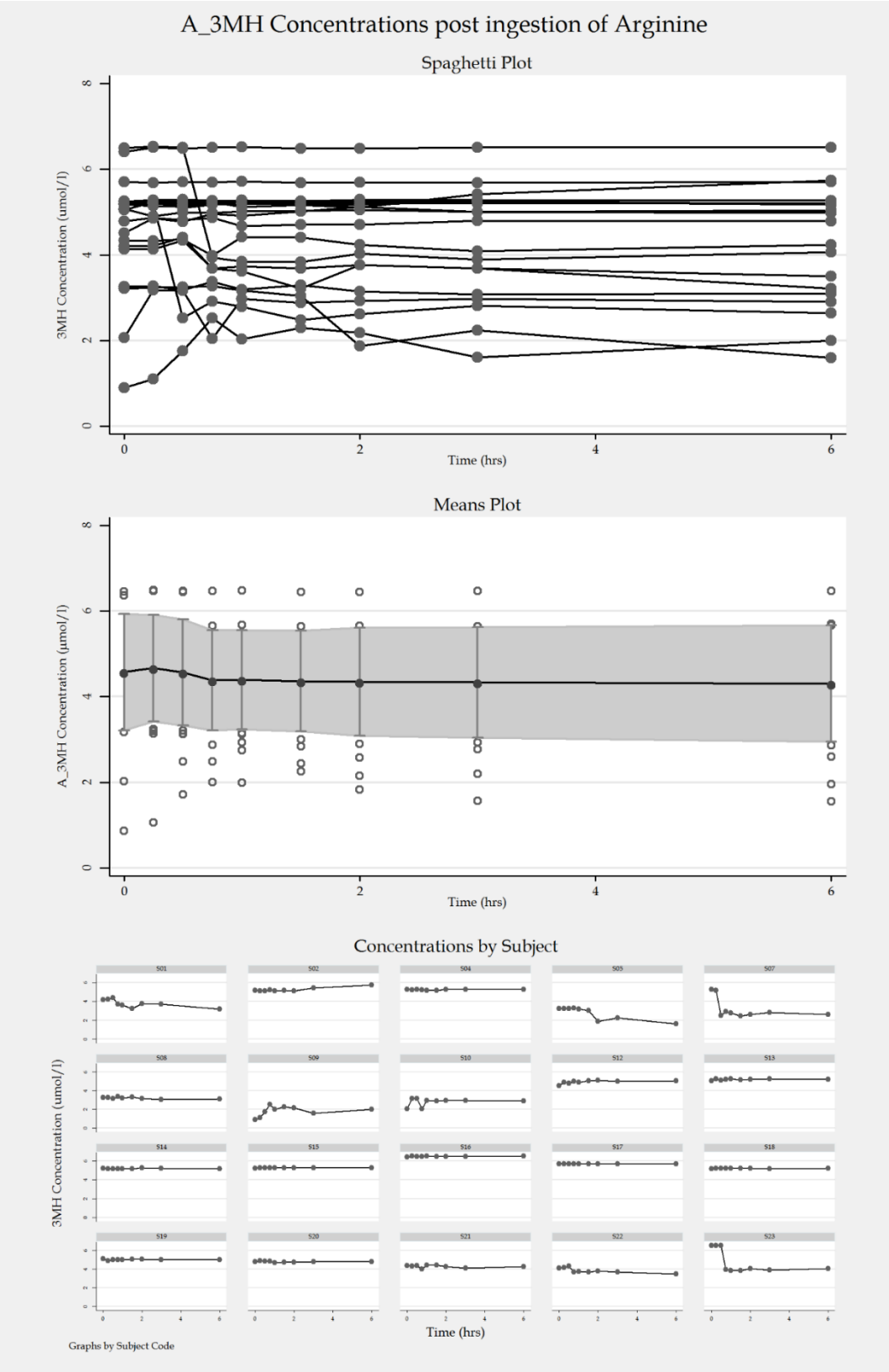


Figure 3.11. 3-Methylhistidine concentrations post ingestion of arginine.

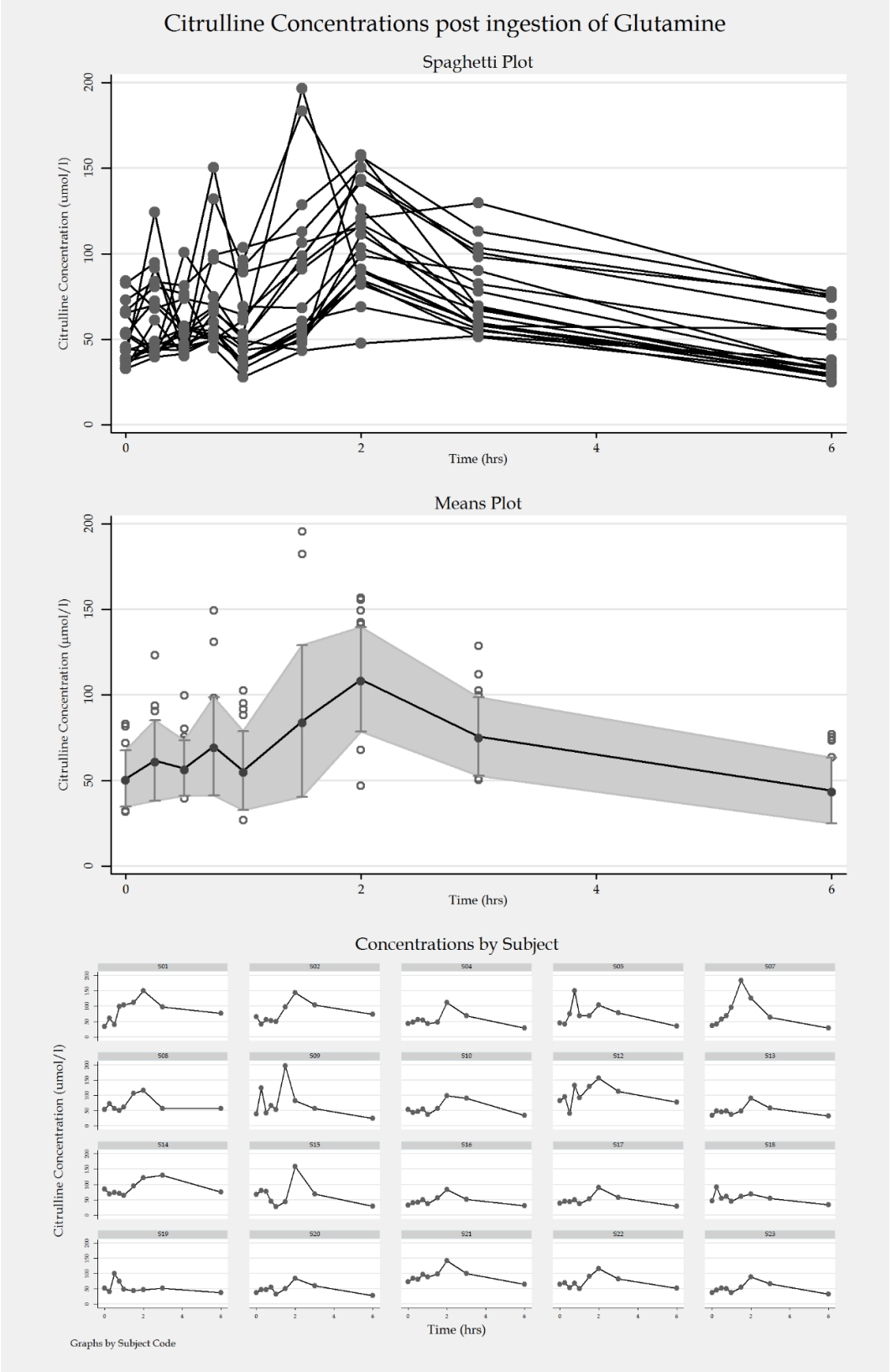


Figure 3.12. Citrulline concentrations post ingestion of glutamine.

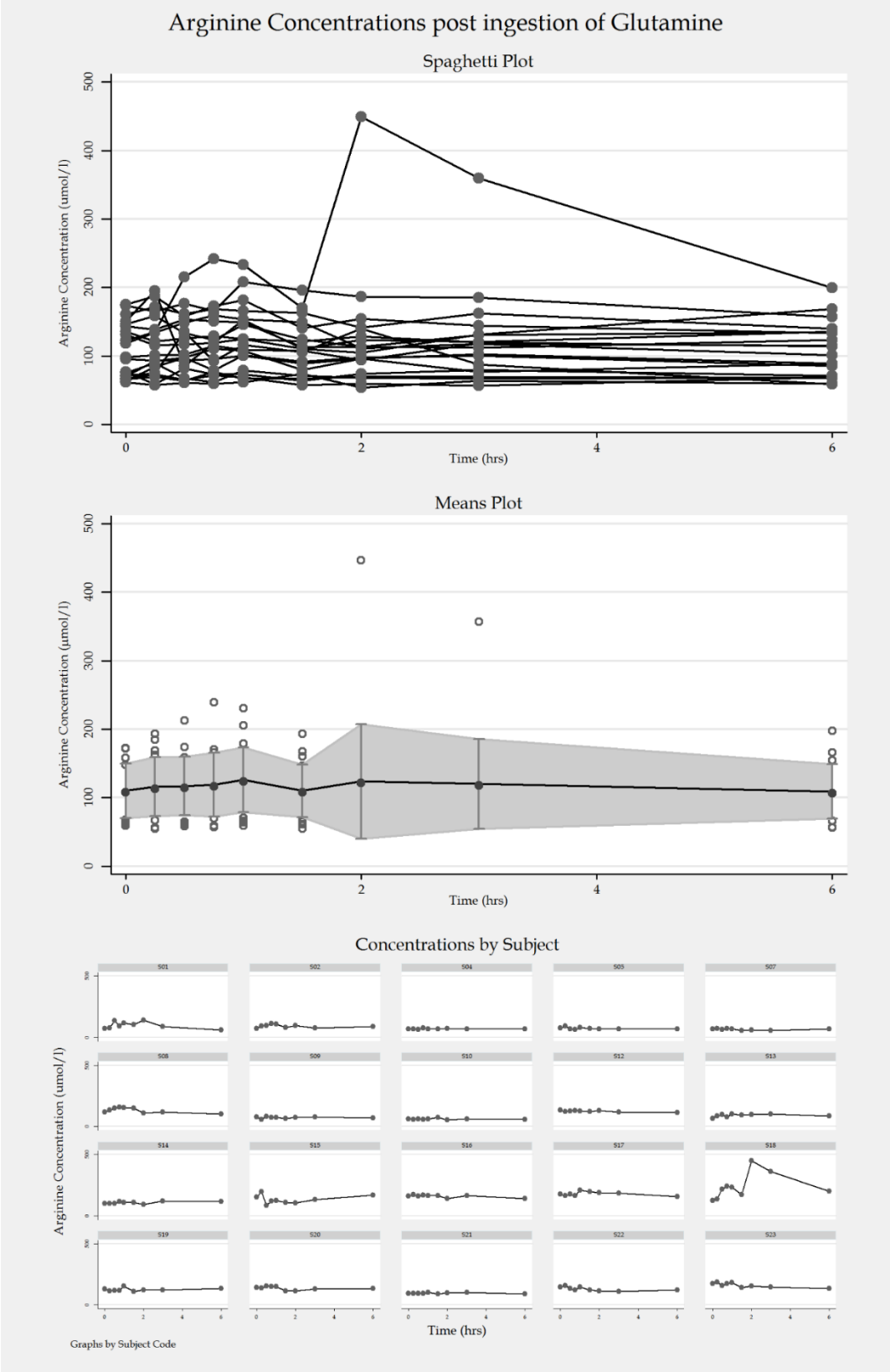


Figure 3.13. Arginine concentrations post ingestion of glutamine.

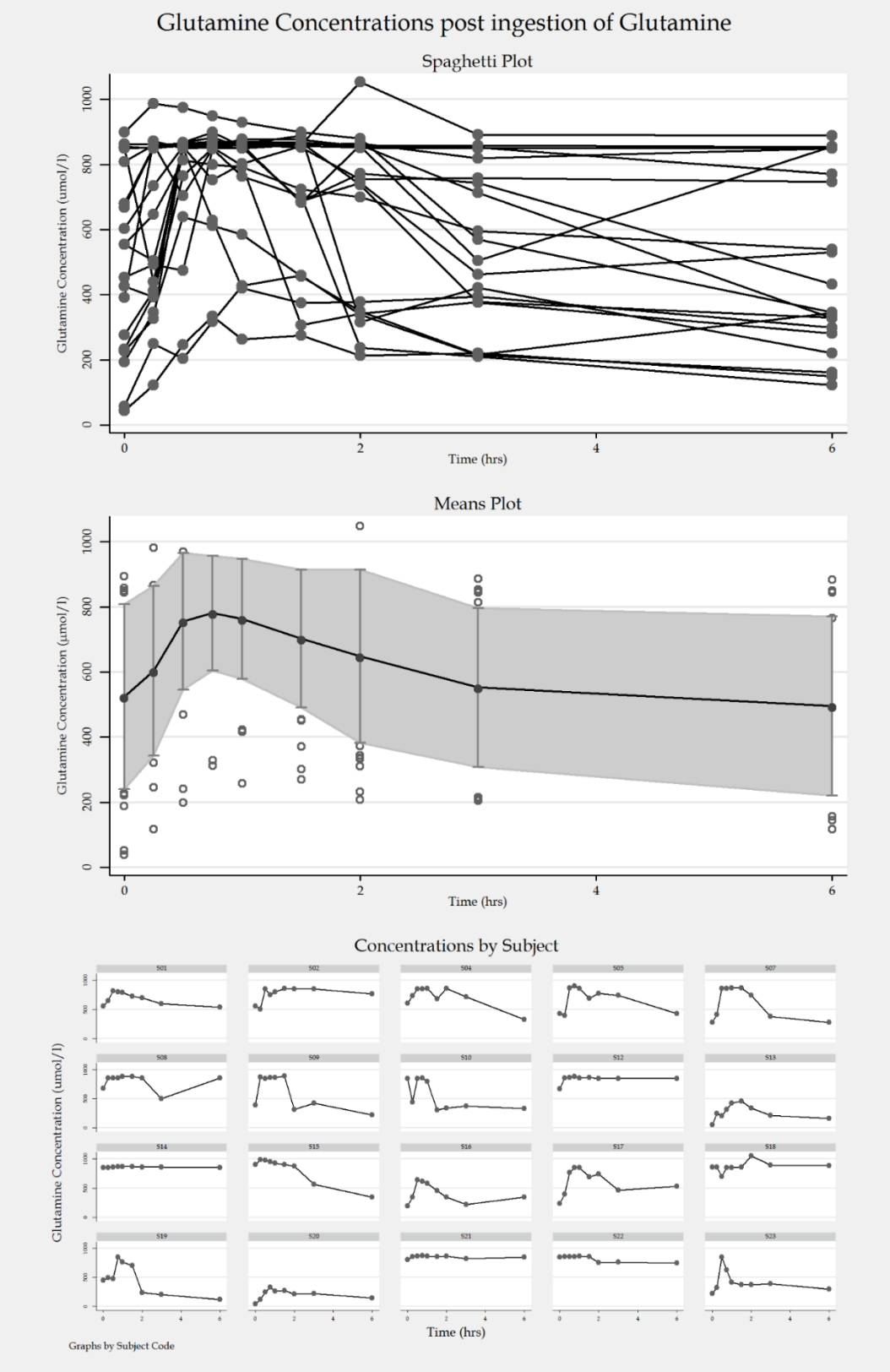


Figure 3.14. Glutamine concentrations post ingestion of glutamine.

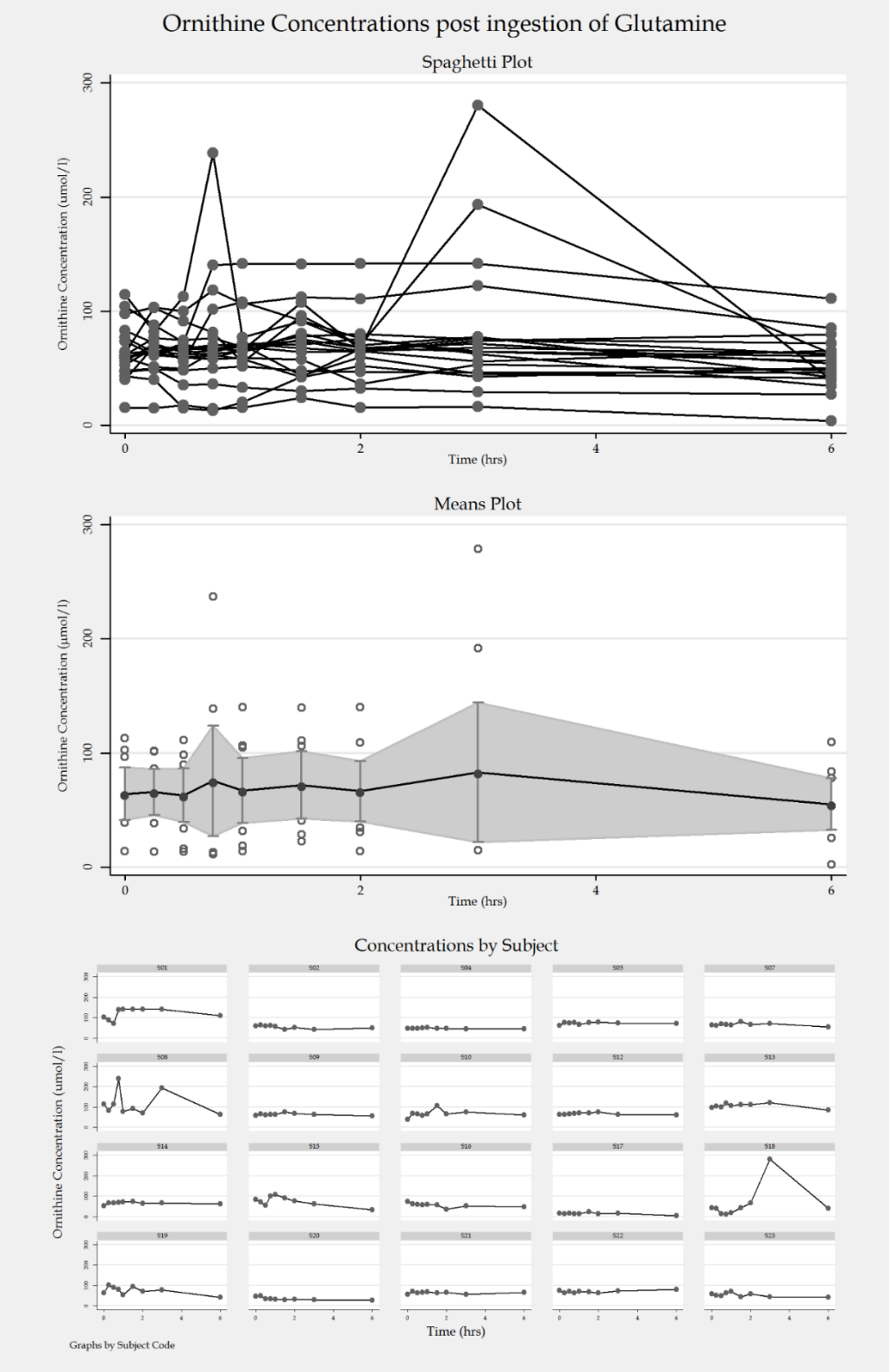


Figure 3.15. Ornithine concentrations post ingestion of glutamine.

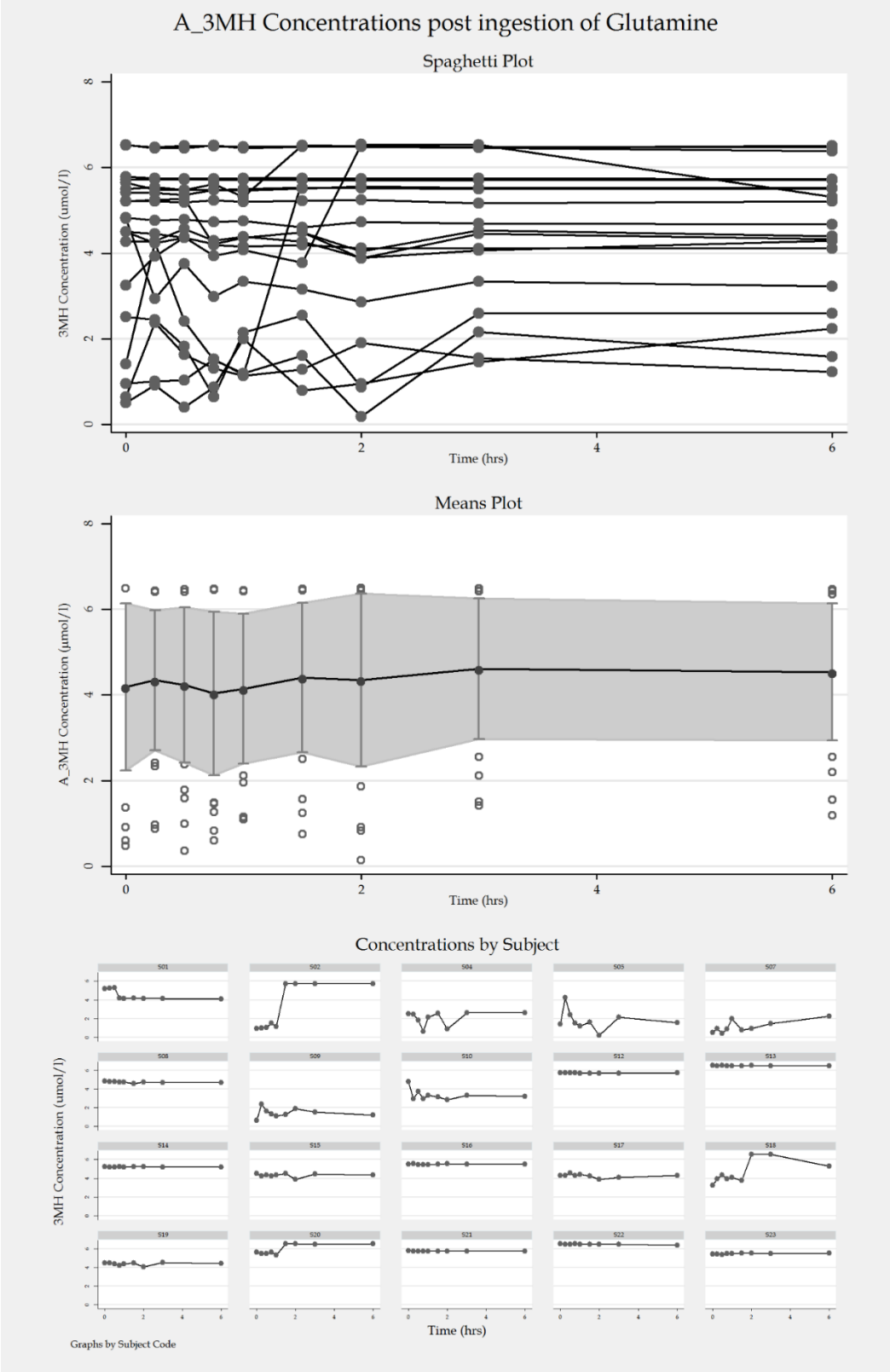


Figure 3.16. 3-Methylhistidine concentrations post ingestion of glutamine.

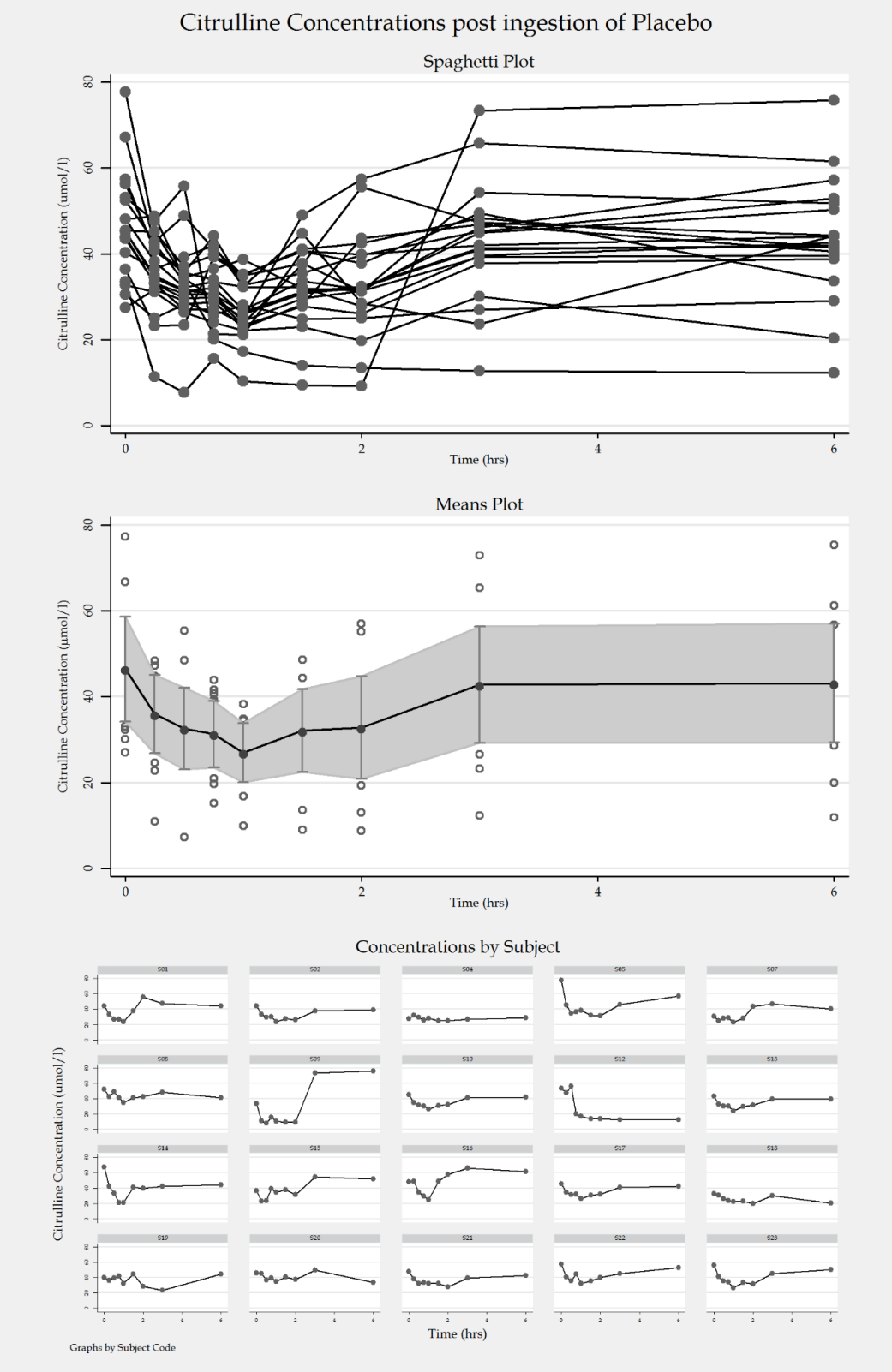


Figure 3.17. Citrulline concentrations post ingestion of placebo.

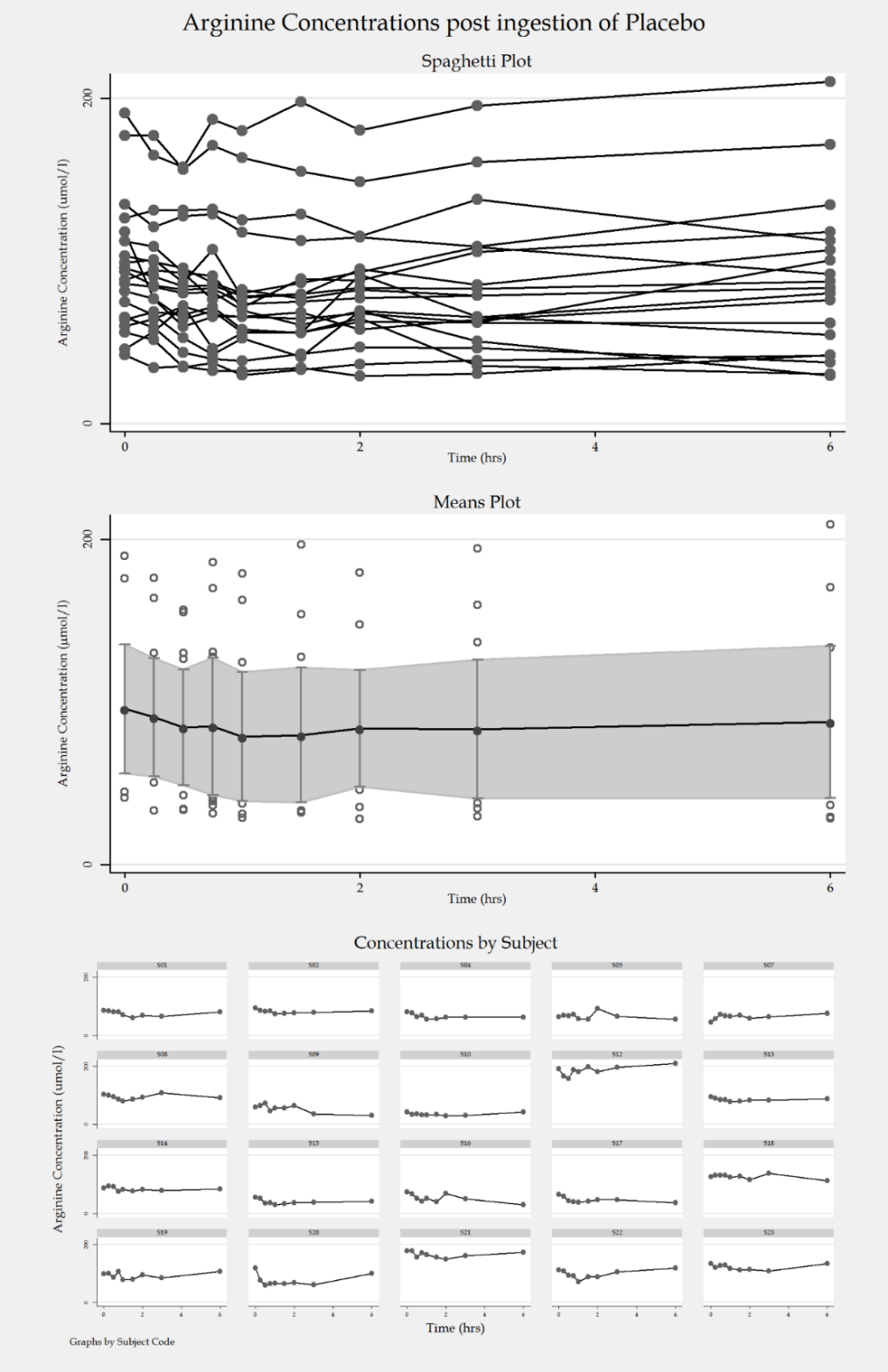


Figure 3.18. Arginine concentrations post ingestion of placebo.

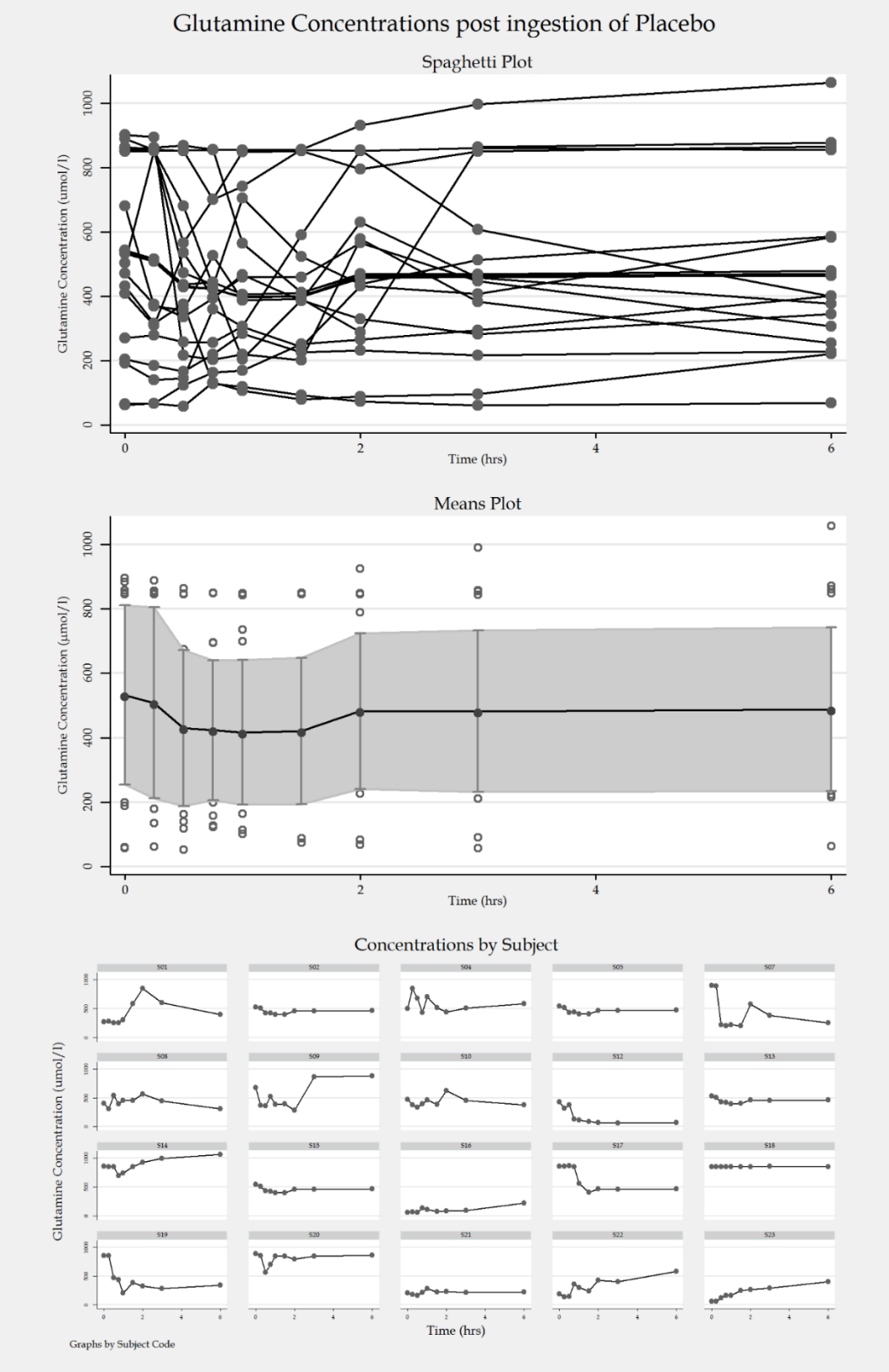


Figure 3.19. Glutamine concentrations post ingestion of placebo.

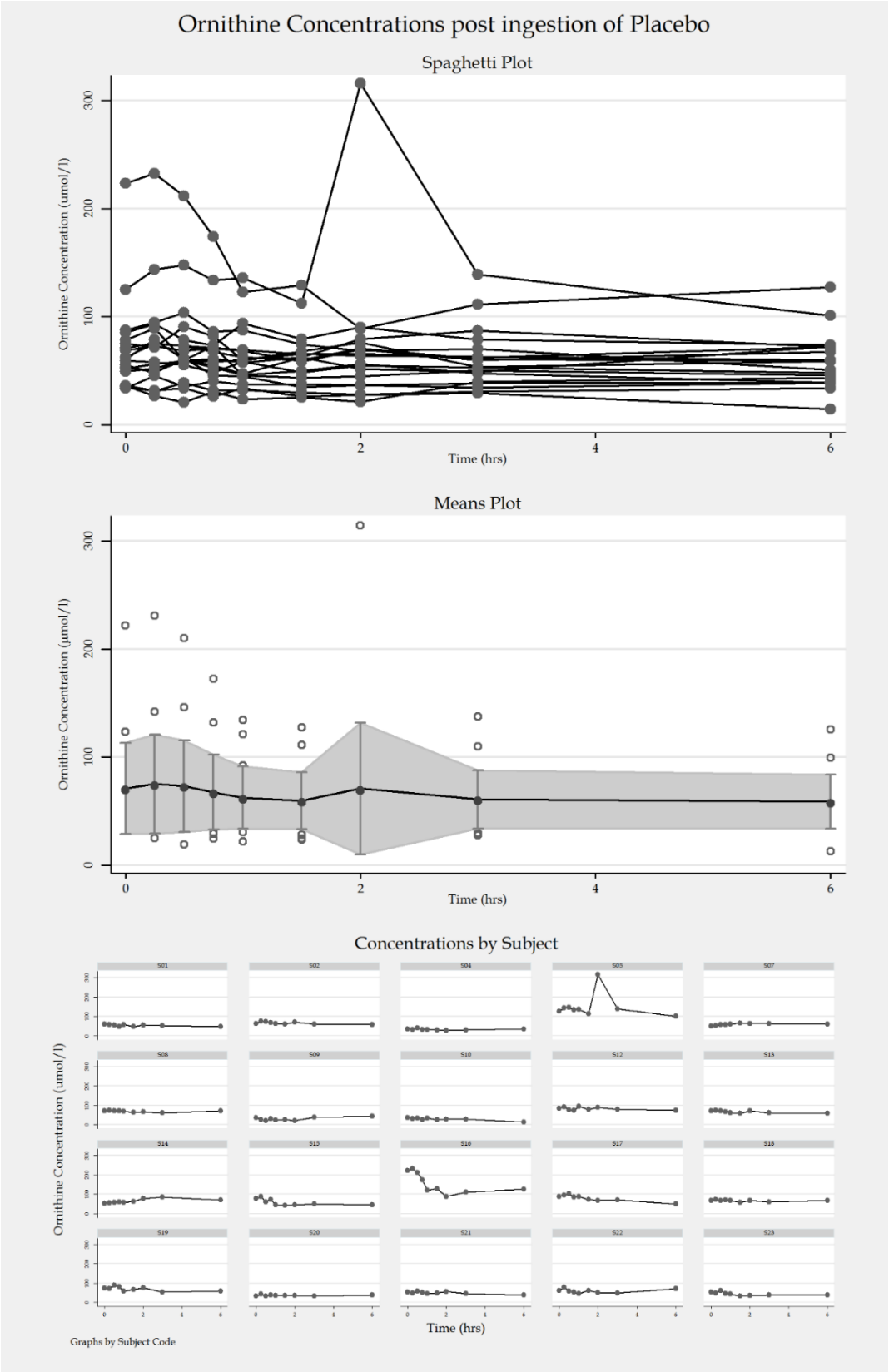


Figure 3.20. Ornithine concentrations post ingestion of placebo.

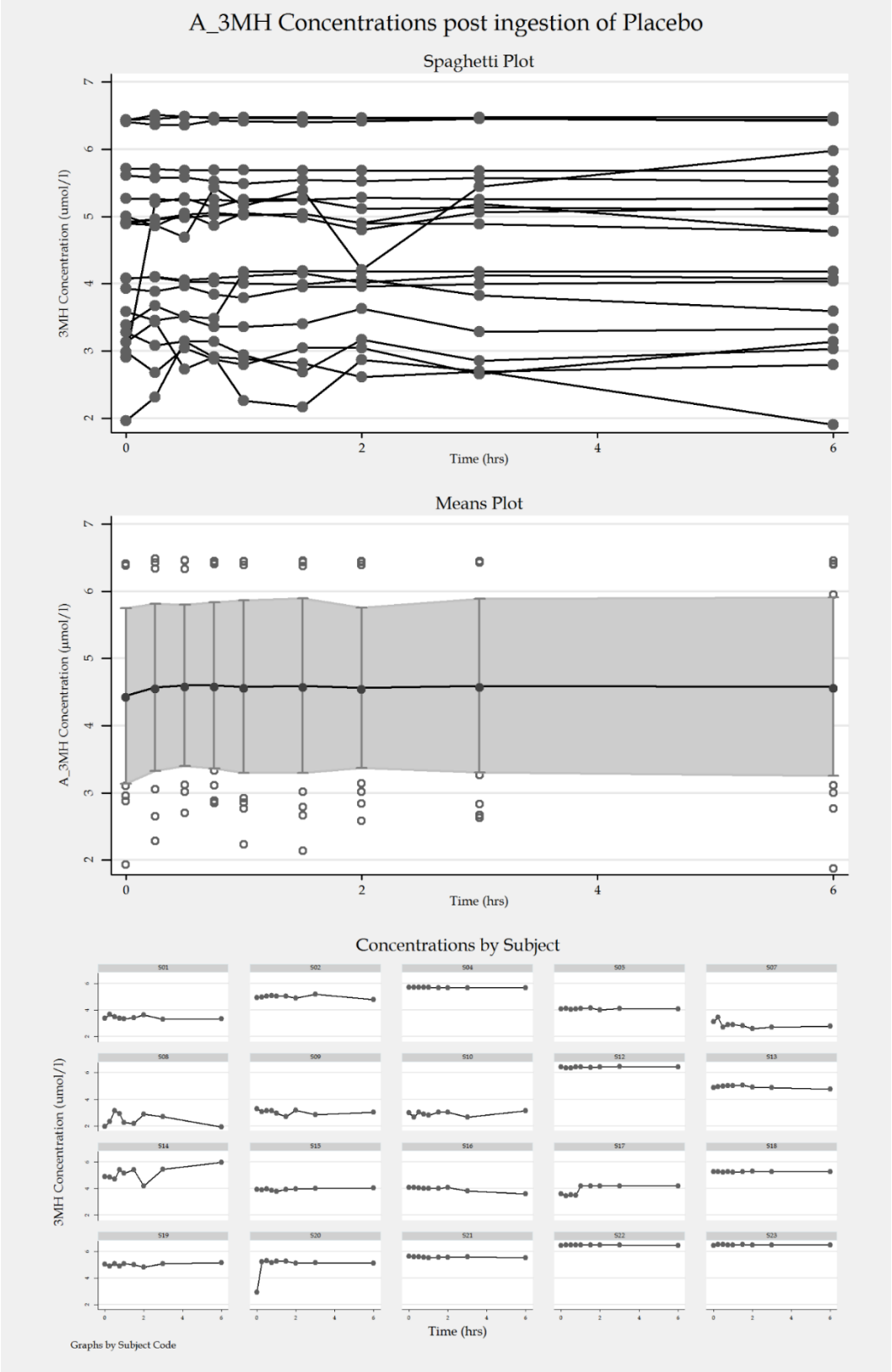


Figure 3.21. 3-Methylhistidine concentrations post ingestion of placebo.

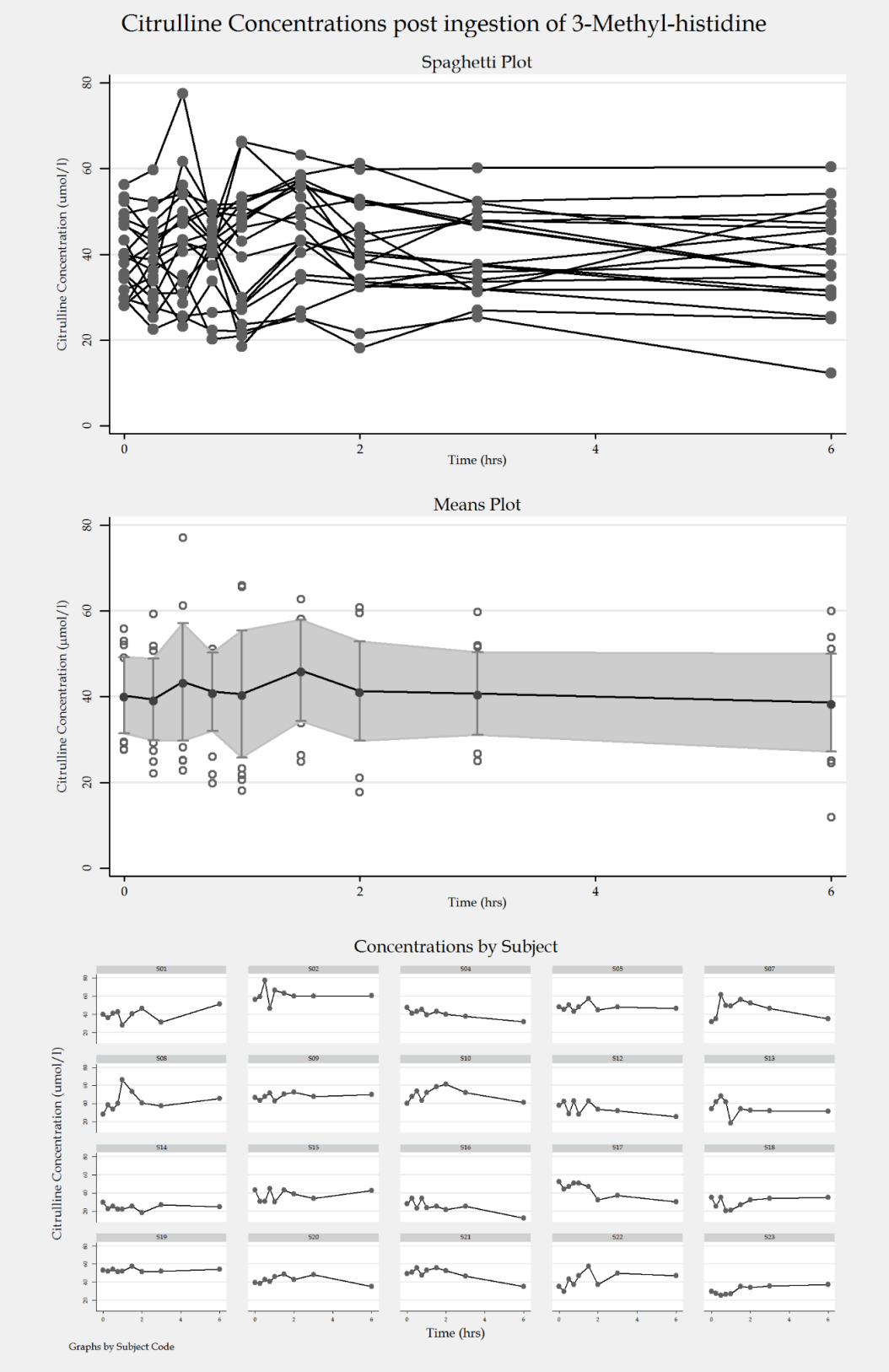


Figure 3.22. Citrulline concentrations post ingestion of 3-methylhistidine.

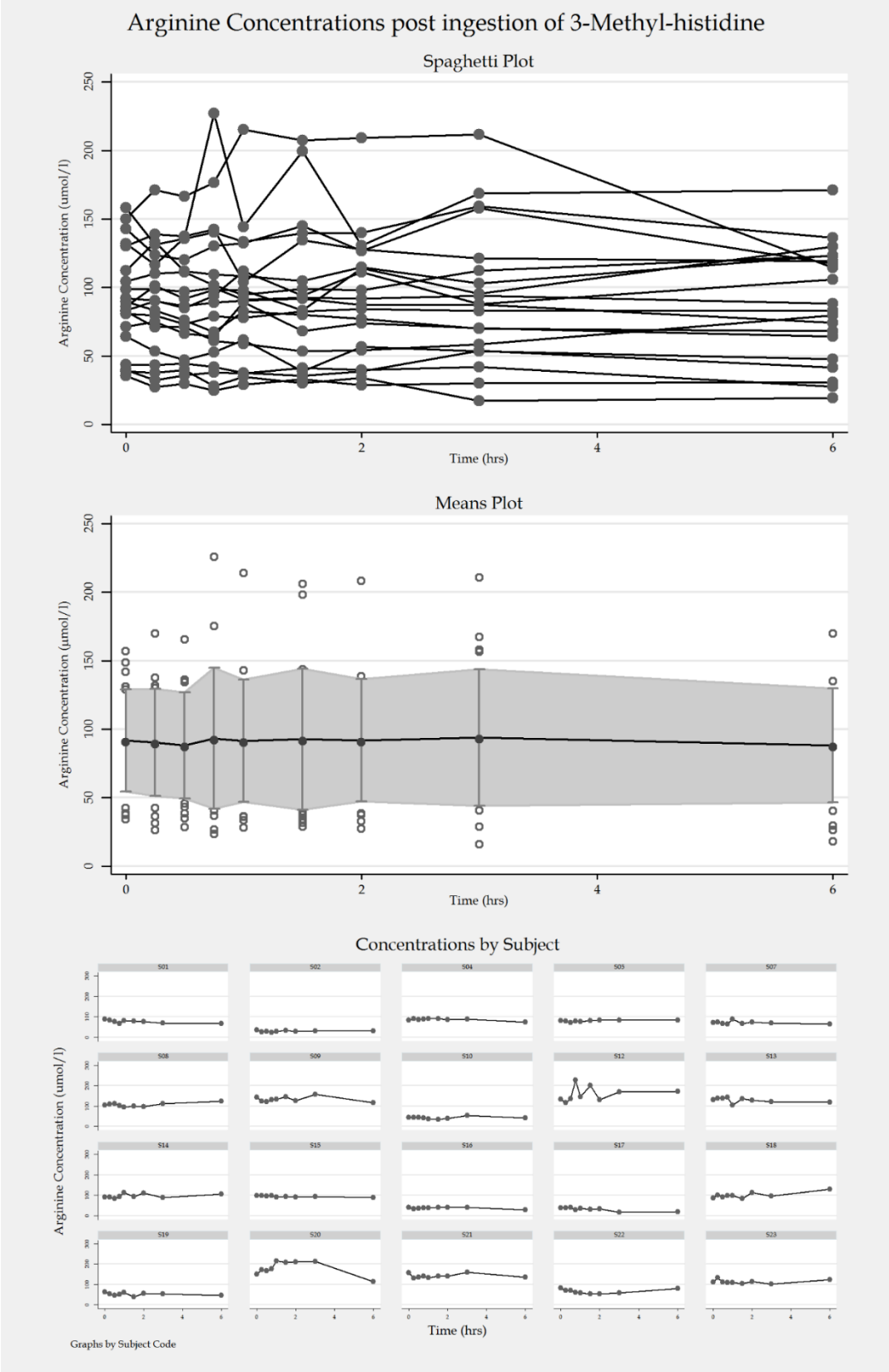


Figure 3.23. Arginine concentrations post ingestion of 3-methylhistidine.

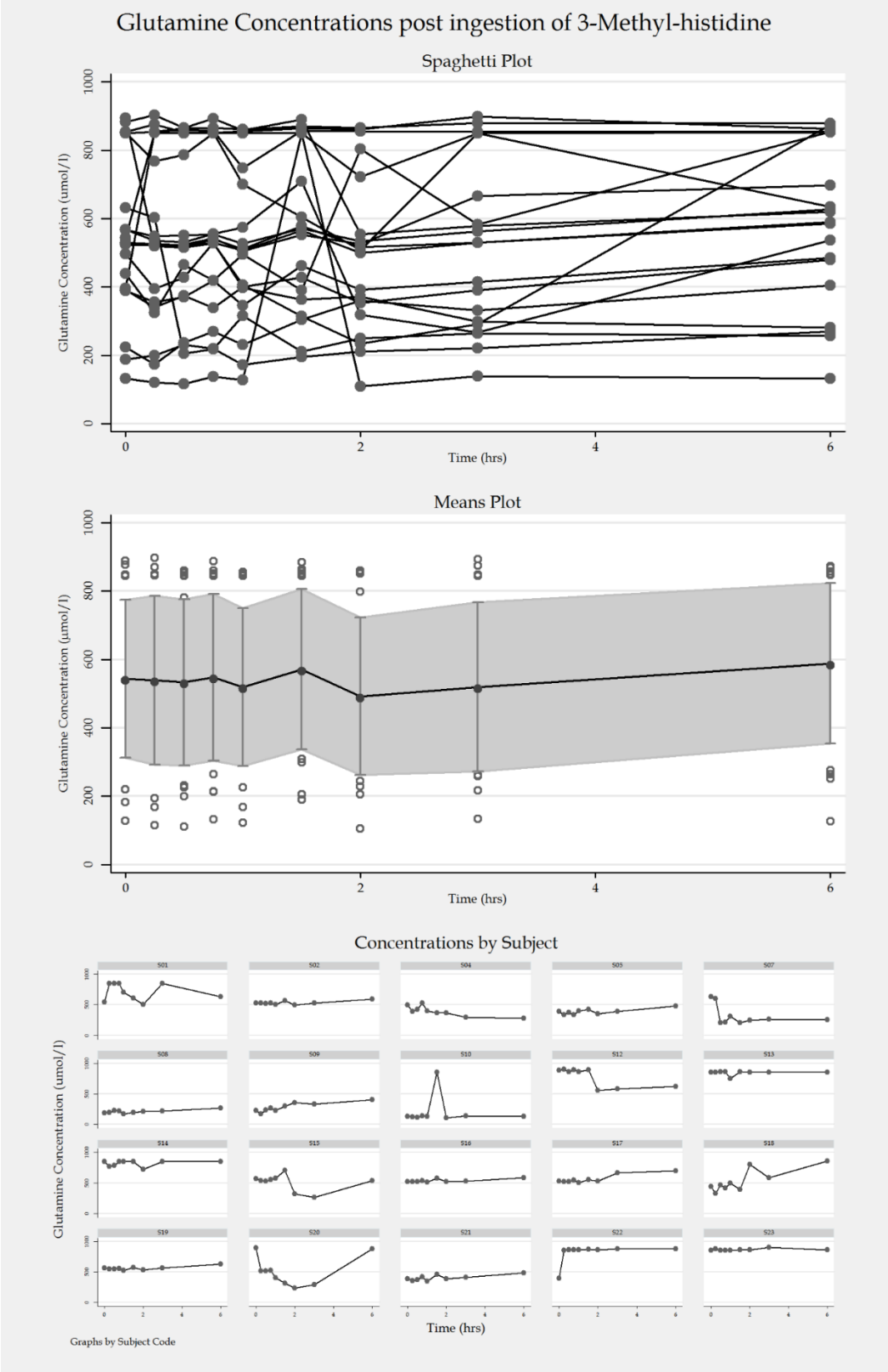


Figure 3.24. Glutamine concentrations post ingestion of 3-methylhistidine.

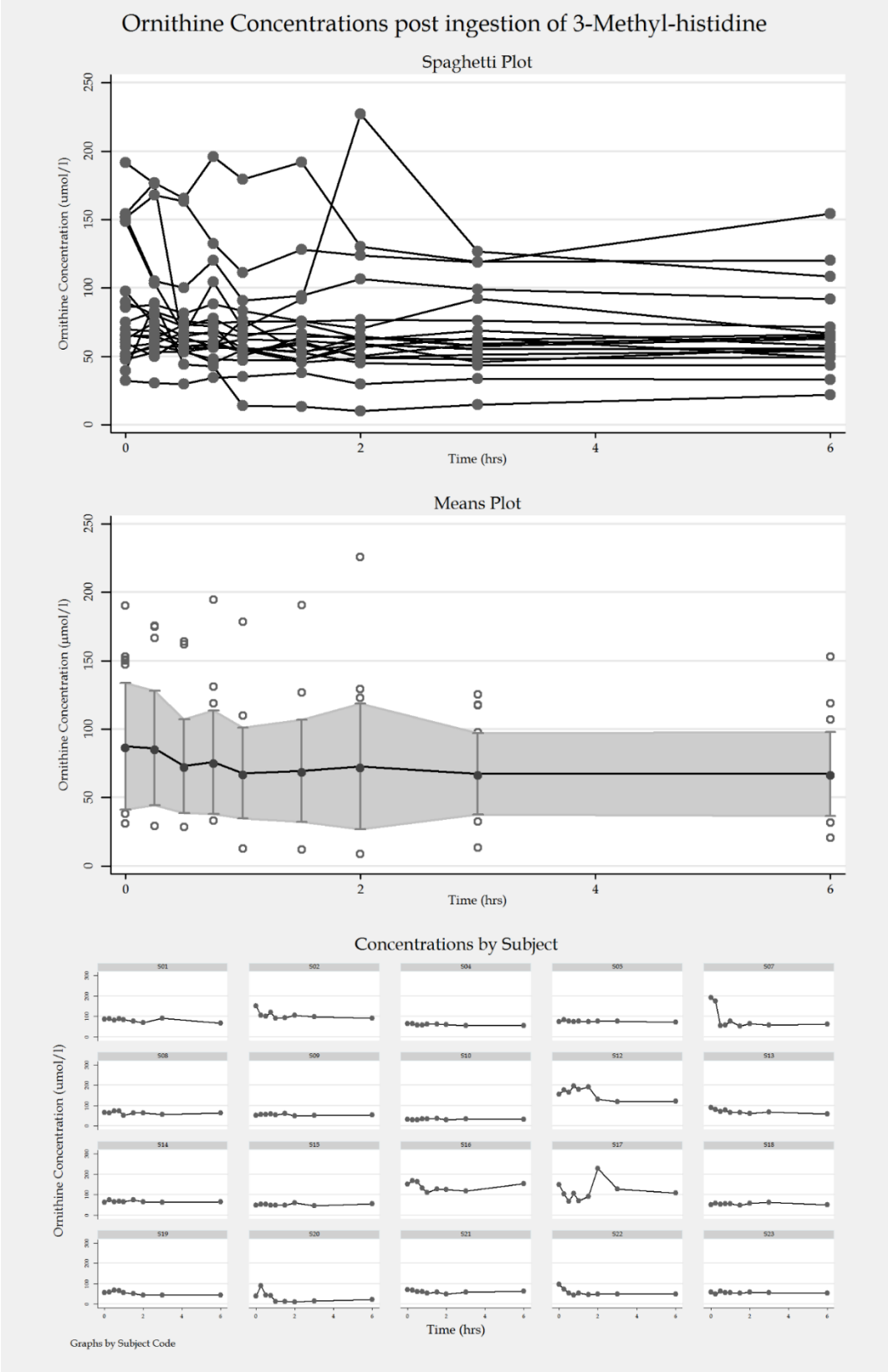


Figure 3.25. Ornithine concentrations post ingestion of 3-methylhistidine.

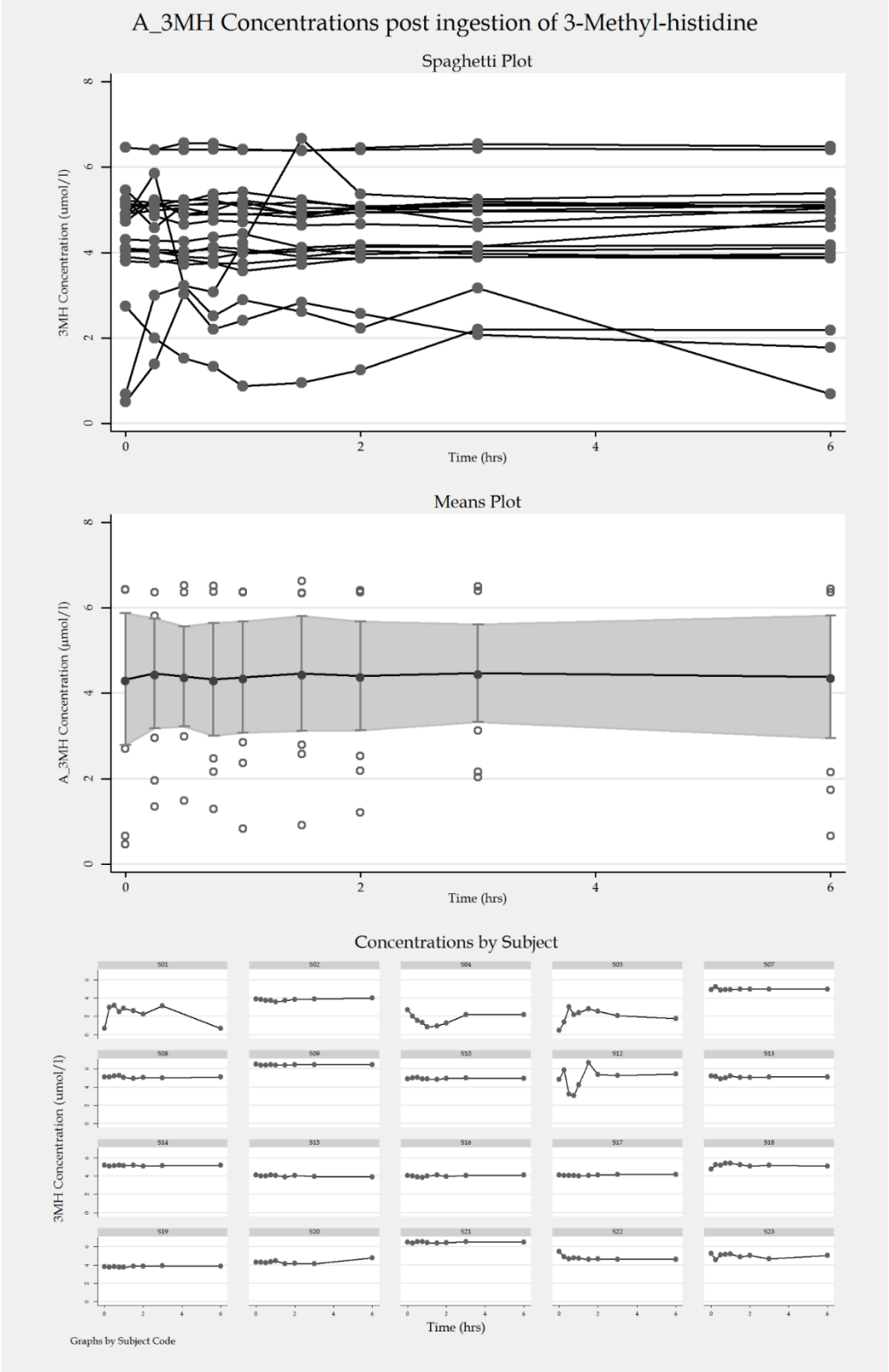


Figure 3.26. 3-Methylhistidine concentrations post ingestion of 3-methylhistidine.

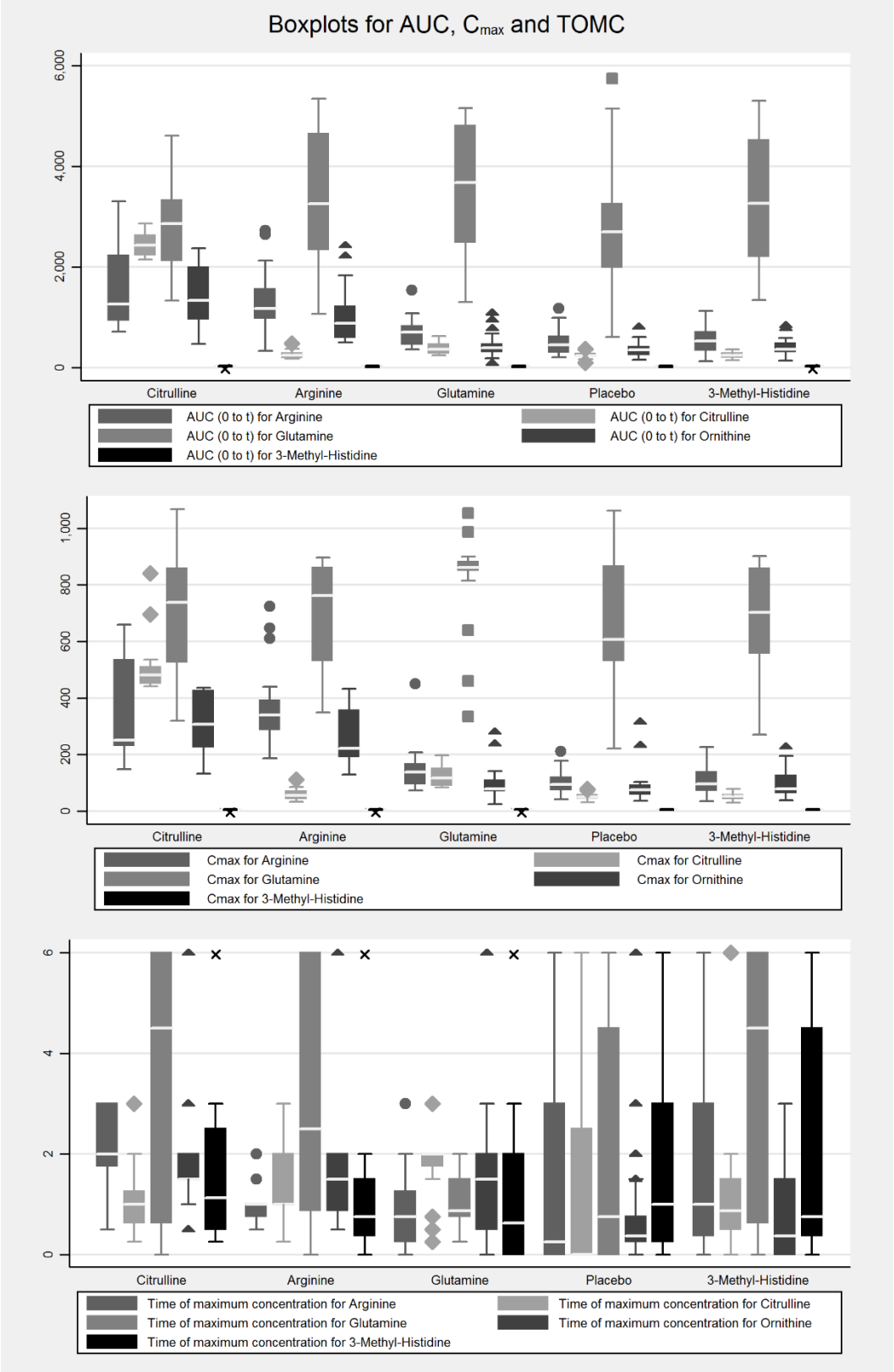


Figure 3.27. Boxplots for AUC, C_{max} and TOMC.

Figure 3.28. DFBETAs for the AUC of each plasma amino acid measured and per treatment.

Chapter 4
Quality of Life in Short Bowel Syndrome Patients

4.1 Introduction

As defined in the previous chapter, intestinal failure occurs when there is reduced intestinal absorption so that macronutrient and/or water and electrolyte supplements are needed to maintain health and/or growth. Short bowel syndrome is a global malabsorption syndrome due to insufficient absorptive capacity and/or disturbed gastrointestinal regulation resulting from extensive small bowel resections. Short bowel syndrome may occur after resection of more than 50 % and is obligatory after resection of more than 70 % of the small intestine or if less than 100 cm of small bowel are left. It is particularly severe after resection of the ileocecal region or if the colon has been removed additionally (Pironi *et al.*, 2015; Pironi *et al.*, 2016). Apart from global malnutrition, clinical symptoms may be caused by specific deficiencies depending on the site of intestinal loss (Keller *et al.*, 2004; Allan and Lal, 2018).

There are three main types of patient with a short bowel: those who have had a jejunoileal resection and a jejunocolic anastomosis (jejunum-colon); those who have had a predominantly jejunal resection, and have more than 10 cm of terminal ileum and the colon remaining (jejunum-ileum); and those who have had a jejunoileal resection, colectomy, and formation of a stoma (jejunostomy). The most common reasons for a short bowel in adults are Crohn's disease, superior mesenteric artery thrombosis, and irradiation damage (Keller *et al.*, 2004; Nightingale and Woodward, 2006; Pironi *et al.*, 2015; Pironi *et al.*, 2016).

The previous chapters discussed citrulline as a diagnostic marker in intestinal diseases and oral challenges of citrulline and other amino acids in healthy volunteers as a proxy of intestinal function. The present chapter will attempt to infer whether citrulline can be used as a proxy of quality of life in patients with short bowel syndrome. This is an interesting question that requires some thought. Citrulline essentially reflects enterocyte mass, disease severity and absorption (see Chapter 2). It is reasonable to think that when citrulline is abnormal, the intestine is not fully functioning and hence this diseased state may possibly lead to impaired

quality of life. Hence, from a clinical point of view, measuring citrulline in daily practice, might reflect the associated level of quality life in short bowel syndrome patients, functioning as a surrogate marker for quality of life. Nevertheless the literature of quality of life in short bowel syndrome is quite variable. Below I review the literature in this field and describe results of a quality of life study performed at University College London Hospitals. In the discussion, I will come back to the question inferred above regarding citrulline as a surrogate marker of quality of life.

4.2 Quality of Life in Short Bowel Syndrome Patients

An important aspect of quality of life is nutrition. Ljungqvist and Pichard (2008) comment on this and say:

> Just because a person becomes a patient should not result in him or her losing the right to enjoy meals as we otherwise would in everyday life. It is, however, striking how often this fundamental and basic right is lost and not fulfilled in the midst of plentiful medical prescriptions and treatments. Despite the obvious insight of the fundamentals of food, the medical profession and caregivers in other situations fail to come to the insight that nutrition is one of the keys to successful outcomes and a better quality of life (p. 320).

Nevertheless, studies on quality of life in patients with short bowel syndrome could be more (Jeppesen *et al.*, 1999; Carlsson *et al.*, 2003b, a; Huisman-de Waal *et al.*, 2007; Kalaitzakis *et al.*, 2008). Short bowel syndrome is a disease that burdens patients' daily lives with intense therapies, complications needing frequent hospital admissions, stressful events, professional challenges, personal challenges and concomitant psychological repercussions. In addition to this, patients have particular aspects to their quality specific for their disease. For example, cancer operations that lead to short bowel syndrome burden patients with additional issues of quality of life related to their cancer (e.g. prognosis and morbidity) (Naghibi *et al.*, 2015; Cotogni *et al.*, 2017).

Overall, patients on with short bowel syndrome seem to report lower quality of life compared with the healthy population and quality of life seems to decline with longer parenteral nutrition-dependency (Avitzur and Miserachs, 2018). Various studies have identified over time a group of main issues that concern patients with short bowel syndrome (Baxter *et al.*, 2010; Winkler, 2010; Wilburn *et al.*, 2017;

Avitzur and Miserachs, 2018). These are shown in Table 4.1. A discussion on certain main studies in the field is given below.

Kalaitzakis *et al.* (2008) used four validated questionnaires to measure aspects of quality of life [Short Form 36 (SF-36)], psychological distress (hospital anxiety and depression scale), fatigue (fatigue impact scale), and gastrointestinal symptoms (gastrointestinal symptom rating scale) in 26 out of 28 patients (93%) attending a short bowel syndrome clinic (median age 62 years, 15 females) at a tertiary referral centre. The control group were people from the general population (n = 286) as well as patients with inflammatory bowel disease (n = 41). Their results showed that short bowel syndrome patients had significantly lower SF-36 physical and mental component summaries than those in the general population as well as significantly lower SF-36 physical but not mental component summaries compared with those of inflammatory bowel disease patients. Fatigue and gastrointestinal symptoms were more severe in short bowel syndrome patients than in inflammatory bowel disease patients ($p < 0.05$). Their conclusion was that patients with short bowel syndrome show poor quality of life compared with that in the general population with fatigue and gastrointestinal symptoms being more severe in patients with short bowel syndrome.

Carlsson *et al.* (2003b) studied 28 short bowel syndrome patients (19 females), mean age 54 years, with eight of them being on home parenteral nutrition. Quality of life was recorded using a visual analogue scale and health-related quality of life was assessed using SF-36 and compared with matched controls. Concerns were assessed using the Rating Form of Inflammatory bowel disease patient concerns. Coping strategies were investigated using the Jalowiec coping scale (Jalowiec *et al.*, 1984) Their results showed that patients' greatest concerns were fear of being a burden (median = 81), having surgery (median = 60) and loss of energy (median = 49). Health-related quality of life was significantly reduced compared to controls ($p < 0.05$), while patients' receiving home parenteral nutrition rated quality of life lower than those without home parenteral nutrition. The presence of a stoma appeared not to influence quality of life negatively but stoma patients expressed more concern. Confrontational coping style was most frequently used.

An important study in the field of short bowel syndrome looked at how the presence of home parenteral nutrition affected patient's daily lives (Baxter *et al.*, 2010). Their

inclusion criterion were patients on home parenteral nutrition and included other causes of intestinal failure in addition to short bowel syndrome. Their sample included 49 patients with short bowel syndrome (out of 100 in total). The authors developed a questionnaire that comprised of 48 items examining body image, coping, ability to eat or drink, employment, emotional problems, fatigue, general health, gastrointestinal symptoms, ability to go on holiday or travel, immobility, pain, physical role, social function, sleep pattern, sexual function, vitality, worry, and weight. These concepts were generally reduced in this patient group.

Olieman *et al.* (2012) examined health related quality of life in children with a history of infantile short bowel syndrome treated in their first year of life. Thirty-one children were included (19 girls, mean age 12 yeas). Their average health related quality of life was significantly lower than that of a control sample of 275 healthy age-matched children and their parents ($p < 0.05$). Physical and school functioning were significantly lower in the children with short bowel syndrome compared to controls and there was also emotional impact on the parents of these children compared to their respective parent controls.

Hence, quality of life is severely affected in patients with short bowel syndrome. Although the there is a literature examining these aspects, multiple aspects are currently unexplored. Namely, there isn't an established quantitative tool that allows to measure the impact of the presence of short bowel syndrome on the quality of life in this group of patients. In pursue of this aim, a questionnaire was developed by a group in Germany and had not been fully validated at the time of the present study (2011-2012). The validation was later published inclusive of our results (Berghöfer *et al.*, 2013). Hence, the aims of the present study were:

1. Investigate the psychometric properties of a newly developed quality of life measure, specific for short bowel syndrome patients, with patients from University College London Hospital.
2. Investigate the main aspects that affect the quality of life in patients with short bowel syndrome.

4.3 University College London Hospital Study

4.3.1 Methods

A short bowel syndrome quality of life scale (SBSQoL^{TM}) containing 17 questions with answers on a visual analogue scale (SBSQoL1-17) was used (Table 4.2). This questionnaire has been developed by a group in Germany and wasn't fully validated at the time of the present study (2011-2012). The validation was later published inclusive of our results (Berghöfer *et al.*, 2013).

The answers of this scale were transformed into a 10-point Likert type, where 1 and 10 means 'not at all' and 'very much' respectively. Only SBSQoL1 went from 'excellent' to 'unbearably bad'. The psychometric properties of the questionnaire (reliability and internal consistency) were examined and exploratory factor analysis was performed in order to examine if the questions could be reduced to a small number of homogeneous factors. Reliability was assessed with Cronbach's alpha (Tsagris *et al.*, 2013). The differences between patients belonging to the upper and lower 25% percentile group of SBSQoL1, which was taken as a general proxy of quality of life, was also examined. All statistical computations were done with SPSS 22.0.

4.3.2 Results

The sample included 15 short bowel syndrome subjects (12 females, 3 males), mean age: 50.7 ± 19.8 years (range 18-88). For SBSQoL1 the mean (± SD) was 4.8 ± 2.7. For all other questions, the mean ranged from 6.1-7.7. Results are shown in Figure 4.1.

Reliability and internal consistency measures of the overall scale were excellent (Cronbach's alpha: 0.907, Standardised Cronbach's alpha: 0.910, Spearman-Brown coefficient: 0.923, Guttman split-half coefficient: 0.920). Exploratory factor analysis was performed with principal component analysis, which yielded four factors, explaining 80% of variance;[1] the scree plot image confirmed this (Figure 4.2). Factor loadings are shown in Table 4.3. The four factors were labelled as:

Factor 1 'General Physical Symptoms and Activities' (11 questions)

[1] Factor analysis usually requires a sample over 50. One recent study though (de Winter *et al.*, 2009) allow for a sample size under 50 for high communalities, which is demonstrated in our sample (Table 4.2).

Factor 2 ‘Daily activities’ (3 questions)

Factor 3 ‘Digestive System Symptoms’ (2 questions)

Factor 4 ‘Stoma Related Symptoms’ (1 question)

Mann-Whitney *U* test was employed to examine differences in questions SBSQoL2-17 between the two percentiles mentioned above; the sample was small and SBSQoL2-17 were not normally distributed (Kolmogorov-Smirnov test was significant for all) (Table 4.4).

A significant difference was detected only in questions 14 (fatigue) and 15 (diarrhoea/increased stomal output). The upper 25% percentile group had higher medians in their answers, while there were borderline differences in questions 12 (sleep), 13 (gastroenterological symptoms), and 16 (musculoskeletal symptoms). Results are shown in Figure 4.3 and Table 4.5.

4.4 Summary and Conclusion

The present study investigated quality of life in patients with short bowel syndrome The SBSQoL^{TM} scale is highly reliable in measuring quality of life in short bowel syndrome patients with a high Cronbach’s alpha. This was also demonstrated in the larger sample, of which the present study was part of (Berghöfer *et al.*, 2013). The differences with the results from the larger study were the number of factors extracted from exploratory factor analysis. Two factors were extracted from the larger study, factor 1 comprised of 11 items and factor of 6 items. The study demonstrated that SBSQoL^{TM} had satisfactory test re-test reliability, responsiveness and construct validity (Berghöfer *et al.*, 2013). The same group proceeded to test whether the growth factor teduglutide would improve quality of life in patients with short bowel syndrome since it decreased the need for parenteral nutrition to a certain degree (Jeppesen *et al.*, 2013). The authors demonstrated significant decrease in the total scale score, factor 1 score and factor 2 score in patients who had received teduglutide. However, when this decrease was compared to placebo, the difference was non-significant. This was attributed to a short observation period, imbalances in oral fluid intake in relation to parenteral solution reductions, large patient and effect heterogeneity and occurrence of gastrointestinal adverse events in a subgroup of teduglutide-treated patients may account (Jeppesen *et al.*, 2013).

The next important finding of the present study was that the main causes of low quality of life were fatigue, diarrhoea/increased stomal output, lack of sleep, gastrointestinal symptoms, and muscle pains. Lack of sleep has been frequently associated with short bowel syndrome predominantly due to long parenteral fluid infusion times and/or a daily infusion schedule because of large-volume fluid requirements, due to pumps and equipment alarms, fear of catheter dislodgement, nocturia, polyuria, need for extra storage space, and complaints of carrying heavy backpacks (Winkler and Smith, 2014). Lack of sleep with associated macronutrient imbalances are frequently associated with fatigue, muscle pains and widespread pain syndromes. These in turn can also affect sleep negatively causing eventually functional impairment and even depression (Winkler, 2005, 2010; Winkler *et al.*, 2010).

Physicians should treat these symptoms as much as possible to ease the burden of patients' disease (e.g. loperamide for diarrhoea, paracetamol or stronger pain killers, zopiclone for sleeping). The importance of addressing these issues is related with better outcomes in patients with short bowel syndrome. Physical symptoms of fatigue, muscle pains, increase stoma output, metabolic bone disease, electrolyte, macronutrient and micronutrient imbalances can all lead to mental health disease, social and financial consequences which are known to decrease life expectancy in general (Marmot *et al.*, 2012).

Next, I would like to address the initial hypothesis that citrulline could function as a surrogate marker of quality of life in patients with short bowel syndrome and by extension in other forms of intestinal failure. The present study demonstrated that quality of life is decreased in patients with short bowel syndrome. The larger study demonstrated that quality of life improved with teduglutide and reductions in parenteral solutions' needs. In Chapter 2, it was shown that lower citrulline levels were a diagnostic marker for parenteral nutrition dependence in patients with short bowel syndrome and extensive mucosal disease. However, in the studies by Berghöfer *et al.* (2013) and Jeppesen *et al.* (2013), citrulline was not measured and correlated with quality of life. Nevertheless, due to the simultaneous association of quality of life and changes in citrulline levels with short bowel syndrome, it is reasonable to advocate based on the transitive property of associations that changes

of citrulline are hence associated with changes with quality of life in patients with short bowel syndrome.

The limitations of the present study include as small sample size, lack of repeated measures over time to assess for re-test reliability and lack of measuring other constructs to assess for construct validity. Another limitation is the inability to discriminate to the effect on quality of life by home parenteral nutrition (which most patients on short bowel are treated with) from reduction of quality of life by short bowel syndrome. There is an obvious overlap of effects of both aspects on quality of life, but ideally a questionnaire should be specific with regards to the patient group and characteristics. Despite these limitations, the similar results found in the present study with the larger study by Berghöfer *et al.* (2013) lends to support to the validity of the current findings.

Finally, the present study provides grounds for certain future studies that should take place. The present study intends to form the basis for investigation of quality of life in a larger sample. This larger study ideally should have repeated measures and associated citrulline measurements as well and be able to differentiate the consequences on quality of life from home parenteral nutrition and short bowel syndrome. Also, future intentions include examining whether the results of exploratory factor analysis can be used to transform the 17-item questionnaire into a 4-item short bowel syndrome quality of life scale (since four factors were identified in the factor analysis) which can be used very easily in clinical settings. A similar study took place recently, by which a patient preference-weighted scoring algorithm for the SBS-QoL^{TM} was developed and was able to estimate a wide range of utility values from patient-level SBS-QoL^{TM} data (Lloyd *et al.*, 2014). This study essentially narrowed down the seventeen items of the initial scale to eight items relates to six health states. The authors advocated using the original scale for more accurate assessment of quality of life, but in the field of questionnaires and psychometric research, short forms of original larger scales is useful for patient reasons initially, who might not be willing to answer lengthy questionnaires, as well as practical reasons of design, sample size and analysis, ensuring powerful enough samples with less items if possible.

4.5 Tables

Table 4.1. Disease-related concerns for patients with short bowel syndrome.

Being a burden on others
Having surgery
Energy level
Loss of sexual drive
Access to quality medical care
Pain or suffering
Dying early
Loss of bowel control
Feeling out of control
Uncertain nature of disease
Achieve full potential
Feeling alone
Developing cancer
Sexual performance
Feelings about my body
Financial difficulties
Effects of medication
Attractiveness
Intimacy
Producing unpleasant odours
Feeling dirty or smelly
Being treated as different
Passing the disease to others
Ability to have a child

Table 4.2. Questions of the SBSQoL™ scale.

SBSQoL™ Scale
SBSQoL1: How did you feel in general during the past week?
SBSQoL2: During the past week, how much did your illness interfere with your everyday activities?
SBSQoL3: During the past week, how much did your illness interfere with your working life / ability to work?
SBSQoL4: During the past week, how much did your illness interfere with your leisure activities?
SBSQoL5: During the past week, how much did your illness affect your social life?
SBSQoL6: During the past week, how much did your illness interfere with your energy level?
SBSQoL7: During the past week, how much did your illness affect your physical health?
SBSQoL8: During the past week, how much did your illness affect your mobility and self-care activities?
SBSQoL9: During the past week, how much were you affected by pain resulting from your illness?
SBSQoL10: During the past week, how much did your illness interfere with your diet, eating and drinking habits?
SBSQoL11: During the past week, how much did your illness affect your emotional life?
SBSQoL12: During the past week, how much did your illness affect your sleep?
SBSQoL13: During the past week, how much were you affected by gastrointestinal symptoms resulting from your illness?
SBSQoL14: During the past week, how much were you affected by fatigue / weakness resulting from your illness?
SBSQoL15: During the past week, how much were you affected by diarrhoea / stomal output resulting from your illness?
SBSQoL16: During the past week, how much were you affected by skeleton / muscle symptoms resulting from your illness?
SBSQoL17: During the past week, how much were you affected by other symptoms / discomfort resulting from your illness?

Table 4.3. Pattern and structure matrix for principal component analysis.

Items	**Pattern coefficients**				**Structure coefficients**				**Communalities**
	Component				Component				
	1	2	3	4	1	2	3	4	
SBSQoL14	**0.978**	-0.100	0.052	-0.051	**0.961**	0.104	0.292	0.109	0.939
SBSQoL6	**0.943**	0.117	-0.009	-0.157	**0.939**	0.303	0.228	0.023	0.916
SBSQoL12	**0.905**	0.176	-0.047	-0.054	**0.922**	0.364	0.174	0.128	0.883
SBSQoL7	**0.854**	-0.098	0.282	-0.113	**0.882**	0.071	0.496	0.011	0.885
SBSQoL9	**0.820**	-0.347	0.317	-0.012	**0.820**	-0.175	0.518	0.077	0.896
SBSQoL8	**0.761**	0.004	-0.234	0.089	**0.786**	0.035	0.517	0.016	0.585
SBSQoL17	**0.745**	-0.114	0.331	-0.083	**0.763**	0.256	0.060	0.529	0.752
SBSQoL16	**0.700**	0.064	-0.084	0.393	**0.722**	0.178	-0.056	0.239	0.754
SBSQoL11	**0.530**	0.358	-0.206	0.061	**0.705**	0.286	0.445	0.543	0.503
SBSQoL1	**0.511**	0.131	0.351	0.461	**0.612**	0.592	-0.099	0.589	0.804
SBSQoL2	**0.483**	0.441	-0.185	0.446	**0.568**	0.479	-0.084	0.206	0.838
SBSQoL3	-0.127	**0.866**	-0.232	-0.019	-0.001	**0.839**	-0.268	0.065	0.788
SBSQoL4	-0.054	**0.850**	0.297	-0.011	0.197	**0.835**	0.278	0.051	0.781
SBSQoL5	0.161	**0.758**	0.316	0.001	0.400	**0.791**	0.350	0.090	0.775
SBSQoL13	0.040	-0.009	**0.917**	0.265	0.306	0.021	**0.910**	0.214	0.904
SBSQoL10	0.109	0.335	**0.725**	-0.252	0.311	0.327	**0.765**	-0.243	0.759
SBSQoL15	-0.176	-0.070	0.088	**0.986**	0.005	-0.005	-0.016	**0.942**	0.927

Table 4.4. Normality tests.

	Kolmogorov-Smirnov[a]			**Shapiro-Wilk**		
	Statistic	df	*p*-value	Statistic	df	*p*-value
SBSQoL2	0.311	9	0.012	0.817	9	0.032
SBSQoL3	0.302	9	0.017	0.805	9	0.023
SBSQoL4	0.234	9	0.169	0.774	9	0.010
SBSQoL5	0.186	9	0.200*	0.855	9	0.085
SBSQoL6	0.355	9	0.002	0.810	9	0.027
SBSQoL7	0.368	9	0.001	0.634	9	0.000
SBSQoL8	0.279	9	0.042	0.768	9	0.009
SBSQoL9	0.233	9	0.172	0.786	9	0.014
SBSQoL10	0.222	9	0.200*	0.881	9	0.160
SBSQoL11	0.235	9	0.164	0.799	9	0.020
SBSQoL12	0.234	9	0.168	0.761	9	0.007
SBSQoL13	0.325	9	0.007	0.708	9	0.002
SBSQoL14	0.238	9	0.150	0.789	9	0.015
SBSQoL15	0.221	9	0.200*	0.840	9	0.057
SBSQoL16	0.192	9	0.200*	0.814	9	0.029
SBSQoL17	0.278	9	0.044	0.715	9	0.002

[a] Lilliefors Significance Correction; * This is a lower bound of the true significance.

Table 4.5. Mann-Whitney *U* test results.

Items	**L25%, n=4[a]**	**U25%, n=5[a]**	**Mann-Whitney *U***	***p*-value**
SBSQoL12	4.0	10.0	3.5	0.095[b]
SBSQoL13	2.0	10.0	3.0	0.059[b]
SBSQoL14	7.5	10.0	0.0	0.012
SBSQoL15	2.5	10.0	2	0.045
SBSQoL16	3.5	9.0	2.5	0.063[b]

[a] Median; [b] Due to a small sample size, these borderline *p*-values should be interpreted with caution.

4.6 Figures

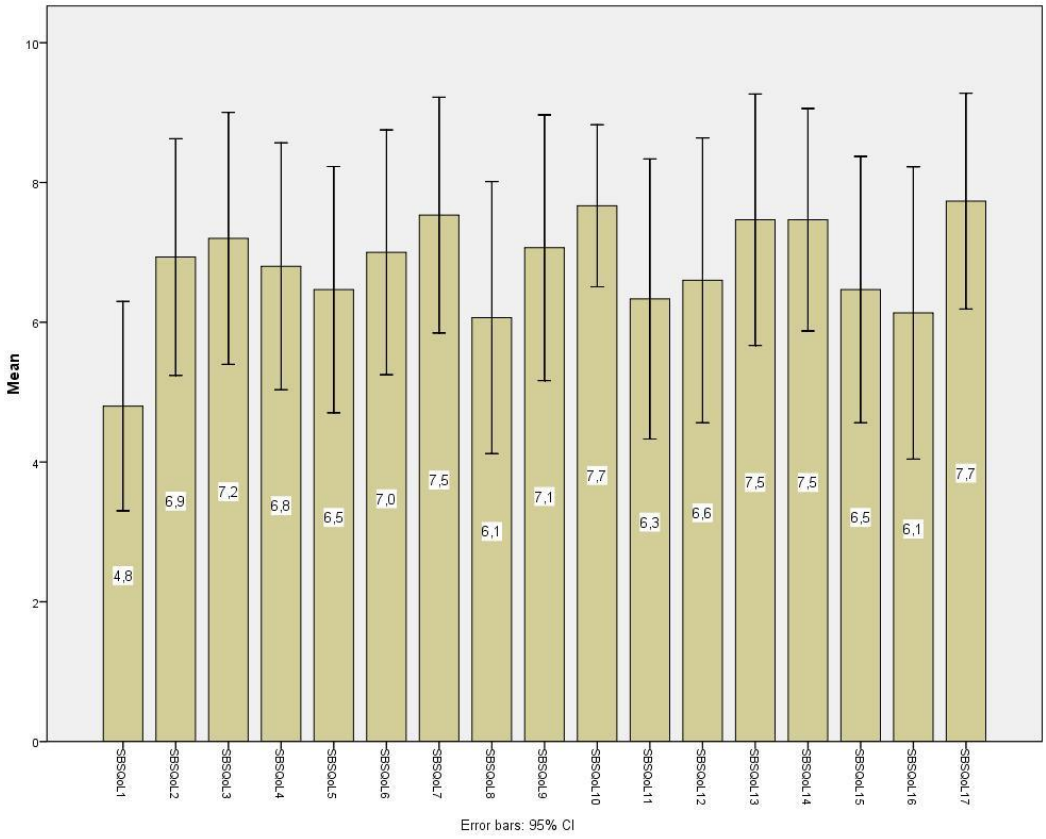


Figure 4.1. Means of answers to questions SBSQoL1-17.

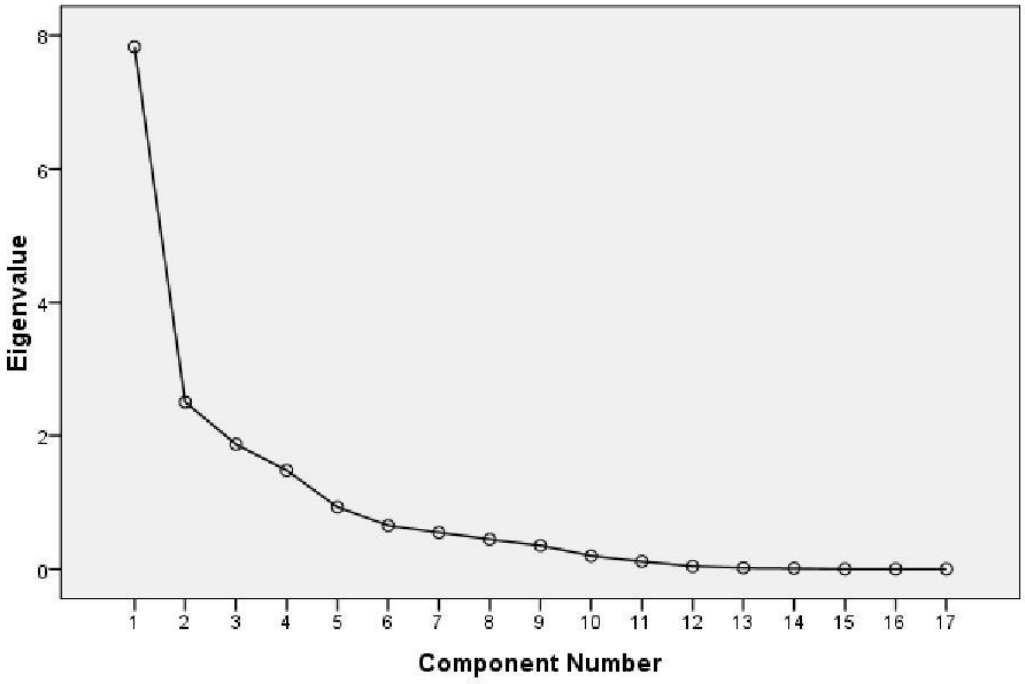


Figure 4.2. Scree plot.

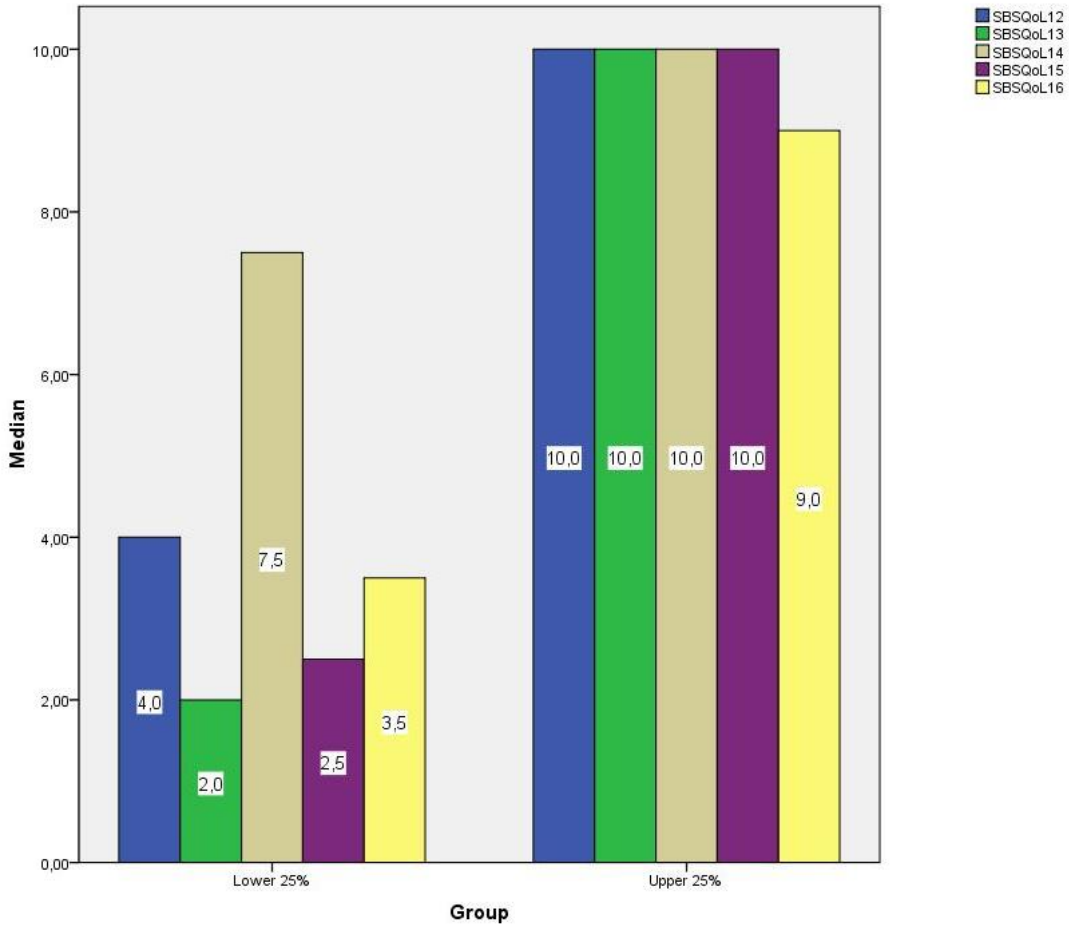


Figure 4.3. Difference in medians for specific SBSQoL^{TM} questions shown in Table 4.5.

Chapter 5
General Discussion, Conclusion and Future Work

5.1 Summary of New Findings from the Thesis

This chapter will try to summarise the important findings from the whole thesis, then try to provide and connect the findings with existing theories and conceptions and finally provide a guide for future work that may be needed. In the preface, the main questions of the thesis were:

1. Is citrulline being used in clinical practice? If so, then how is it being used (e.g. diagnostically, therapeutically, which particular conditions etc.)?
2. How is citrulline related to the intestine?
3. How can existing knowledge provide grounds to utilize citrulline as a marker to reflect intestinal function (absorption and possibly others)?
4. Can citrulline be a surrogate marker of other consequences related to bowel disease?

Before I answer them, I am going to present a summary of the main findings from the studies in the present thesis. Hence, the summary of new findings of the present thesis is:

1. The first important finding was that citrulline as a term has been used at the end of the 19th century-beginning of the 20th century to describe an extract of the *C. colocynthis* which was used as a subcutaneous laxative. A comprehensive search was performed which corroborates this finding with extensive old literature mentioning this term. The authors who had described this laxative were German clinical researchers Hiller (1882) and Kohlstock (1892) with multiple citations to their work (see Appendix A). The next new finding was the fact that citrulline (non protein amino acid) was first described as an amino acid by Koga and Ohtake (1914) and not by Wada (1930a) which is the common belief. This was also published in a history of medicine journal (Fragkos and Forbes, 2011a).
2. The second original finding of this thesis focuses on the findings from the second chapter, the systematic review and meta-analysis of human studies on

citrulline and the intestine. This chapter is an extensive review of the literature on citrulline in relation to the intestine (role, function, anatomy) and a statistical analysis of their results. Most importantly, meta-analyses showed that:

a. Citrulline levels are correlated strongly with small bowel length in short bowel syndrome patients (correlation coefficient 0.68).

b. Citrulline is strongly negatively correlated (correlation coefficient -0.56) with intestinal disease severity with regards to enteropathies (coeliac disease, tropical enteropathy, mucositis, and acute rejection in intestinal transplantation; but nor Crohn's disease).

c. Citrulline cut-off levels have an overall sensitivity and specificity of 80 %. There was however variability in the cut-off levels, but the majority of studies used cut-off level of 20 μmol/L.

d. Citrulline levels in untreated coeliac disease patients compared to controls were reduced by 10 μmol/L. Citrulline levels increase with gluten-free diet and with improvement of the enteropathy.

e. Citrulline levels are decreased in critical illness and sepsis. The existing literature indicates that it could possibly be attributed to use of nitric oxide and arginine in sepsis/inflammation leading to a transient decrease in citrulline; similar to the behaviour of albumin as a negative inflammatory marker. Secondly, it could also indicate enteropathy of acute illness.

f. These findings suggest that citrulline be considered a marker of possible acute intestinal injury or intestinal insufficiency.

3. The next finding stems from the clinical study (Chapter 3).

   a. The design of the clinical study aims to answer which of the four amino acids citrulline, arginine, glutamine and 3-methylhisitidine and placebo affect post absorptive concentrations of citrulline, glutamine, arginine, ornithine, and 3-methyl-histidine in a more potent manner. The design for this was a fully balanced Williams design crossover study minimizing the effects of carryover or imbalance possibilities.

b. The original findings from the results were that citrulline was the most potent stimulator for all other amino acids, contrary to beliefs of glutamine challenges.

c. There was no effect of age, gender and BMI on the AUC, C_{max} and TOMC. This essentially indicates that basic anthropometric characteristics and age have no effect on pharmacological post-ingestion measures.

d. Citrulline, arginine and ornithine were more affected by the treatment overall (crossover ANOVA $p < 0.05$, Tables 3.7 and 3.8).

e. There was no carryover and period effect for all treatments, except for 3-methylhistidine which had a significant period effect (possibly due to small concentrations, $p < 0.05$, Tables 3.7 and 3.8). This confirms that the study design maintained low likelihood for carryover or period effects.

f. Citrulline increased arginine concentrations more significantly compared to glutamine, placebo and 3-methylhistidine but not arginine ($p < 0.05$ from Tukey multiple comparisons; Table 3.9).

g. Arginine increased arginine concentrations more significantly than glutamine, placebo and 3-methylhistidine ($p < 0.05$ from Tukey multiple comparisons) but with a less prominent effect than citrulline (Table 3.9, see also Table 3.6, Figure 3.27).

h. Citrulline increased citrulline concentrations more significantly compared to arginine, glutamine, placebo and 3-methylhistidine but not arginine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9), while arginine increased citrulline significantly less than glutamine only ($p < 0.05$ from Tukey multiple comparisons, Table 3.9).

i. Citrulline increased ornithine levels significantly compared to arginine, glutamine, placebo and 3-methylhisitidine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9).

j. Arginine also increased ornithine levels compared to glutamine, placebo and 3-methylhistidine ($p < 0.05$ from Tukey multiple comparisons, Table 3.9) but less prominently than citrulline ($p < 0.05$ from Tukey multiple comparisons, Table 3.9).

a. No treatment was equivalent to treatment with citrulline for producing equivalent post-absorptive concentrations ($p > 0.05$). With respect to all other concentrations, equivalence was reached with all treatments (Anderson-Hauck test $p < 0.05$).

b. My results raise the interesting prospect that citrulline challenges could be useful in intestinal failure but also in liver failure where urea cycle pathways including glutamine, arginine and ornithine are implicated. Furthermore, gastric emptying and intestinal transit factors were not measured in the present study. This study is among the few studies to trial a challenge test in healthy volunteers with many post-absorptive measurements. Similar studies in the literature include those by Peters *et al.* (2007a, 2007b); Peters *et al.* (2007c); Peters *et al.* (2008b) and Pinto Costa *et al.* (2013) which have citrulline stimulation tests by glutamine. A different category of studies which investigate citrulline physiology also exists and includes studies by Castillo *et al.* (1995); Fujita and Yanaga (2007); Rougé *et al.* (2007); Van De Poll *et al.* (2007a); Van De Poll *et al.* (2007b); Thibault *et al.* (2011).

4. The final study (Chapter 4) was investigation of quality of life in short bowel syndrome patients. This was part of a larger international study. In the UCLH cohort of patients the SBSQoL^{TM} scale is highly reliable in measuring quality of life in patients with short bowel syndrome. Main causes of low quality of life are fatigue, diarrhoea/increased stomal output, lack of sleep, GI symptoms, and muscle pains.

5.2 Limitations

Limitations throughout the thesis and the relevant studies exist. These are:

1. From the meta-analysis, these stem from the increased heterogeneity and possibility of publication bias, detection bias and confounding bias that was documented. It was a pattern in many studies not to analyse confounding factors such as other amino acids, renal function (citrulline's pathways involve a renal component) and inflammatory state. Also, different measuring methods for citrulline (IEC, HPLC, TMS, ELISA), sample preparation, population parameters, different scales for documenting disease severity, absorption, small

bowel length and so on. Each biological method and clinical assessment scale has inherent variability which is added up when a meta-analysis is performed and this leads to heterogeneity. The random effects models take heterogeneity into account allowing for reliable interpretation of significant associations.

2. From the clinical study, limitations included the open-label design and short duration of follow-up. It was conducted in a small sample of healthy volunteers, who have different bioavailability profiles to patients. Thus, the clinical significance of the findings – namely, challenges in patients for functional reasons – must be clarified in additional studies involving patients with intestinal or liver failure. Additional limitations included the variability in measurements from the subjects which produced increased confidence intervals for the areas under the curves. Renal impairment also plays a significant role when interpreting citrulline plasma values especially in patients – where acute or chronic kidney disease can be invariably present.

3. The SBSQoL^{TM} questionnaire had seventeen items. These need to be correlated with other quality of life questionnaires and explored in larger sample sizes. Also the longitudinal analysis of quality of life needs to be examined. Finally, the present study was not able to differentiate reduced quality of life due to home parenteral nutrition from reduced quality of life due to short bowel syndrome.

5.3 Concluding Remarks, Advice for Practitioners and Future Work

Hence, answering the dissertations general aims and hypotheses, citrulline is currently being used as a marker of intestinal injury in many clinical conditions. Research initially started in animals but since the early 2000s it has expanded to human research quite extensively. Research remains mainly within the field of intestinal failure and associated disciplines and as such measuring citrulline in daily clinical practice has still not been actually realised. Main reasons for this are variability in measurement techniques and non-straightforward interpretation of levels requiring possibly experts in the field.

The intriguing part of citrulline's metabolism is unique site of production in the intestine. Although intuitively this would indicate that any changes in citrulline would reflect intestinal function, the systematic review from Chapter 2 shows quite

a difference in worldwide practice. Although there was a convergence in practices and results, variability exists. Many studies didn't analyse confounding factors such as other amino acids, renal function (citrulline's pathways involve a renal component) and inflammatory state. Hence, for future studies it is suggested that nitric oxide, full amino acid profiles (or at least arginine, glutamine and ornithine) are measured when citrulline is measured, renal function and inflammatory state.

I think an interesting field involves the studies of citrulline in the context of critical illness. Studying patients in intensive care settings has multiple advantages: patients are monitored intensely; there are frequent blood measurements, even multiple times per day; clinical scores are established in the filed which indicate severity of organ failure and specifically the gut; and patients are expected to respond eventually or succumb to their illness. Hence, intensive care settings provide a certainty and finality in the clinical settings, that cross-sectional measurements in other clinical settings have proven heterogeneous. Hence, I believe that there is prospect in measuring citrulline in context of critical illness. It will allow clarifying the role of sequential of citrulline measurements and citrulline's role as a marker of acute intestinal injury. One disadvantage so far obvious are the different study designs amongst studies of citrulline and critical illness.

Chapter 3 essentially demonstrated that citrulline can function successfully as an oral challenge test and better than glutamine. Trying to transfer this knowledge into practice will be challenging but not impossible. Measuring citrulline in clinical practice is difficult because frequent blood sampling in daily practice is always an arduous task. However, as I mentioned before, I think intensive care settings will be more accessible to this type of testing. The usefulness of a challenge test in conditions where these is extensive mucosal enteropathy will be more of use in the short term. These are conditions were patients can undergo intestinal adaptation over a period of time before becoming malnourished. A citrulline challenge test would be possibly useful to assess early-on the extent to which the bowel is compromised so as to intervene sooner.

In Chapter 4, I presented results regarding quality of life in patients with short bowel syndrome. Four factors were extracted from the 17-item scale: general physical symptoms and activities, daily activities, digestive system symptoms, and stoma related symptoms. The main causes of low quality of life were fatigue,

diarrhoea/increased stomal output, lack of sleep, gastrointestinal symptoms, and muscle pains. Due to the transitive property of associations, the findings from this quality of life study suggest that changes of plasma citrulline levels are associated with changes with quality of life in patients with short bowel syndrome, due to the simultaneous association of quality of life and changes in citrulline levels with short bowel syndrome.

The present dissertation investigated various aspects relating to citrulline and the intestine, namely, history, physiology, intestinal disease, and psychosocial effects. Overall, citrulline is currently an important aspect of intestinal pathophysiology. As a marker, citrulline needs to be used more by practitioners who deal with enteropathies (coeliac disease, tropical enteropathy, mucositis, Crohn's disease and intestinal transplantation) as well as surgical conditions where extensive bowel resection might be needed leading up to short bowel syndrome. Citrulline in these cases has shown to be a marker of severity, small bowel length and need for parenteral nutrition. If ones combines this association with the satisfactory sensitivity and specificity of citrulline, this shows that its measurement would benefit practitioners in decision making for therapies and interventions.

Quality of life is decreased in short bowel syndrome patients and this shows the amount of research still needed in short bowel syndrome. The clinical study shows an interesting potential for a citrulline stimulation test with citrulline instead of glutamine in enteropathy and short bowel syndrome patients. Endocrinologists use challenge tests as part of their daily practice to investigate insulin, cortisol or other hormone insufficiencies. The gastroenterology field would benefit from this approach as well with challenge tests for the intestine.

Future work could include:

1. A clinical study with citrulline challenges in enteropathy / short bowel syndrome / mucositis patients.
2. A larger study for quality of life correlating quality of life with other psychosocial parameters and other quality of life scales. Also longitudinal studies to examine responsiveness and other questionnaire related parameters.
3. Studies with sequential measurements of citrulline in the context of critical illness that will correlate citrulline with organ failure severity scores.

4. A topic that the present thesis did not discuss thoroughly was citrulline and cellular studies. There is a good amount of literature investigating the proliferative effects of citrulline on muscle cells. This topic is intriguing in the sense it provides potential for the therapeutic effects in conditions where muscle loss is a problem, such as sarcopenia. Sarcopenia is an issue in elderly patients, cancer patients and also obesity with sarcopenic obesity (Osowska *et al.*, 2004; Osowska *et al.*, 2006; Perez-Guisado and Jakeman, 2010; Boutry *et al.*, 2012; Faure *et al.*, 2012). Future studies in this filed would be interesting in skeletal or smooth muscle cells investigating proliferative and cytotoxic effects of citrulline on these cells.

Hence, in a time when intestinal failure research is growing and intestinal physiology and function still has unresolved mysteries, this thesis' results could not be more desired. This has been a research endeavour which investigated citrulline in the context of normal physiology with the absorption study, in the context of abnormal conditions with the meta-analysis and the quality of life in short bowel syndrome patients. Results involved aspects of the clinical significance and diagnostic potential of plasma citrulline levels and within the limitations of the present results, new avenues for further research have been suggested.

Appendix A

Bibliography on Citrulline Resin

Table A.1. A bibliographic list of the sources mentioning the term "citrulline" from 1882 to 1930, before the discovery of the modern amino acid citrulline.

No.	Source
	Dutch Texts
1	Nederlandstijdschriftvoorgeneeskunde. Houten: BohnStafleuvanLoghum; 1894. vol. 38, p. 1010 (Pinkhof, 1894)
2	Ritsema IC, Sack J, Greshoff M. Index phytochemicus. Amsterdam: Uitgave van het Laboratorium; 1905. p. 12 (Haarlem. Koloniaal museum. [from old catalog] *et al.*, 1905).
	English Texts
3	Morse WH. New therapeutical agents. Detroit: Davis; 1882. p. 183 (Morse, 1882).
4	The Cincinnati Lancet-Clinic. Cincinnati: J.C. Culbertson; 1882. vol. 48, p. 136 (French, 1882)
5	The London Medical Record. London: Hart; 1882. vol. 10, p. 490 (1882d)
6	American Medical Digest. New York; 1882. p. 156 (1882a).
7	The Louisville Medical News. Louisville: Ky; 1882. Vol. 14 pp. 117-8 (1882b)
8	Medical Record. New York: W. Wood; 1882. vol. 22, p. 604 (1882c).
9	Chicago Medical Review. Chicago: Chandler & Engelhard; 1882. vol. 5-6, p. 563 (1882e)
10	The Medical Press & Circular. London; 1882. p. 268
11	The North Carolina Medical Journal. Charlotte, N. C.; 1883. vol. 11, p. 45 (1883a)
12	The Medical Times and Register. Philadelphia: Medical Publishing Company; 1883. vol. 13, p. 71 (1882f)
13	Pharmaceutical Journal and Transactions. London: Churchill; 1883. p. 343 (1883b)
14	Therapeutic Gazette. G. S. Davis; 1883. vol. 7, p. 380
15	Lescher FH. Recent materia medica: notes on their origin and therapeutics. London: Churchill; 1884. p. 27 (Lescher, 1884)
16	Bruce JM. Materia medica and therapeutics, 1st ed. London: Cassell; 1884. p. 297 (Bruce, 1884)
17	Medical and Surgical Reporter. Philadelphia; 1889. vol. 60, p. 498 (1889b)
18	Wood's Medical and Surgical Monographs. New York: W. Wood; 1889. vol. 3, p. 504 (1889a)
19	Meyer Druggist. St. Louis; 1891. p. 358 (1891)
20	Braithwaite JO. Yearbook of Pharmacy. London: J. & A. Churchill; 1893. p. 220 (Braithwaite, 1893)
21	American Veterinary Review. USA; 1893. vol. 17, p. 138 (Middleton, 1893)
22	The Medical Age. Detroit: E. G. Swift; 1893. vol. 11, p. 322 (Stockwell, 1893).
23	Therapeutic Gazette. G. S. Davis; 1893. vol. 17, p. 704 (1893b)
24	Bulletin of Pharmacy. E.G. Swift; 1893. vol. 7, pp. 181, 279, 286 (1893c)
25	American Journal of Pharmacy. Philadelphia: Philadelphia College of Pharmacy; 1893. vol. 65, p. 225 (Moerk, 1893)
26	The Medical Bulletin. Philadelphia: F. A. Davis; 1893. vol. 15, p. 310 (1893e)
27	Proceedings of the American Pharmaceutical Association at the Annual Meeting. Scio, Ohio; 1893. vol. 41, p. 450 (1893a)
28	Western Druggist. Chicago; 1893. vol. 15, p. 150
29	Chemist & Druggist. London: Morgan Brothers; 1893. vol. 42, p. 380
30	Yearbook of Pharmacy. London: J. & A. Churchill; 1893. p. 220
31	Pacific Medical Journal. San Francisco; 1893. vol. 36, p. 562
32	The Cincinnati Lancet and Clinic. Cincinnati: Culbertson; 1893. vol. 70, p. 540
33	The Western Medical Reporter. Chicago; 1893. vol. 15, issue 1, p. 278
34	The Journal of the British Homoeopathic Society. London; 1893. vol. 1, p. 101
35	Proceedings of the ... annual meeting of the Kansas Pharmaceutical Association. Marysville: Hoadley & Hackman; 1893. vols. 14-20, p. 119
36	Stevens AA. A Manual of Therapeutics. Philadelphia: Saunders; 1894. p. 126
37	The Medical Age. Detroit: E. G. Swift; 1894. vol. 12, pp. 31, 115, 532
38	Annual of the Universal Medical Sciences. Philadelphia: F.A. Davis; 1894. vol. 5, p. A76
39	The Year-book of Treatment. Lea Brothers; 1894. vol. 10, p. 124
40	The Veterinary Magazine. Philadelphia; 1894. vol. 1, p. 223
41	Shoemaker JV. A practical treatise on materia medica and therapeutics: with especial reference to the clinical application of drugs, 3rd ed. Philadelphia: F.A. Davis; 1895. p. 359
42	Boas I. Diseases of the intestines (Trans. S Basch). New York: Appleton; 1901. p. 253
43	Therapeutic Monthly. Philadelphia: Medical Journal Union; 1901. vols. 1-2, p. 88
44	The Pharmaceutical Era. New York: D. O. Haynes; 1902. vol. 27, p. 15
45	Fantus B. A text book on prescription writing and pharmacy. Chicago: Chicago Medical Book Co.; 1905. p. 251
46	Gould GM. A dictionary of new medical terms. Blakiston; 1905. p. 185

No.	Source
47	Wilcox RW. Materia medica and pharmacy. Philadelphia: Blakiston; 1905. pp. 543-4
48	Wilcox RW. Pharmacology and therapeutics, 6th ed. Philadelphia: Blakiston; 1905. p. 706
49	de Médicis Sajous CE. Sajous's analytical cyclopædia of practical medicine, 3rd ed. Philadelphia: F.A. Davis; 1906. vol. 2, p. 275
50	Culbreth DMR. A Manual of materia medica and pharmacology, 4th ed. Philadelphia: Lea Brothers; 1906. p. 602
51	Wood HC. Therapeutics: its principles and practice, 13th ed. Philadelphia: Lippincott; 1906. p. 651
52	Croftan AC. Clinical Therapeutics. Chicago: Cleveland Press; 1907. p. 456
53	Pharmaceutical Era. The Era Dose Book. Philadelphia: Haynes; 1907. p. 7
54	Merck's 1907 Index. Merck & Co.; 1907. p. 143
55	MacEwan P. The art of dispensing: a treatise on the methods and processes involved in compounding medical prescriptions, 8th ed. London: Chemist and Druggist; 1908. p. 421
56	Coblentz V. The newer remedies, including their synonyms, sources, tests, solubilities, incompatibles, medicinal properties and doses as far as known, together with such proprietaries as have similar titles, 4th ed. Apothecary Publishing Co.; 1908. p. 36
57	The Cincinnati Lancet and Clinic. Cincinnati: Culbertson; 1908. vol. 100, pp. 136, 366
58	Billings F. Diseases of the digestive system. New York and London: Appleton; 1910. p. 729
59	Therapeutic Gazette. Detroit: G. S. Davis; 1910. vol. 34, p. 556
60	The Encyclopaedia Britannica: a dictionary of arts, sciences, literature and general information. Encyclopaedia Britannica; 1910. vol. 6, p. 696
61	Beal JH. Elementary Principles of the Theory and Practice of Pharmacy: Animal and vegetable drugs. Scio, Ohio: J.H. Beal; 1911. vol. 3, p. 149
62	Bandler SW. Medical gynecology, 3rd ed. Philadelphia and London: Saunders; 1914. p. 470
63	Veterinary Materia Medica and Therapeutics, 8th ed. Chicago: American Veterinary Publishing Co.; 1919. p. 416
64	Prinz H. Dental materia medica and therapeutics: with special reference to the rational application of remedial measures to dental diseases: a textbook for students and practitioners, 5th ed. St. Louis: C.V. Mosby; 1920. p. 612
65	Aaron CD. Diseases of the digestive organs, with special reference to their diagnosis and treatment, 3rd ed. Philadelphia: Lea & Febiger; 1921. p. 227
66	Gardner W. Chemical synonyms and trade names: a dictionary and commercial handbook, 3rd ed. C. Lockwood and Son; 1926. p. 89
	French Texts
67	L'Union Pharmaceutic. Paris; 1882. vol. 23, p. 563
68	Revue des Sciences Médicales en France et a l'étranger. Paris: Masson; 1883. vol. 21, pp. 517-8
69	Répertoire de Pharmacie. Paris; Baillière; 1883. vol. 11, p. 422
70	Journal de Médecine, de Chirurgie et de Pharmacologie. Bruxelles: Lamertain; 1883. vol. 77, p. Page 598
71	Bulletin Général de Thérapeutique. Paris: Bureau du Journal; 1884. p. 430
72	Journal de Médecine, de Chirurgie et de Pharmacologie. Bruxelles: Lamertain; 1884. vol. 79, p. 174
73	Bourneville, Bricon. Manuel des injections sous-cutanées. Paris: Progrés Medical; 1885. p. 187
74	Bouchut E. Compendium Annuaire de Therapeutique. Paris; 1885. p. 69
75	Dujardin-Beaumetz G, Egasse E. Les plantes médicinales indigènes et exotiques. Paris: O. Doin; 1889. p. 201
76	Bouley H-M, Sanson A, Reynal J. Nouveau dictionnaire pratique de médecine, de chirurgie, et d'hygiène vétérinaires. Paris: Labé; 1890. p. 366
77	Revue Médicale. Louvain: Charles Peeters; 1892. vol. 11, p. 516
78	L'Union Pharmaceutic. Paris; 1892. vol. 33, p. 504
79	Hayem G. Leçons de thérapeutique Paris: Masson; 1893. vol. 4, p. 531
80	Gazette de Gynécologie. Paris: Odoin; 1893. vol. 8, p. 159
81	Bulletin général de thérapeutique médicale, chirurgicale, obstétricale et pharmaceutique. Paris: G. Doin & cie; 1893. vols. 124-125, p. 568
82	de Maurans G. Compendium moderne de médecine pratique. Paris: Maloine; 1894. p. 414
83	Recueil de Médecine Vétérinaire. Paris: Vigot Éditions; 1894, vol. 71, pp. 71-3
84	Journal d'Hygiène. Paris; 1894. vol. 20, p. 96
85	Haller A. L'Industrie Chimique. Paris: Baillière; 1895. p. 204
86	Revue de Médecine Vétérinaire. Toulouse (France). Écoles nationales vétérinaires de Lyon et de Toulouse; 1895. vol. 52, p. 707
87	Planchon G, Collin E. Les drogues simples d'origine végétale. Paris: O. Doin; 1896. p. 300
88	Cagny P. Formulaire des vétérinaires practiciens. Paris: Baillière; 1897. p. 107
89	Stokvis BJ. Leçons de pharmacothérapie. Haarlem, Bohn & Paris: Octave Doin; 1898. vol. 2, pp. 267, 274-5
90	Le Moniteur Scientifique. Paris: G. Quesneville; 1900. vol. 56, p. 722
91	Manquat A. Traité élémentaire de thérapeutique de matière médicale et de pharmacologie. Paris: J.-B. Bailliere et fils; 1903. vol. 1, p. 732
92	Cohin E. Traité de toxicologie végétale. Paris: O. Doin; 1907. p. 76
	German Texts

No.	Source
93	Zeitschrift für Klinische Medicin. Berlin: A. Hirschwald; 1882. vol. 4, pp. 481-97
94	Kongress für Innere Medizin. Verhandlungen. Wiesbaden; 1882. vols. 1-2, p. 217
95	Zentralblatt (Centralblatt) für Klinische Medizin. Leipzig; 1882. vol. 3, p. 159; vol. 14, pp. 455-6
96	Medizinische Neuigkeiten fuer Praktische Aerzte. Erlangen: Palm & Enken; 1882. vol. 32, issue 28, p. 222-3
97	Centralblatt für die Medicinischen Wissenschaften. Berlin; 1882. vol. 20, p. 603
98	Archiv der Pharmazie. Berlin: Deutschen Apotheker-Vereins; 1882. vol. 220, issue 9, p. 688
99	Deutsche Medizinal-Zeitung. Berlin: Grosser; 1882. vol. 3, p. 310
100	Harnack E. Lehrbuch der Arzneimittellehre und Arzneiverordnungslehre. Hamburg: Voss; 1883. p. 356
101	Karsten H. Illustrirtes Repetitorium der Pharmaceutisch-Medicinischen Botanik und Pharmacognosie. Berlin: Springer; 1883. p. 214
102	Jahresbericht über die Leistungen und Fortschritte in der Gesammten Medicin. Berlin: A. Hirschwald; 1883. p. 317
103	Jahrbuch für Practische Aerzte. Berlin: A. Hirschwald; 1883. vol. 6, p. 143
104	Nothnagel H, Rossbach MJ. Handbuch der Arzneimittellehre, 5th ed. Berlin: A. Hirschwald; 1884. pp. 572, 582-3
105	Boehm R. Lehrbuch der allgemeinen und speciellen Arzneiverordnungslehre fur studirende, Aerzte und Apotheker. Jena: Fischer; 1884. p. 502
106	Medizinisch-Chirurgische Rundschau. Wien: Urban und Schwarzenberg; 1884. vol. 23, p. 593
107	Jahresbericht über die Fortschritte der Pharmakognosie, Pharmacie und Toxikologie. Göttingen: Vandenhoeck und Ruprecht; 1884. vols. 16-17, p. 948
108	Jahresbericht der Pharmazie. Göttingen: Vandenhoeck & Ruprecht; 1886. vols. 18-19, p. 948
109	Ewald CA. Handbuch der allgemeinen und speciellen Arzneiverordnungslehre. Berlin, Hirschwald; 1887. p. 753
110	Koch A. Encyklopädie der gesammten Thierheilkunde und Thierzucht. Wien: Perles; 1888. vol. 5, p. 79
111	Liebreich MEO. Compendium der Arzneiverordnung: nach dem Arzneibuch für das deutsche Reich und den neuesten fremden Pharmacopoeen. Berlin: Fischer; 1891. p. 703
112	Geissler E, Moeller J. Real-encyclopädie der gesammten pharmacie. Wien und Leipzig: Urban & Schwarzenberg; 1891. vol. 10, p. 916
113	Charité-Annalen. Berlin: Hirschwald; 1892. vol. 17, pp. 283-94 (Kohlstock, 1892)
114	Pharmazeutische Zentralhalle für Deutschland. Berlin: Dresden Steinkopff; 1892. vol. 33, p. 689
115	Zeitschrift für therapie: mit einbeziehung der electro- und hydrotherapie. Wien: Weiss; 1892. vol. 10, issue 22, p. 173-4
116	Leipziger populäre Zeitschrift für Homöopathie. Leipzig: W. Schwabe; 1892. vol. 23, p. 222
117	Wissenschaftliche Drogenkunde. Berlin: Gaertner; 1892. vol. 2, p. 425
118	Cloetta A, Filehne W. Dr. A. Cloetta‘s Lehrbuch der Arzneimittellehre und Arzneiverordnungslehre, 8th ed. Leipzig: Mohr; 1893. p. 232
119	Schmidt's Jahrbücher der in- und ausländischen gesammten Medizin. Leipzig: Wigand; 1893. vol. 238, issue 1, p. 17
120	Excerpta Medica. Leipzig: Sallman; 1893. vol. 2, p. 141
121	Therapeutische Monatshefte, Berlin: Springer; 1893. vol. 7, p. 34 (1893d)
122	Pharmaceutische Rundschau. New York: Pharmaceutical Review Pub. Co.; 1893. vol. 11, p. 20
123	Archiv für wissenschaftliche und practische Thierheilkunde. Berlin: A. Hirschwald; 1893. vol. 19, p. 427 (Baum, 1893)
124	New Yorker Medicinische Monatsschrift. New York: Medical Monthly Pub. Co.; 1893. vol. 5, p. 278
125	Jahrbuch der praktischen Medizin: Kritischer Jahresbericht für die Fortbildung der praktischen Ärzte. Stuttgart: F. Enke; 1893. p. 289
126	Zentralblatt für die Gesamte Therapie. Wien: M. Perles; 1893. vol. 11, p. 16
127	Archiv für wissenschaftliche und practische Thierheilkunde. Berlin: A. Hirschwald; 1894. vol. 20, pp. 10, 11, 21
128	Jahresbericht über die Leistungen auf dem Gebiete der Veterinär-Medicin. Berlin: A. Hirschwald; 1894. vols. 13-14 - pp. 151, 179, 181
129	Jahrbuch der praktischen Medizin: Kritischer Jahresbericht für die Fortbildung der praktischen Ärzte. Stuttgart: F. Enke; 1894. p. 772
130	Karsten H. Flora von Deutschland, Oesterreich und der Schweiz: Gera-Untermhaus: Köhler; 1895. vol. 2, p. 459
131	Deutsche Zeitschrift für Tiermedicin und vergleichende Pathologie. Leipzig: Vogel; 1895. vols. 21-22, p. 356
132	Penzoldt F. Handbuch der speciellen Therapie der innerer Krankheiten in sechse Baenden. Jena: G. Fischer; 1896. vol. 4, p. 526
133	Real-Encyclopadie der gesammten Heilkunde. Berlin: Urban & Schwarzenberg; 1896. vol. 11, p. 604
134	Thoms HFM. Die Arzneimittel der organischen Chemie: für Ärzte, Apotheker, und Chemiker. Berlin: Springer; 1897. p. 53
135	Eulenburg A. Lehrbuch der allgemeinen Therapie und der therapeutischen Methodik. Wien: Urban & Schwarzenberg; 1898. vol. 1, p. 166

No.	Source
136	Liebreich MEO. Encyklopaedie der Therapie. Berlin: A. Hirschwald; 1898. vol. 2, p. 809
137	Nevinný J. Allgemeine und specielle Arzneiverordnungslehre für Studirende und Aerzte. Leipzig: Deuticke; 1900. p. 182
138	Ebstein W, Schwalbe J, Braun. Handbuch der praktischen Medicin. Stuttgart: F. Enke; 1900. vol. 2, p. 616
139	Andreae JM. Zusammenstellung neuer Arzneimittel: mit kurzen Bemerkungen über Herkommen, Zusammensetzung, Wirkung und Dosirung, 3rd ed. Leipzig: W. Engelmann; 1901. p. 39
140	Kionka H. Grundriss der Toxicologie. Leipzig: Veit; 1901. p. 110
141	Die Therapie der Gegenwart: Medizinisch-chirurgische Rundschau für praktische Ärzte. Wien: Urban & Schwarzenberg; 1901. p. 311
142	Zeitschrift des allgemeinen Oesterreichischen Apotheker-Vereines. Wien: Im Selbstverlage des Vereines; 1901. vol. 39, p. 26
143	Ewald CA. Klinik der Verdauungskrankheiten. Berlin: Hirschwald; 1902. vol. 3, p. 121
144	Mindes J. Manuale der neuen Arzneimittel: für Apotheker, Ärzte und Drogisten, 4th ed. Zürich: Art Institut Orell Füssli; 1902. p. 66
145	Pharmazeutische Zentralhalle für Deutschland. Berlin: Dresden Steinkopff; 1902. vol. 43, p. 318
146	E. Merck (Firm). Preis-Liste der chemischen Fabrik von E. Merck in Darmstadt: Vorzugspreise; 1903. p. 81
147	Arends G. Neue arzneimittel und pharmazeutische spezialitaten, einschliesslich der neuen drogen, organ-und serumpraparate. Berlin: Springer; 1903. p. 114
148	Pamphlets on Biology: Kofoid collection. 1903. vol. 409, pp. 33, 80
149	von Lengerken OFF. Arzneibuch für Mediziner: Handbuch zur Beurteilung und zur selbständigen Aufstellung von Rezepten, im Anschluss an das Arzneibuch für das Deutsche Reich, 4. Ausg. Leipzig: Veit; 1904. p. 204
150	Fortschritte der praktischen und wissenschaftlichen Pharmazie. Berlin: Urban & Schwarzenberg; 1905. p. 87
151	Schnirer MT. Taschenbuch der Therapie mit besonderer Berücksichtigung der Therapie an den Berliner, Wiener u.a. deutschen Kliniken, 2nd ed. Wien; 1906. p. 187
152	Rodari P. Grundriss der medikamentösen Therapie der Magen- und Darmkrankheiten. Wiesbaden: Bergmann; 1906. p. 152
153	Kobert R. Lehrbuch der intoxikationen. Stuttgart: F. Enke; 1906. vol. 2(Pt. 2), p. 561
154	Leyden E, Llemperer F. Deutsche Klinik am Eingange des zwanzigsten Jahrhunderts. Berlin: Urban & Schwarzenberg; 1907. p. 499 (Leyden and Klemperer, 1907).
155	von Lengerken OFF. Handbuch neuerer Arzneimittel. Frankfurt: Alt; 1907. p. 199
156	Zweig W. Die Therapie der Magen- und Darmkrankheiten. Berlin & Wien: Urban & Schwarzenberg; 1907. p. 288
157	Penzoldt F. Lehrbuch der klinischen Arzneibehandlung. Jena: G. Fischer; 1908. p. 561
158	Hager H. Hager's Handbuch der pharmaceutischen Praxis für Apotheker, Ärzte, Drogisten und Medicinalbeamte. Berlin: Springer; 1908. p. 231
159	Fröhner E. Lehrbuch der Arzneimittellehre für Tierärzte, 8th ed. Stuttgart: Enke; 1909. p. 440
160	Kahane M. Der Arzneitherapie der Gegenwart: Die neuesten Arzneimittel und ihre Anwendung in der ärztlichen Praxis. Berlin: Urban & Schwarzenberg; 1910. p. 224
161	Wegele C. Die Therapie der Magen- und Darmerkrankungen. Jena: G. Fischer; 1911. p. 141
162	Roth O. Die Arzneimittel der heutigen Medizin: mit therapeutischen Notizen zusammengestellt für praktische Ärzte und Studierende der Medizin, 11th ed. Würzburg: Kabitzsch; 1911. p. 137.
163	Zörnig H. Arzneidrogen als nachschlagebuch für den gebrauch der apotheker, ärzte, veterinärärzte, drogisten und studierenden der pharmazie. Leipzig: W. Klinkhardt; 1913. vol. 1, p. 205
164	Muspratt JS. Encyklopädisches Handbuch der technischen Chemie. F. Vieweg & Sohn; 1915. vol. 3(Pt. 1), p. 92
165	Heinigke C. Handbuch der homöopathischen Arzneiwirkungslehre. Leipzig: Schwabe; 1922. p. 204
166	Heffter A, Heubner W. Handbuch der experimentellen Pharmakologie. Berlin: Springer; 1924. vol. 2 (Pt. 2), p. 1654
167	Skandinavisches Archiv für Physiologie. Berlin: Walter De Gruyter & Co.; 1926. vols. 49-50, p. 158(Hollander, 1927)
168	Winterstein EH, Trier G. Die Alkaloide: eine Monographie der natürlichen Basen. Berlin: Borntraeger; 1927. p. 814
	Italian Text
169	Balsamo-Crivelli R. Boccacino: racconto, 2nd ed. Bari: Laterza; 1921. p. 27
	Swedish Text
170	Rosendahl HV. Lärobok i farmakognosi. Upsala: Schultz; 1897. p. 400

Appendix C

Supplementary Materials for Chapter 3 (Pharmacodynamic Study)

C.1 Supplementary Tables

Table C.1. General descriptive statistics regarding mass spectrometry measurements for plasma amino acids.

	N	Mean		SD	Range	Minimum	Maximum	Variance	Skewness		Kurtosis	
	Statistic	Statistic	Std. Error	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Arginine Retention Time	888	3.06248	0.00298	0.0888	1.177	2.903	4.08	0.008	9.533	0.082	103.409	0.164
Arginine Area	888	11787351	419839.1	12510924	76185358	5896	76191254	1.57E+14	1.885	0.082	3.991	0.164
Arginine Height	888	953484.9	33570.98	1000393	5645841	998	5646839	1E+12	1.836	0.082	3.657	0.164
Arginine ISTD area	888	23695844	557403	16610234	1.01E+08	8359	1.01E+08	2.76E+14	0.771	0.082	0.338	0.164
Citrulline Retention Time	888	3.63984	0.005628	0.167711	0.958	2.97	3.928	0.028	-2.938	0.082	8.692	0.164
Citrulline Area	888	20617686	1825535	54399723	3.47E+08	2258	3.47E+08	2.96E+15	3.441	0.082	12.092	0.164
Citrulline Height	888	1301878	103936.4	3097235	14964580	796	14965376	9.59E+12	2.837	0.082	7.13	0.164
Citrulline ISTD area	887	23361111	564571.4	16814373	1.01E+08	5827	1.01E+08	2.83E+14	0.768	0.082	0.292	0.164
Glutamine Retention Time	888	3.73369	0.03005	0.89546	5.042	2.605	7.647	0.802	3.856	0.082	14.07	0.164
Glutamine Area	888	10649962	358610.2	10686343	83215004	11547	83226551	1.14E+14	1.532	0.082	3.753	0.164
Glutamine Height	888	834739.9	28643.51	853557.3	6405744	1598	6407342	7.29E+11	1.584	0.082	3.856	0.164
Glutamine ISTD area	888	23228928	567076.6	16898502	1.01E+08	8359	1.01E+08	2.86E+14	0.766	0.082	0.26	0.164
Ornithine Retention Time	889	6.56434	0.005906	0.1761	0.824	6.36	7.184	0.031	1.796	0.082	2.959	0.164
Ornithine Area	889	6002900	244112.8	7278491	61966512	36960	62003472	5.3E+13	3.058	0.082	12.855	0.164

Table C.1. *(Continued)*

	N	Mean		SD	Range	Minimum	Maximum	Variance	Skewness		Kurtosis	
Ornithine Height	889	437073.8	16419.15	489555	3743128	3380	3746508	2.4E+11	2.647	0.082	9.05	0.164
Ornithine ISTD area	889	21431772	455407	13578461	66258685	121785	66380470	1.84E+14	0.517	0.082	-0.485	0.164
3-MH Retention Time	888	4.48536	0.323307	9.634325	112.944	3.292	116.236	92.82	10.053	0.082	100.772	0.164
3-MH Area	879	700723.6	21839.69	647501.6	5189498	1837	5191335	4.19E+11	2.117	0.082	7.559	0.165
3-MH Height	888	58351.34	1666.424	49658.3	437650	1087	438737	2.47E+09	2.326	0.082	9.496	0.164
3-MH ISTD area	889	23745127	557594.1	16625283	1.01E+08	41301	1.01E+08	2.76E+14	0.752	0.082	0.322	0.164

Table C.2. Dates samples were analysed.

Date	Frequency	Valid Percent
07-Oct-13	17	1.4
08-Oct-13	28	2.3
11-Oct-13	50	4.2
14-Oct-13	23	1.9
16-Oct-13	63	5.2
18-Oct-13	256	21.3
25-Oct-13	89	7.4
04-Nov-13	84	7
05-Nov-13	119	9.9
08-Nov-13	80	6.7
11-Nov-13	18	1.5
22-Jan-14	135	11.2
27-Jan-14	180	15
30-Jan-14	60	5
Total	1202	100

Table C.3. Crossover ANOVA for plasma amino acid concentrations (before conversion into AUC).

	CITRULLINE					**ARGININE**				
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**Partial SS**	**df**	**MS**	**F**	**p**
Intersubjects										
Sequence effect	8172.45	9	908.05	0.33	0.9431	954748	9	106083.1	1.45	0.2841
Residuals	27202.57	10	2720.26	0.52	0.8774	730645	10	73064.5	9.58	0.0001
Intrasubjects										
Treatment effect	1.59E+07	4	3.98E+06	759.7	**0.0001**	4.38E+06	4	1.09E+06	143.49	**0.0001**
Carryover effect	13438.89	4	3359.72	0.64	0.6332	128323.3	4	32080.83	4.21	0.0022
Period effect	8047.77	4	2011.94	0.38	0.8202	295352.1	4	73838.01	9.69	0
Residuals	4.55E+06	868	5239.54			6.62E+06	868	7622.9		
Total	2.15E+07	899				1.31E+07	899			
	GLUTAMINE					**ORNITHINE**				
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**	**Partial SS**	**df**	**MS**	F	p
Intersubjects										
Sequence effect	2.99E+06	9	332054.6	0.63	0.7502	338865.8	9	37651.76	1.63	0.2281
Residuals	5.27E+06	10	527026.7	12.71	0.0001	230752.6	10	23075.26	4.29	0.0001
Intrasubjects										
Treatment effect	3.05E+06	4	763333.2	18.41	**0.0001**	3.39E+06	4	847934.7	157.77	**0.0001**
Carryover effect	1.35E+06	4	338558.7	8.16	0.0001	93981.73	4	23495.43	4.37	0.0017
Period effect	2.68E+06	4	669092.4	16.13	0.0001	122995.8	4	30748.96	5.72	0.0002
Residuals	3.60E+07	868	41473.28			4.66E+06	868	5374.4		
Total	5.27E+07	899				8.96E+06	899			
	3-METHYL-HISTIDINE									
Source of Variation	**Partial SS**	**df**	**MS**	**F**	**p**					
Intersubjects										
Sequence effect	279.55	9	31.06	0.82	0.6129					
Residuals	379.21	10	37.92	39.49	0.0001					
Intrasubjects										
Treatment effect	15.75	4	3.94	4.1	**0.0027**					
Carryover effect	7.34	4	1.83	1.91	0.1067					
Period effect	161.83	4	40.46	42.13	0.0001					
Residuals	833.55	868	0.96							
Total	1696.61	899								

Note for all models: Omnibus measure of separability of treatment and carryover = 77.6393%

Table C.4. MANOVA results including age, gender and BMI. Adjusted R^2 = [a] 39.8%; [b] 98.0%; [c] 0.0%; [d] 53.3%; [e] 0.0%.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	*$AUC_{0\text{-}6}$ for Arginine*	20112174.199[a]	7	2873168	10.368	**<0.001**
	$AUC_{0\text{-}6}$ for Citrulline	75684972.210[b]	7	10812139	682.918	**<0.001**
	$AUC_{0\text{-}6}$ for Glutamine	9900089.380[c]	7	1414298	0.904	0.507
	$AUC_{0\text{-}6}$ for Ornithine	19136356.236[d]	7	2733765	17.141	**<0.001**
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	211.089[e]	7	30.156	0.477	0.849
Intercept	*$AUC_{0\text{-}6}$ for Arginine*	1529131	1	1529131	5.518	0.021
	$AUC_{0\text{-}6}$ for Citrulline	1023489	1	1023489	64.646	**<0.001**
	$AUC_{0\text{-}6}$ for Glutamine	23252995	1	23252995	14.857	**<0.001**
	$AUC_{0\text{-}6}$ for Ornithine	506418.6	1	506418.6	3.175	0.078
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	1205.488	1	1205.488	19.07	**<0.001**
Gender	*$AUC_{0\text{-}6}$ for Arginine*	147.066	1	147.066	0.001	0.982
	$AUC_{0\text{-}6}$ for Citrulline	280.487	1	280.487	0.018	0.894
	$AUC_{0\text{-}6}$ for Glutamine	23429.59	1	23429.59	0.015	0.903
	$AUC_{0\text{-}6}$ for Ornithine	197850.3	1	197850.3	1.241	0.268
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	110.901	1	110.901	1.754	0.189
Age	*$AUC_{0\text{-}6}$ for Arginine*	4071.496	1	4071.496	0.015	0.904
	$AUC_{0\text{-}6}$ for Citrulline	13367.74	1	13367.74	0.844	0.361
	$AUC_{0\text{-}6}$ for Glutamine	304934.6	1	304934.6	0.195	0.66
	$AUC_{0\text{-}6}$ for Ornithine	184052.5	1	184052.5	1.154	0.286
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	53.932	1	53.932	0.853	0.358
BMI	*$AUC_{0\text{-}6}$ for Arginine*	21803.02	1	21803.02	0.079	0.78
	$AUC_{0\text{-}6}$ for Citrulline	23241.44	1	23241.44	1.468	0.229
	$AUC_{0\text{-}6}$ for Glutamine	1094899	1	1094899	0.7	0.405
	$AUC_{0\text{-}6}$ for Ornithine	23860.67	1	23860.67	0.15	0.7
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	1.8	1	1.8	0.028	0.866
treatment	*$AUC_{0\text{-}6}$ for Arginine*	20080849	4	5020212	18.115	**<0.001**
	$AUC_{0\text{-}6}$ for Citrulline	75646032	4	18911508	1194.492	**<0.001**
	$AUC_{0\text{-}6}$ for Glutamine	8139282	4	2034821	1.3	0.276
	$AUC_{0\text{-}6}$ for Ornithine	18734575	4	4683644	29.367	**<0.001**
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	30.471	4	7.618	0.121	0.975
Error	*$AUC_{0\text{-}6}$ for Arginine*	25495504	92	277125		
	$AUC_{0\text{-}6}$ for Citrulline	1456568	92	15832.26		
	$AUC_{0\text{-}6}$ for Glutamine	1.44E+08	92	1565139		
	$AUC_{0\text{-}6}$ for Ornithine	14672880	92	159487.8		
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	5815.592	92	63.213		

Table C.4. *(Continued)*

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Total	*$AUC_{0\text{-}6}$ for Arginine*	1.34E+08	100			
	$AUC_{0\text{-}6}$ for Citrulline	1.29E+08	100			
	$AUC_{0\text{-}6}$ for Glutamine	1.15E+09	100			
	$AUC_{0\text{-}6}$ for Ornithine	89697886	100			
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	79273.21	100			
Corrected Total	*$AUC_{0\text{-}6}$ for Arginine*	45607678	99			
	$AUC_{0\text{-}6}$ for Citrulline	77141540	99			
	$AUC_{0\text{-}6}$ for Glutamine	1.54E+08	99			
	$AUC_{0\text{-}6}$ for Ornithine	33809236	99			
	$AUC_{0\text{-}6}$ for 3-Methyl-Histidine	6026.681	99			

C.2 Supplementary Figures

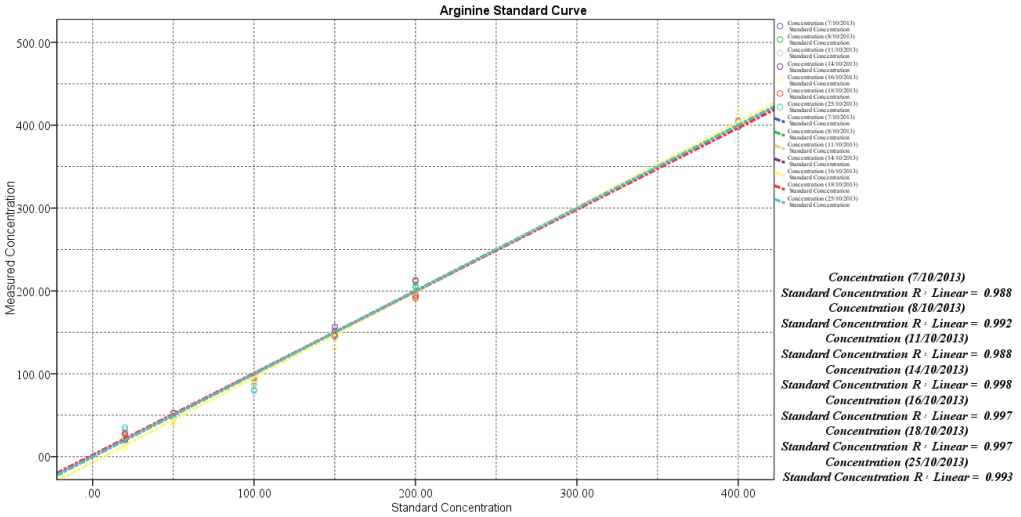


Figure C.1. Internal standard curves for measuring arginine with LC-MS.

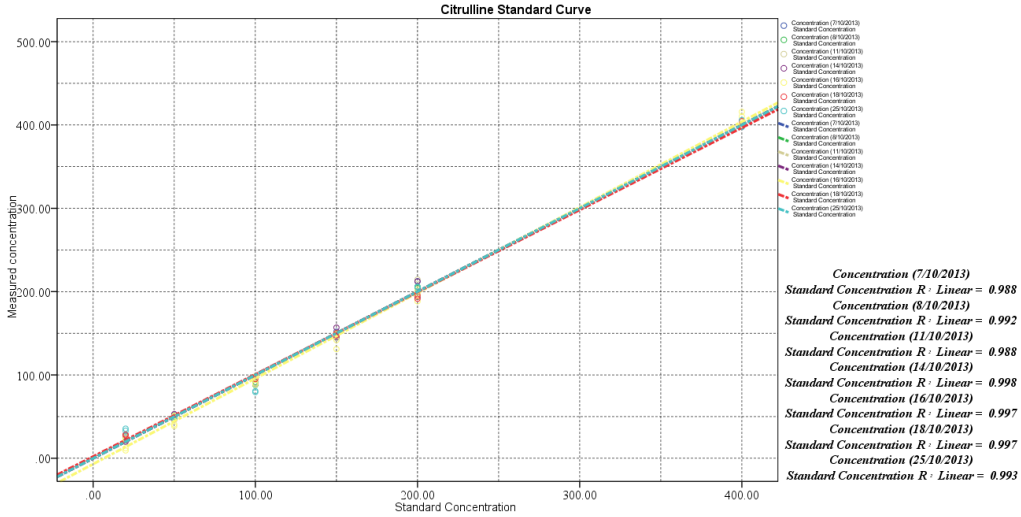


Figure C.2. Internal standard curves for measuring citrulline with LC-MS.

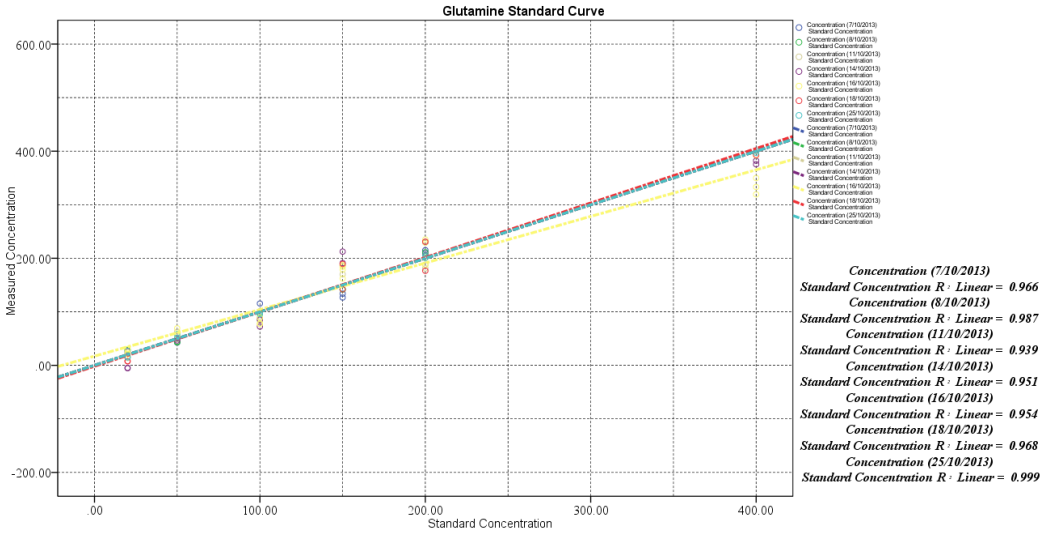


Figure C.3. Internal standard curves for measuring glutamine with LC-MS.

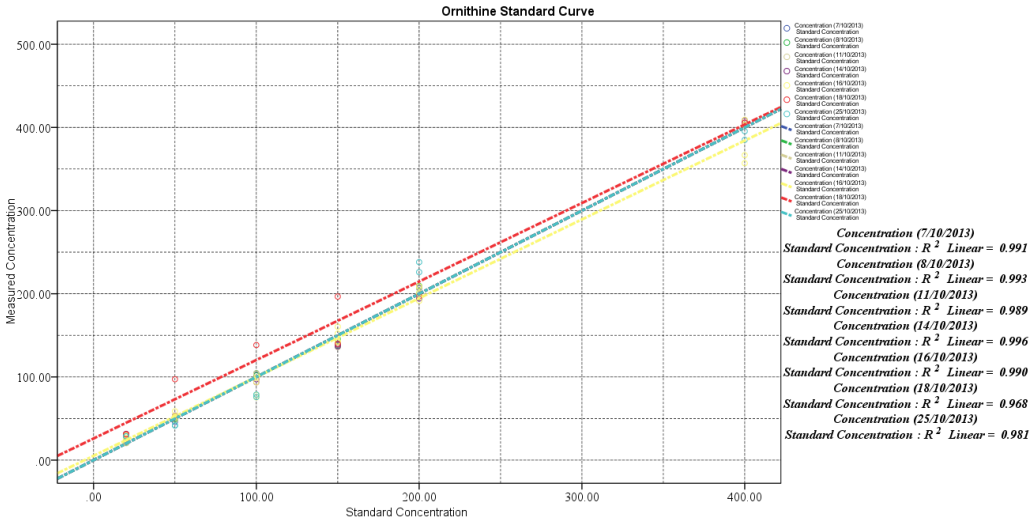


Figure C.4. Internal standard curves for measuring ornithine with LC-MS.

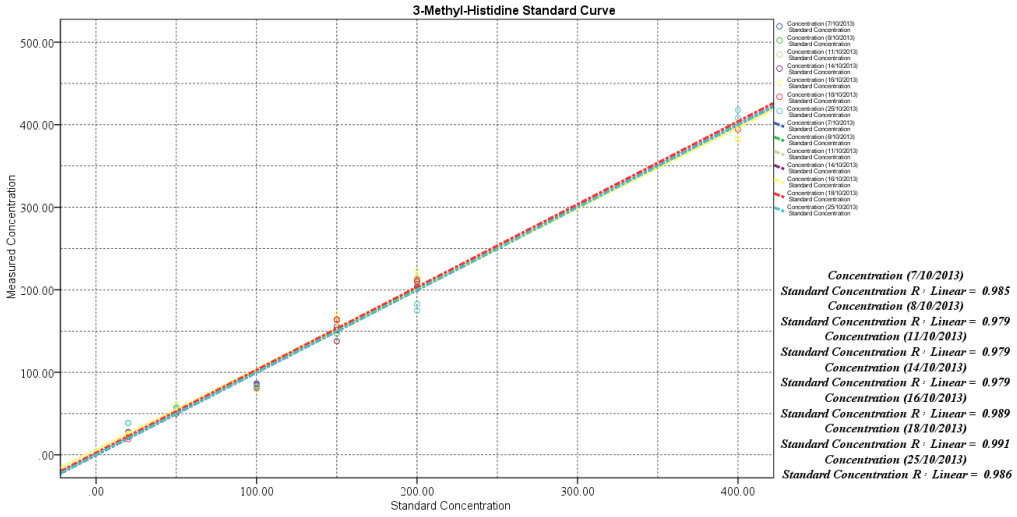


Figure C.5. Internal standard curves for measuring 3-methylhistidine with LC-MS.

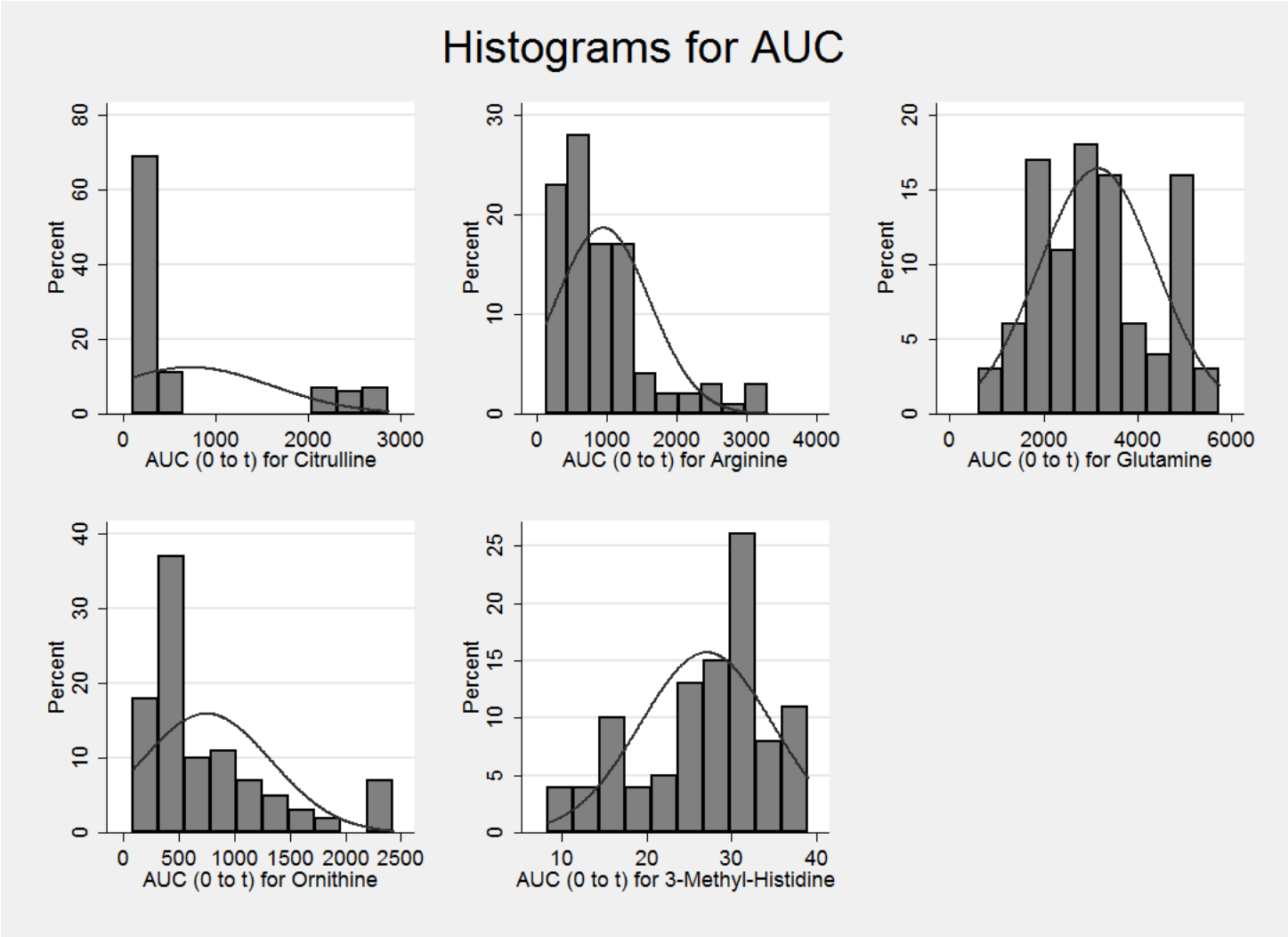


Figure C.6. Histograms showing distribution of AUC.

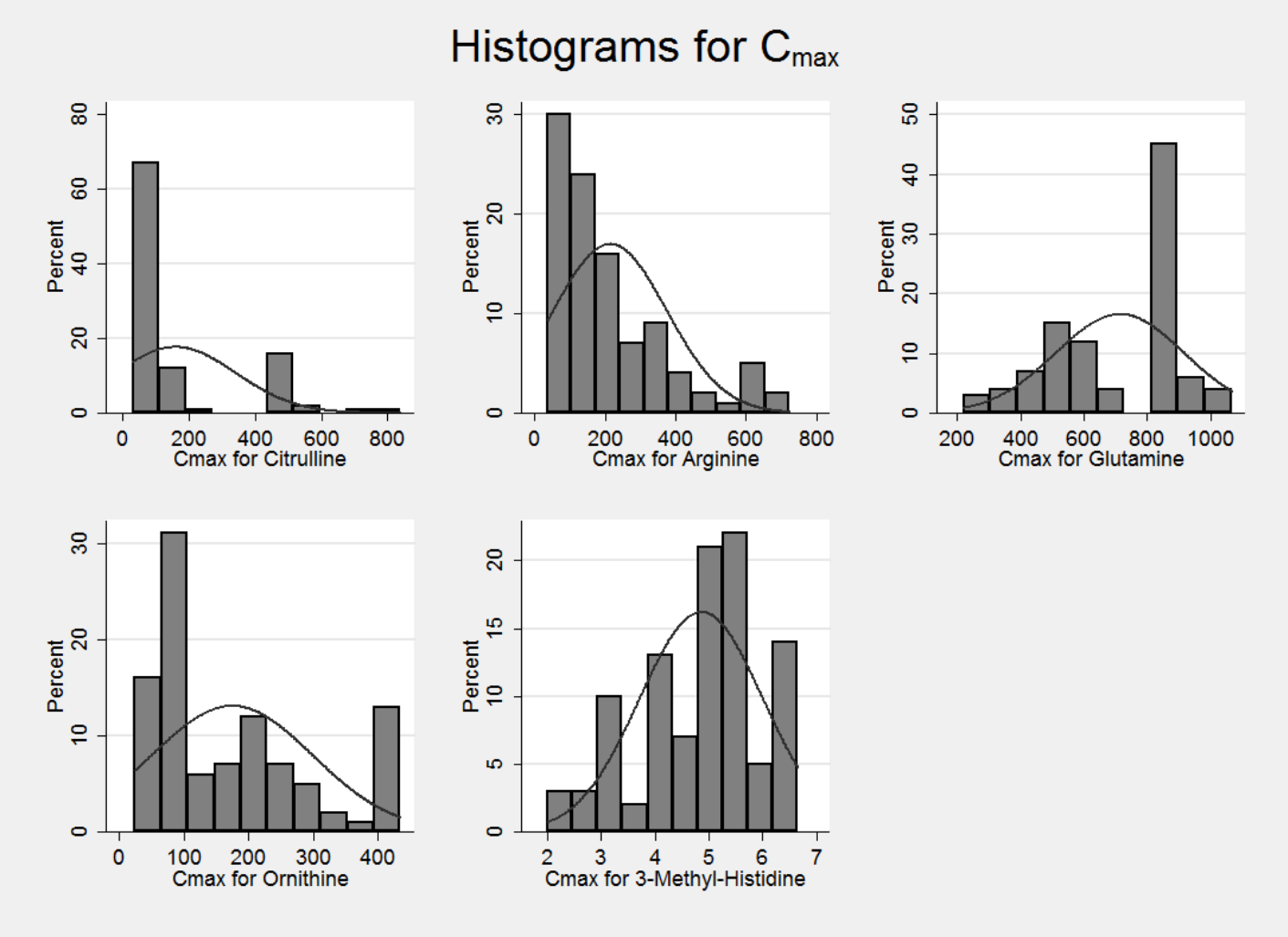


Figure C.7. Histograms showing distribution of C_{max}.

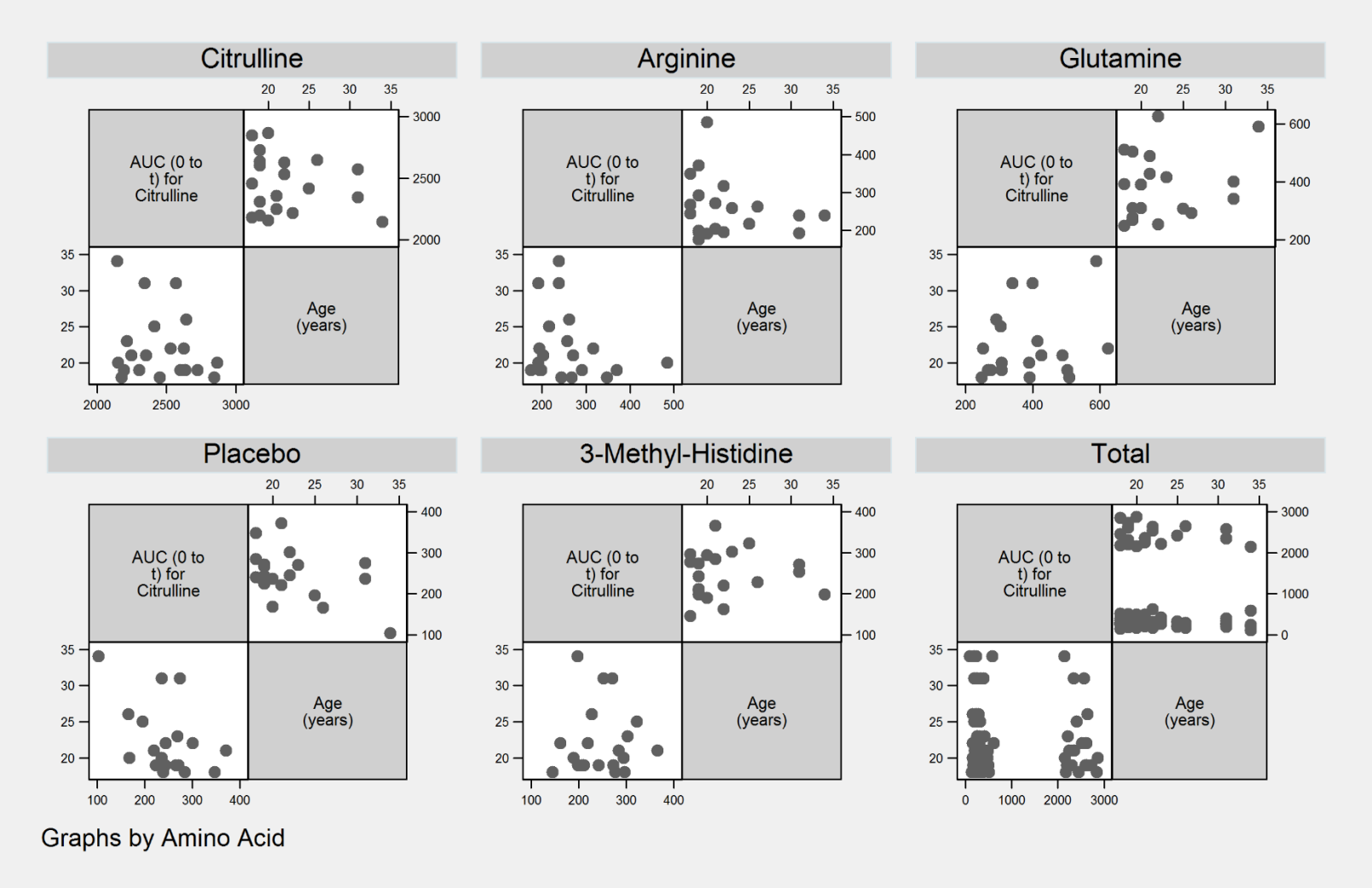


Figure C.8. Scatterplot of citrulline AUC with age by treatment and in total. No differences noted.

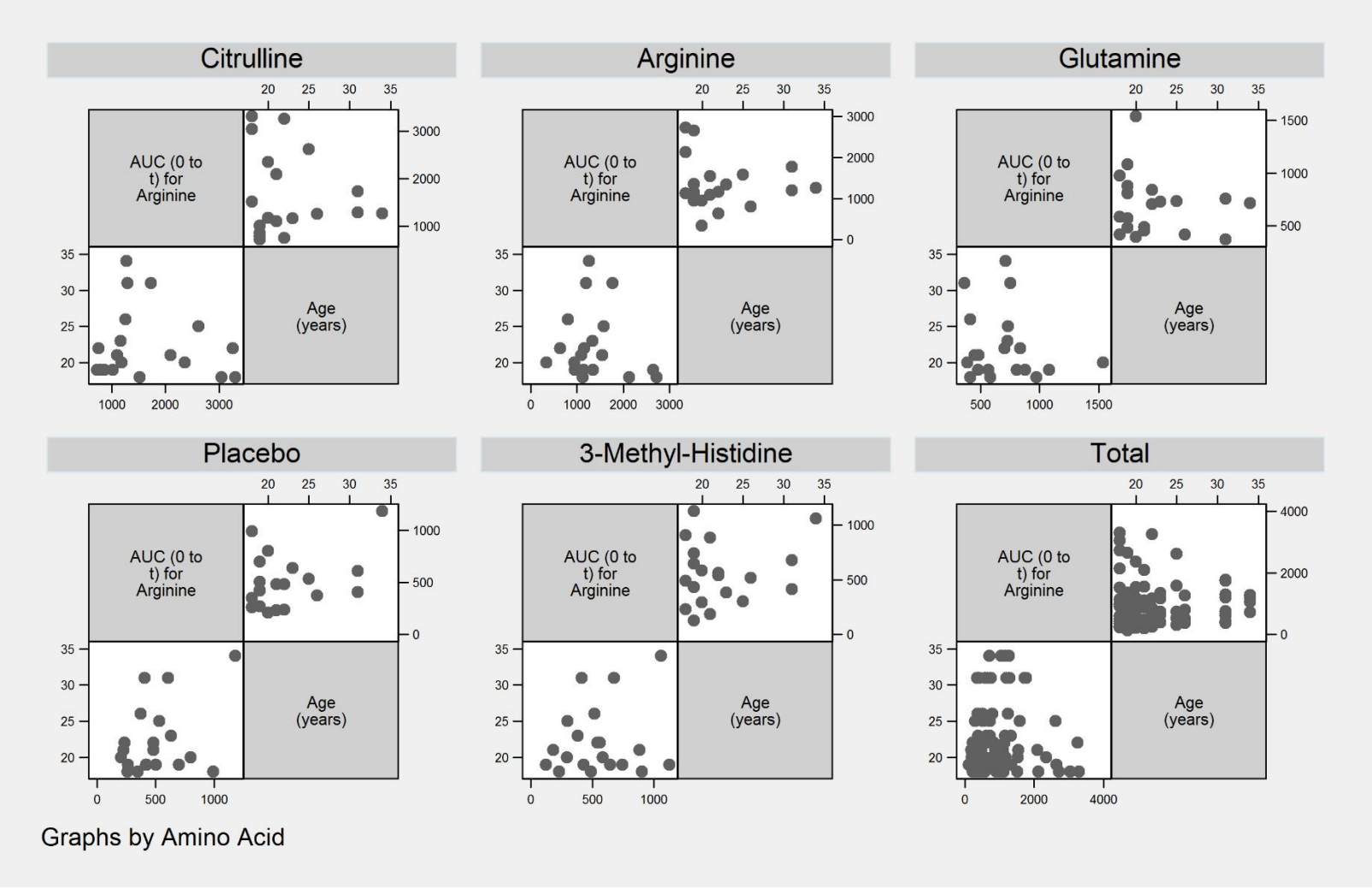


Figure C.9. Scatterplot of arginine AUC with age by treatment and in total. No differences noted.

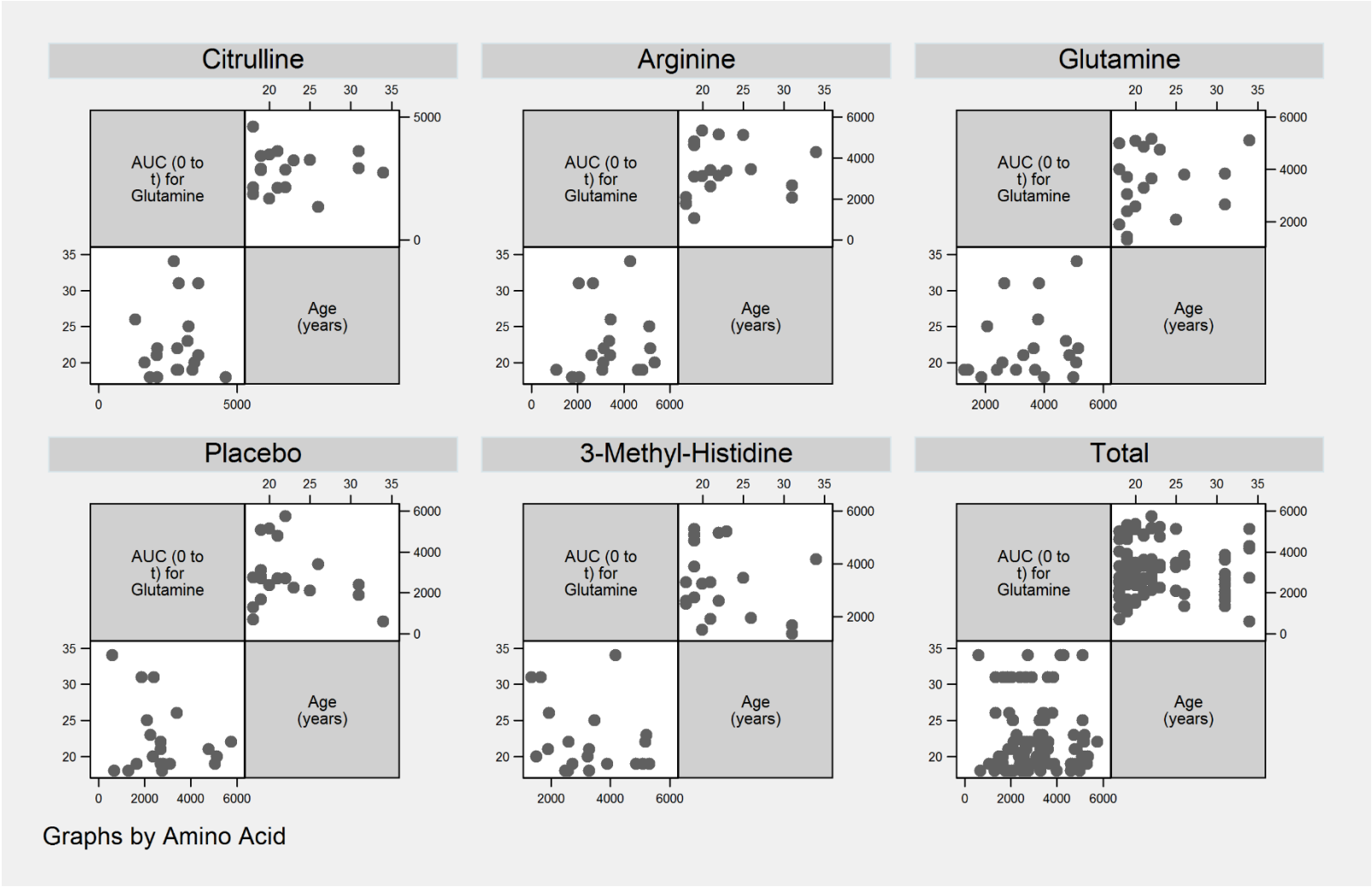


Figure C.10. Scatterplot of glutamine AUC with age by treatment and in total. No differences noted.

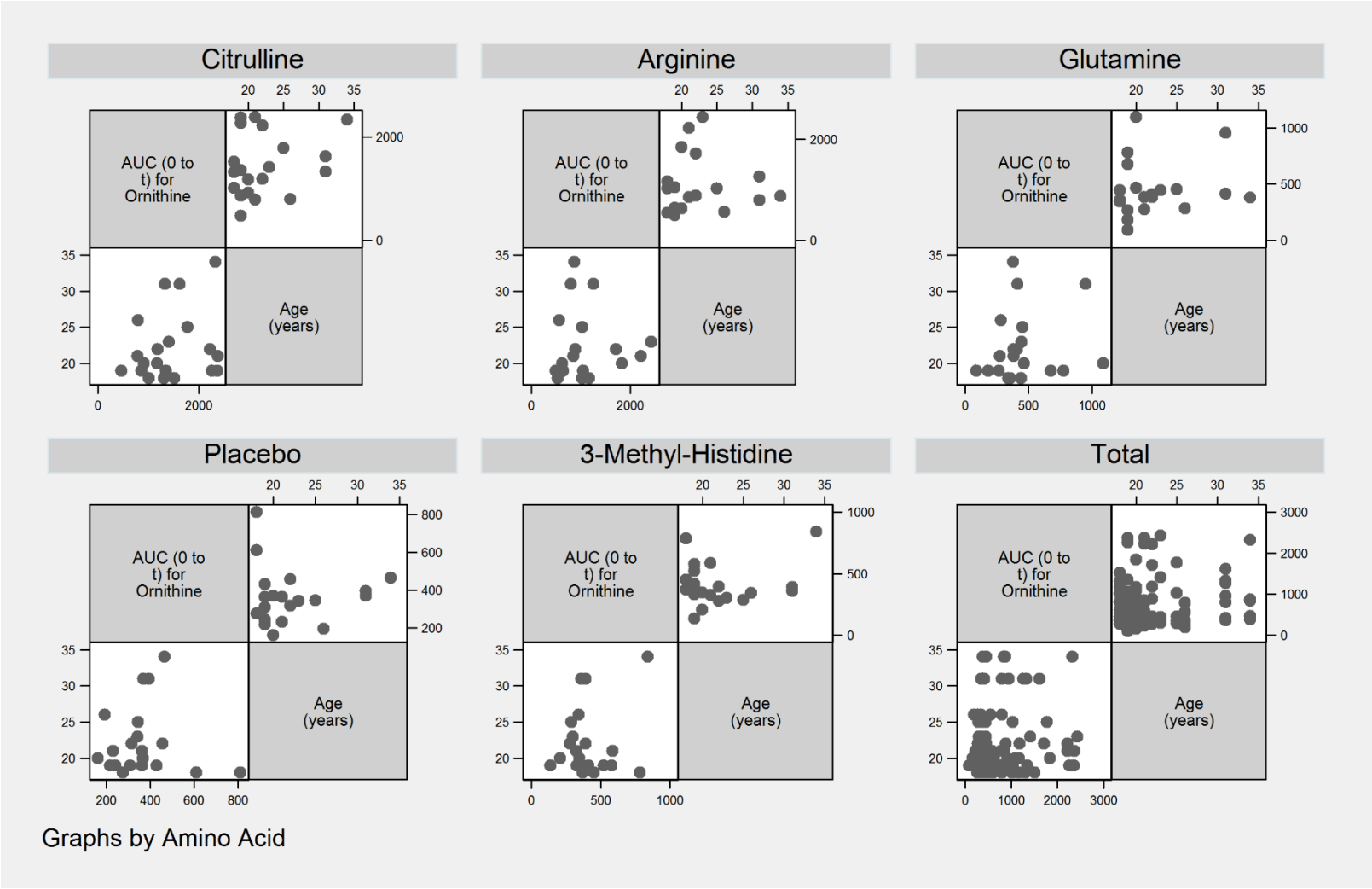


Figure C.11. Scatterplot of ornithine AUC with age by treatment and in total. No differences noted.

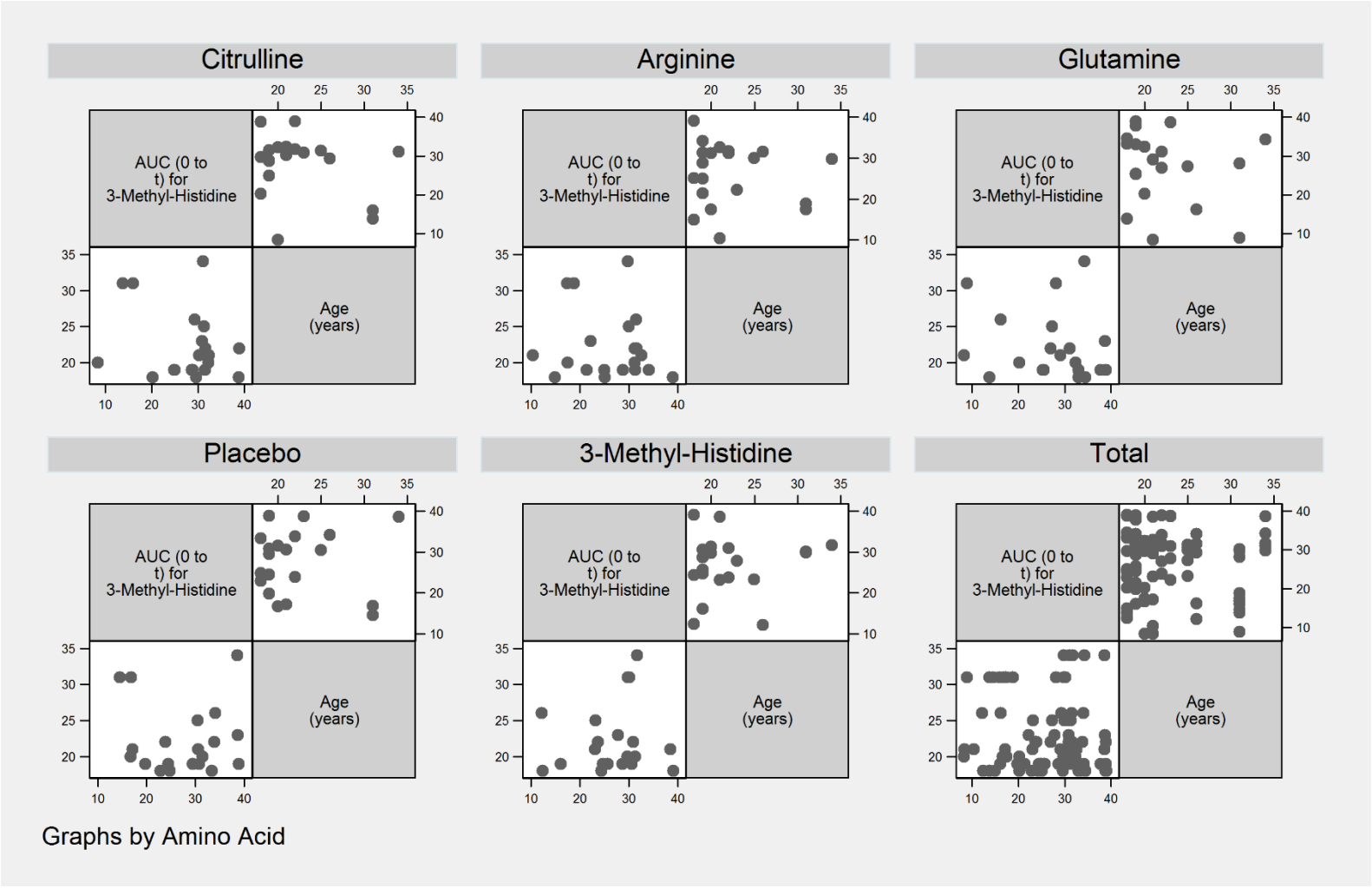


Figure C.12. Scatterplot of 3-methylhistidine AUC with age by treatment and in total. No differences noted.

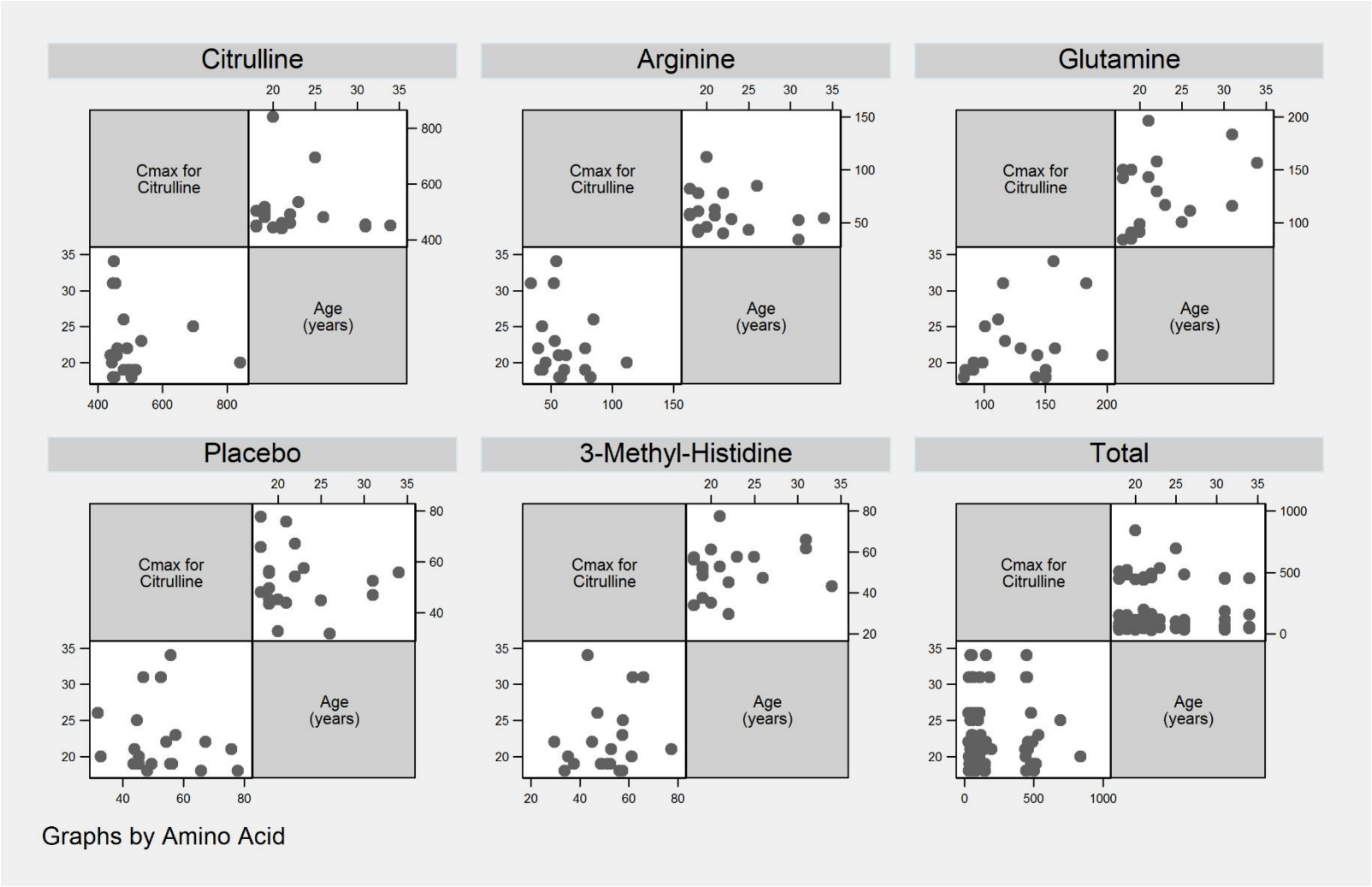


Figure C.13. Scatterplot of citrulline C_{max} with age by treatment and in total. No differences noted.

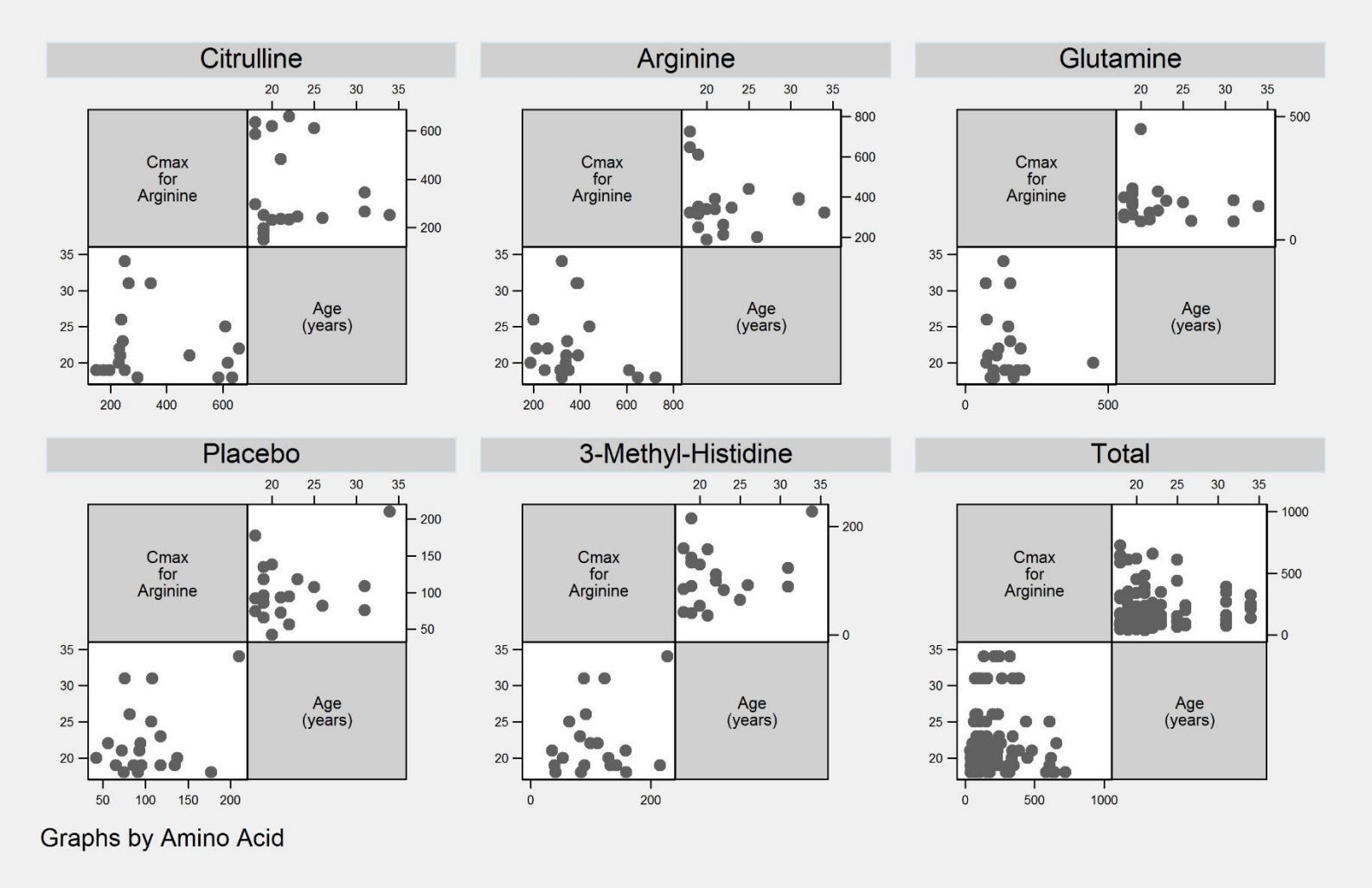


Figure C.14. Scatterplot of arginine C_{max} with age by treatment and in total. No differences noted.

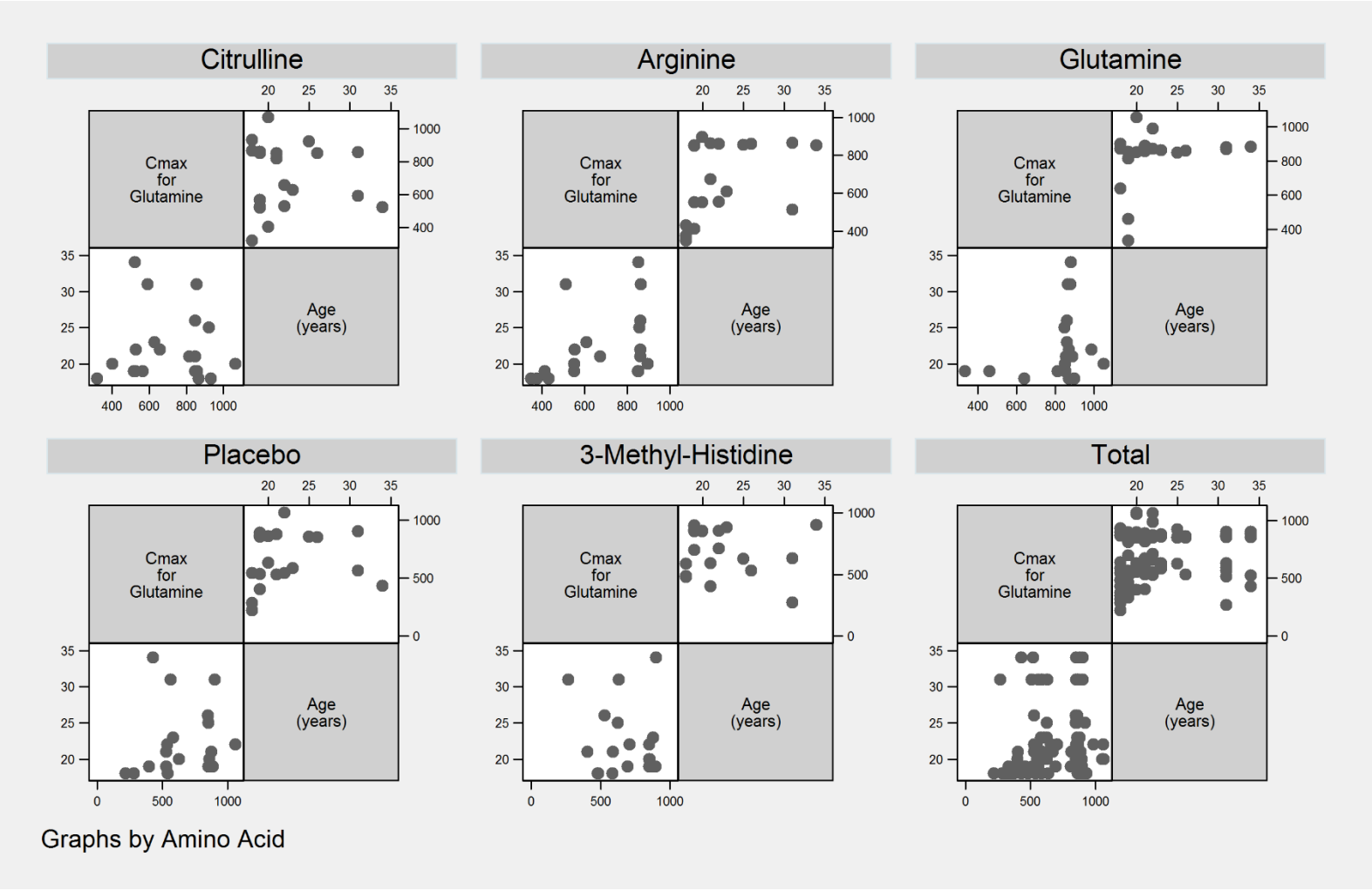


Figure C.15. Scatterplot of glutamine C_{max} with age by treatment and in total. No differences noted.

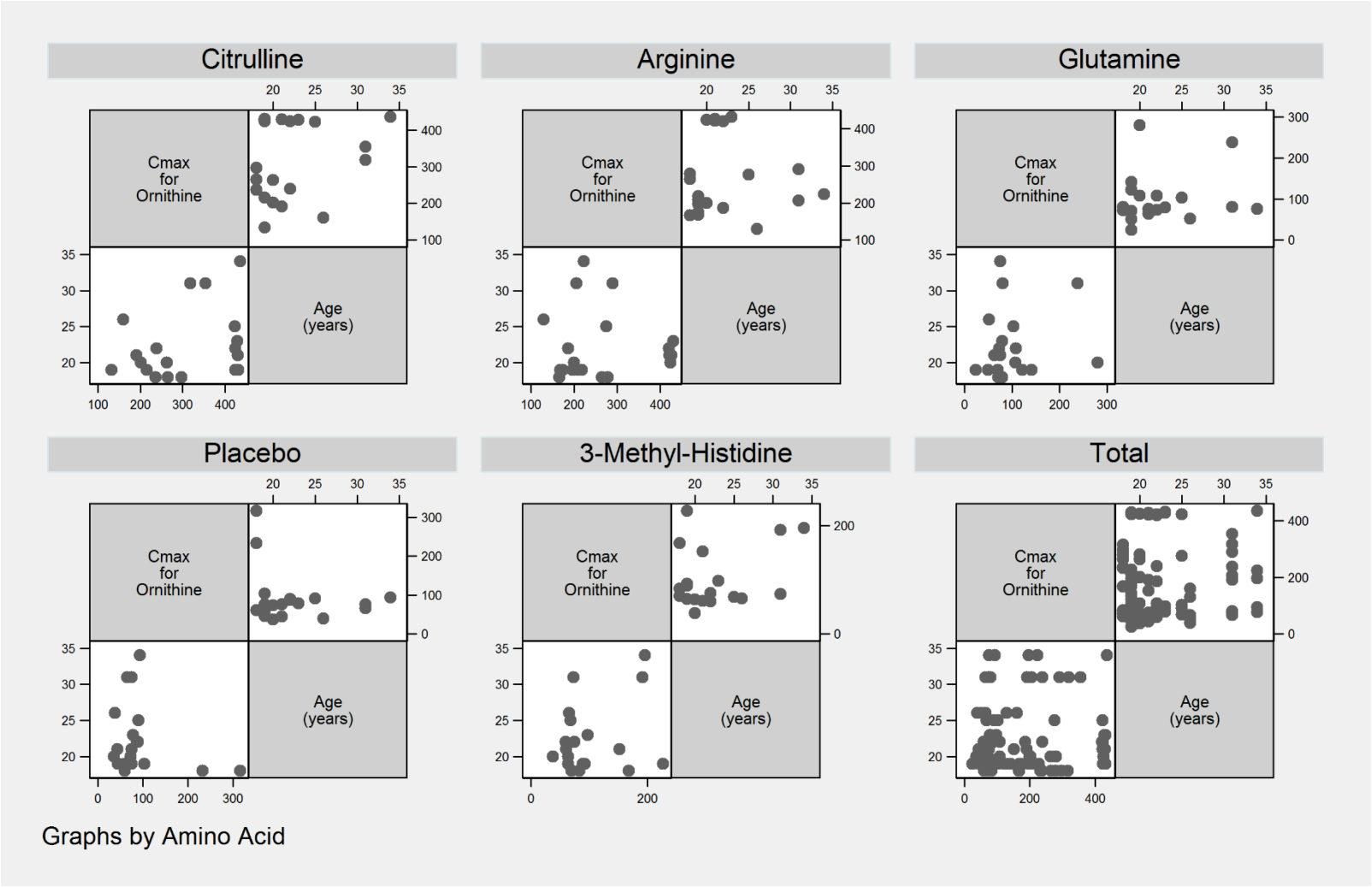


Figure C.16. Scatterplot of ornithine C_{max} with age by treatment and in total. No differences noted.

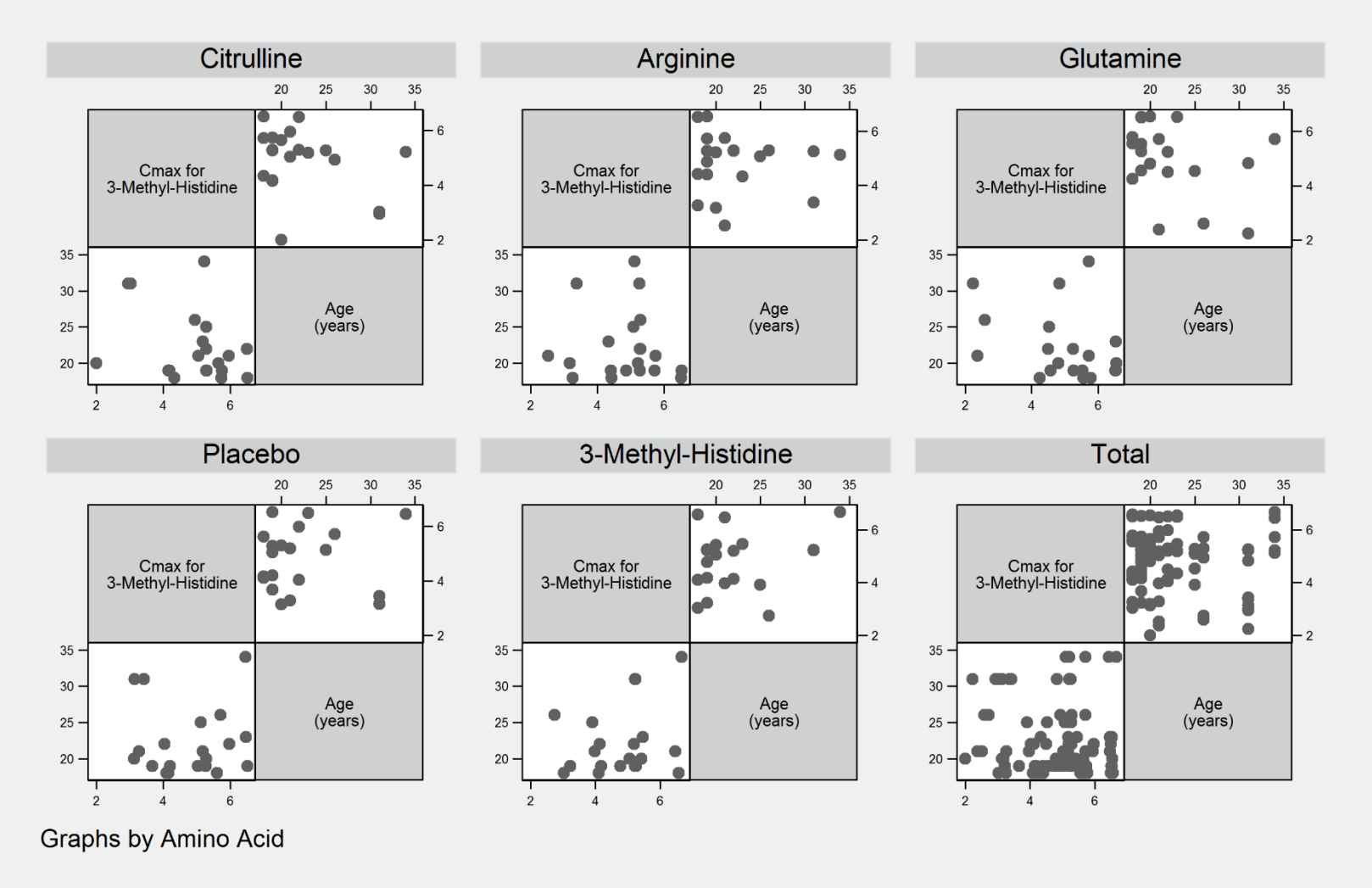


Figure C.17. Scatterplot of 3-methylhistidine C_{max} with age by treatment and in total. No differences noted.

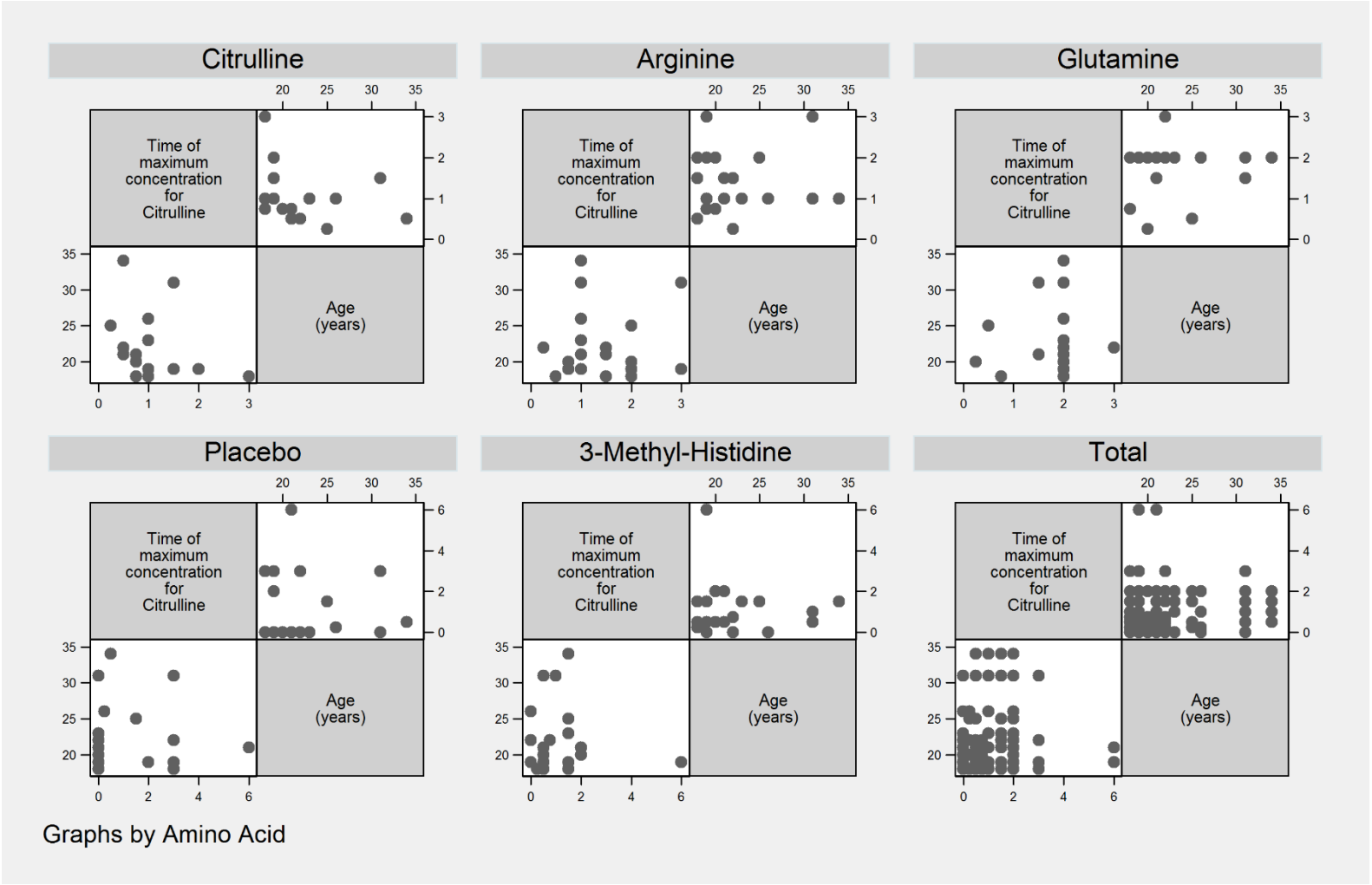


Figure C.18. Scatterplot of citrulline TOMC with age by treatment and in total. No differences noted.

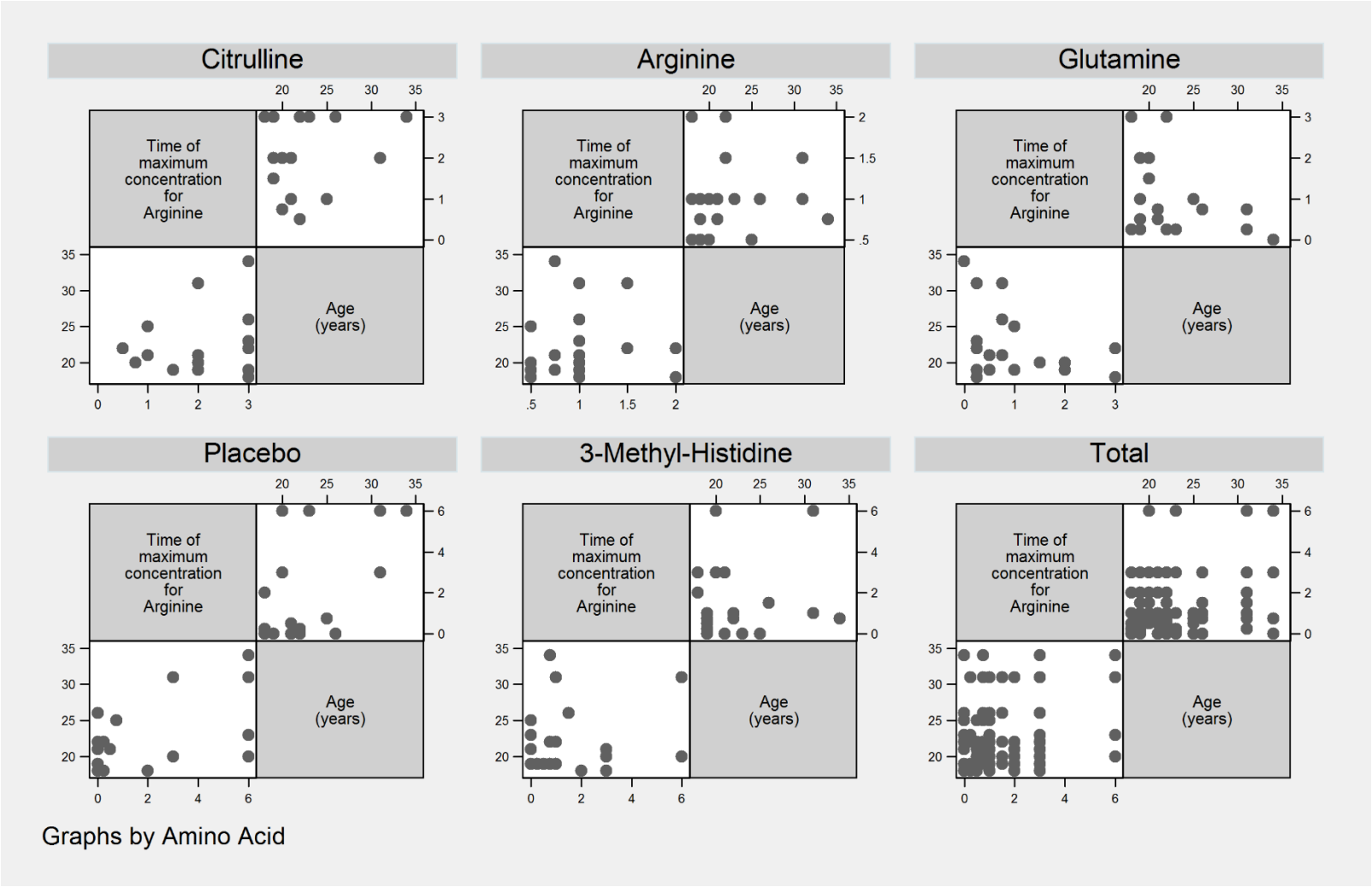


Figure C.19. Scatterplot of arginine TOMC with age by treatment and in total. No differences noted.

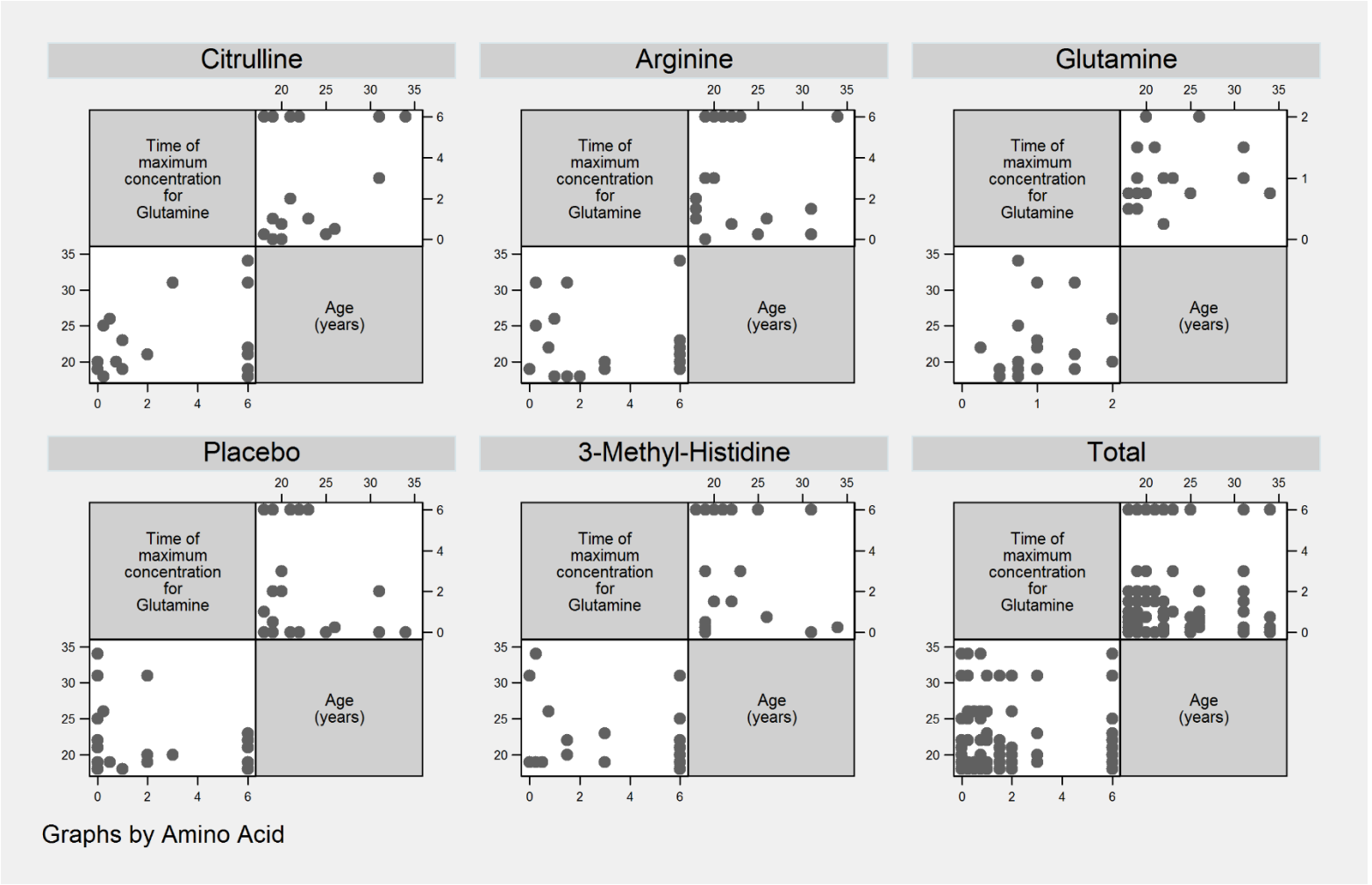


Figure C.20. Scatterplot of glutamine TOMC with age by treatment and in total. No differences noted.

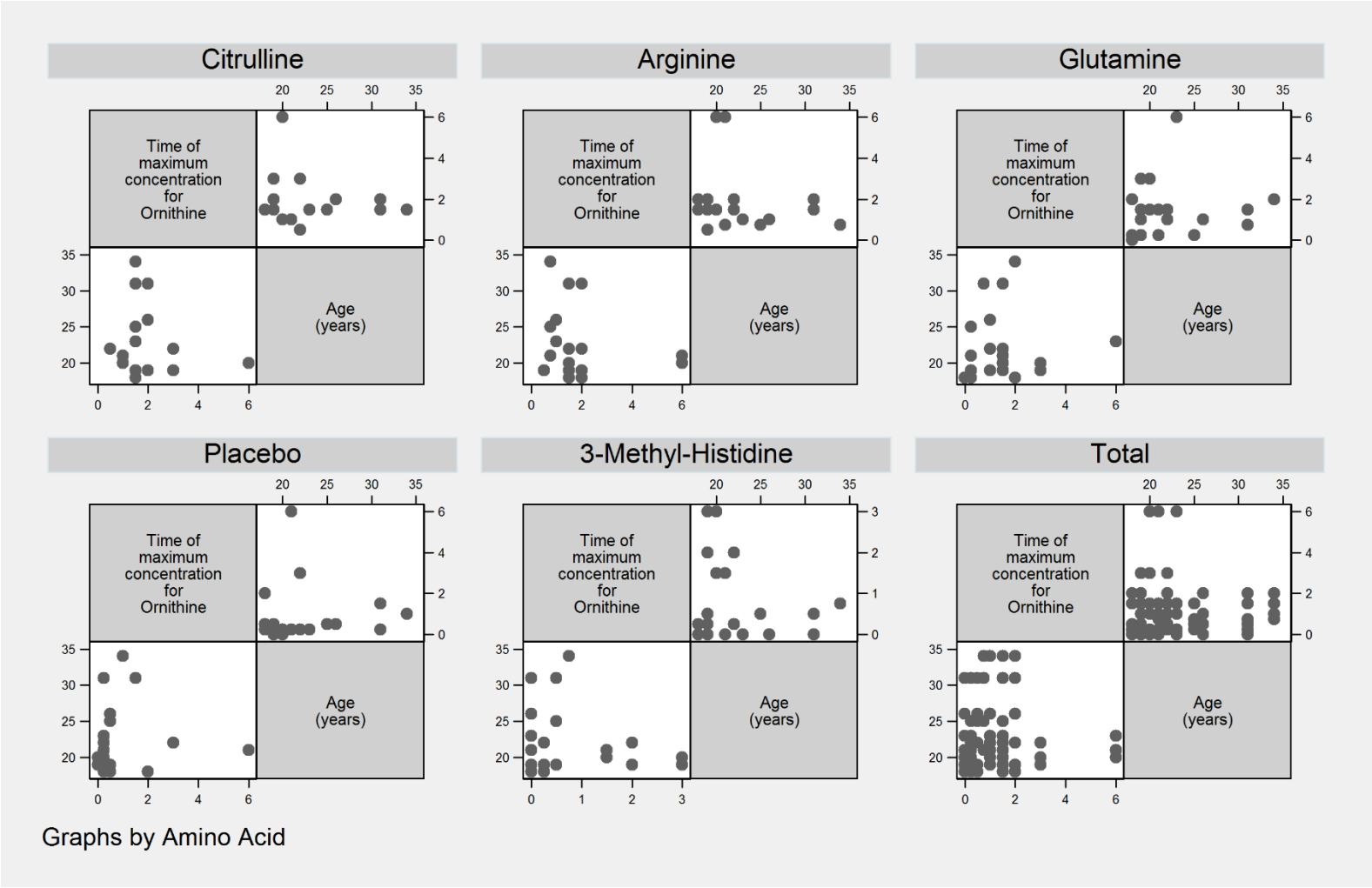


Figure C.21. Scatterplot of ornithine TOMC with age by treatment and in total. No differences noted.

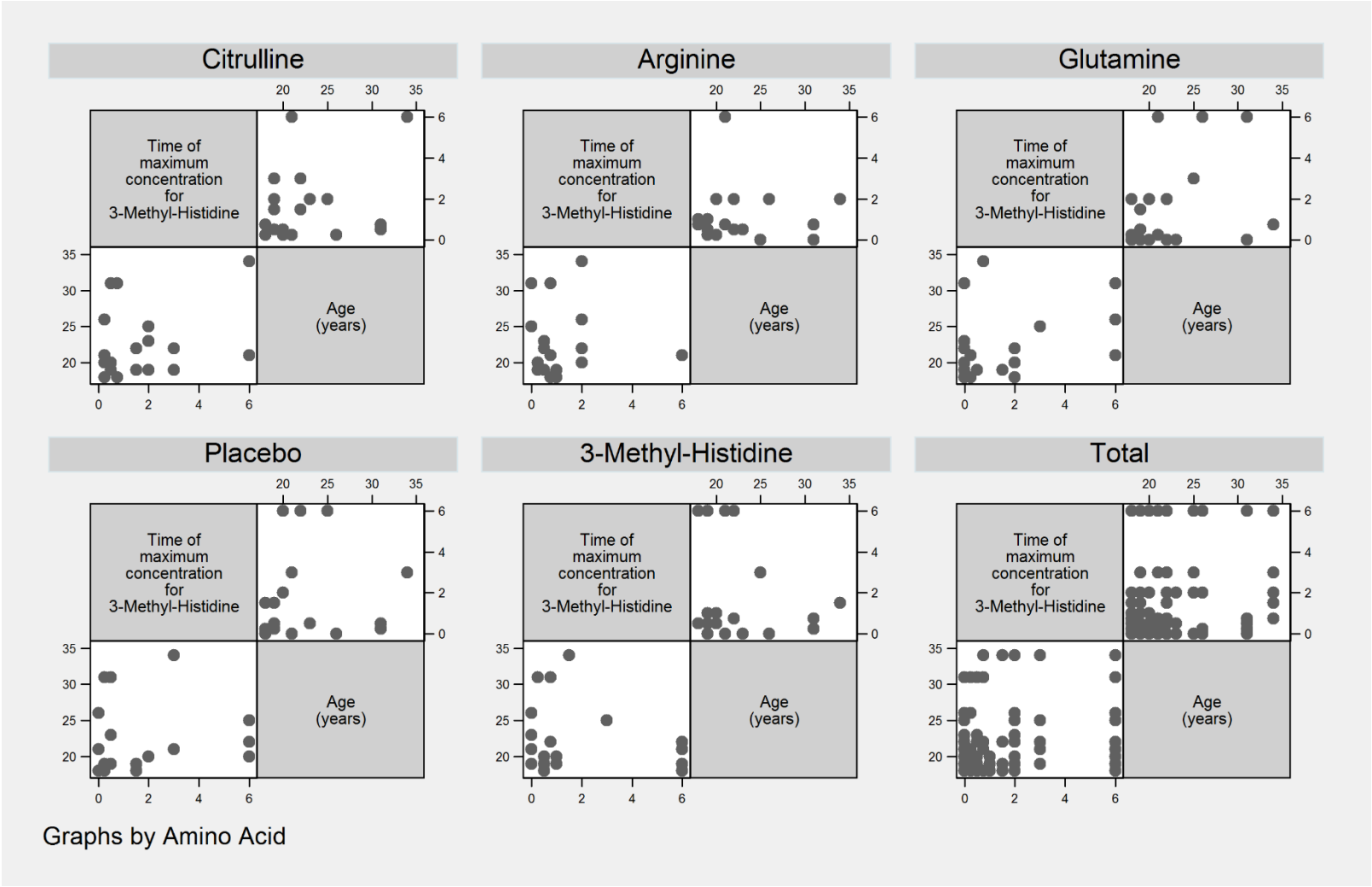


Figure C.22. Scatterplot of 3-methylhistidine TOMC with age by treatment and in total. No differences noted.

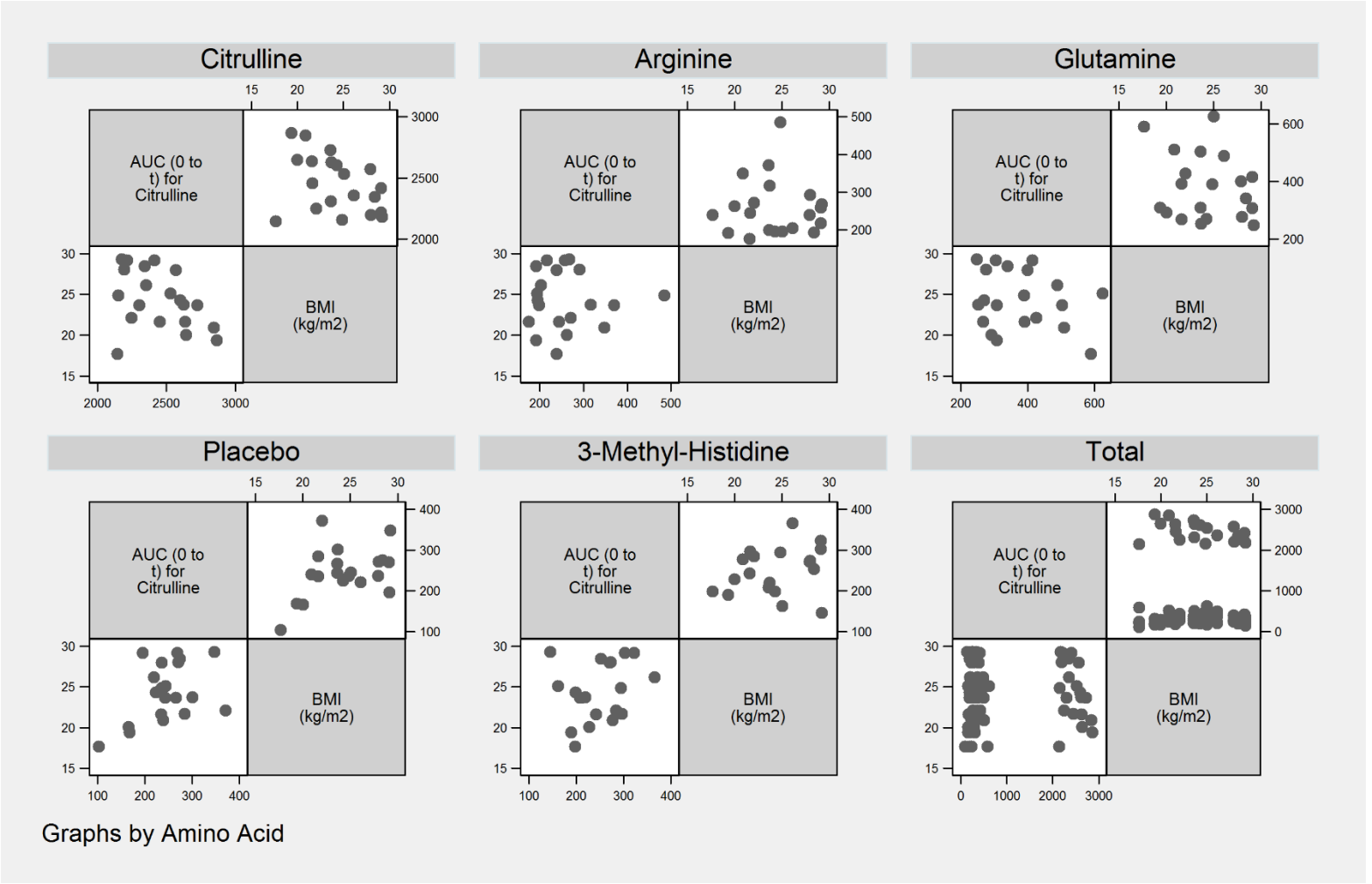


Figure C.23. Scatterplot of citrulline AUC with BMI by treatment and in total. No differences noted.

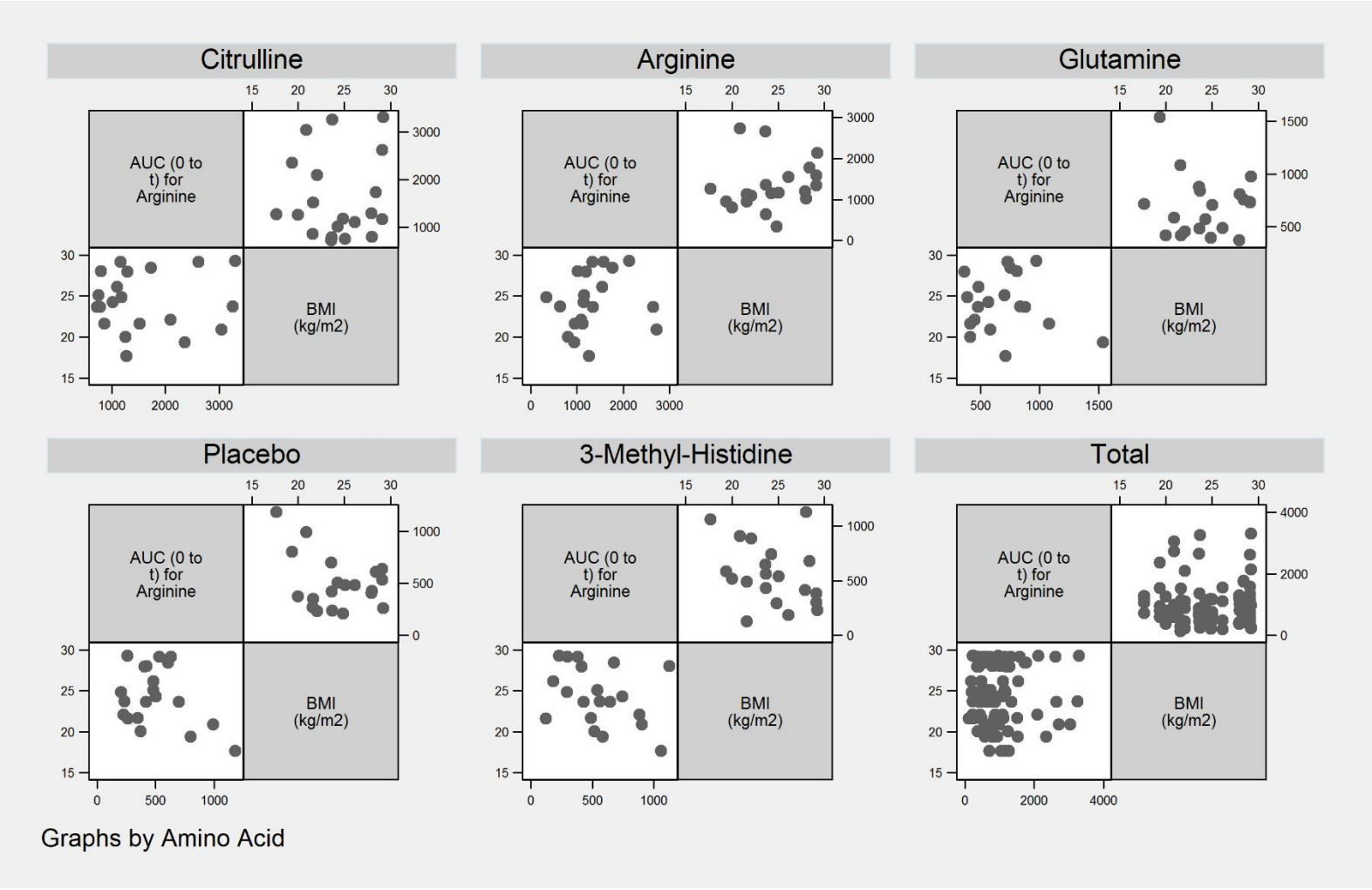


Figure C.24. Scatterplot of arginine AUC with BMI by treatment and in total. No differences noted.

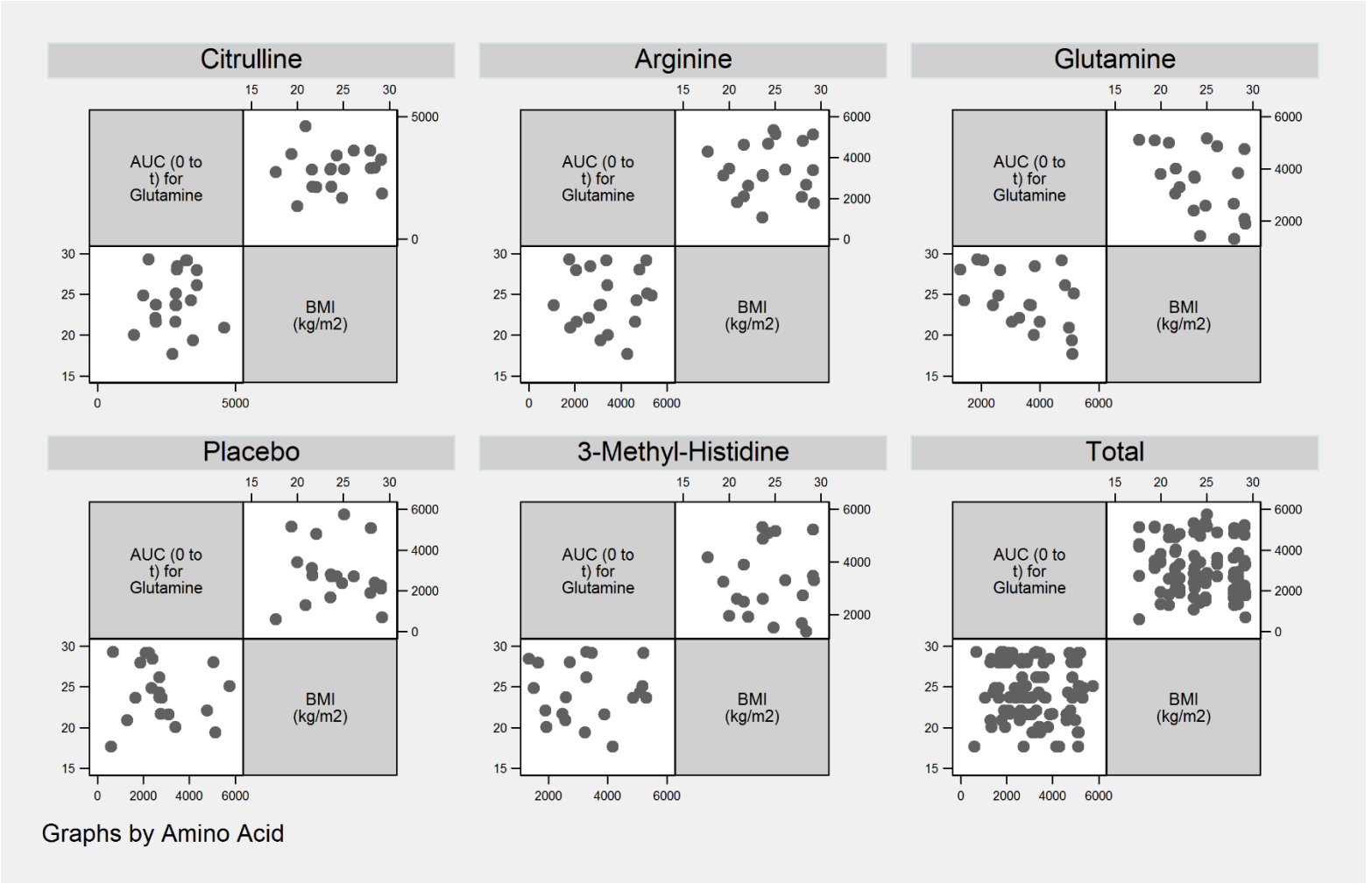


Figure C.25. Scatterplot of glutamine AUC with BMI by treatment and in total. No differences noted.

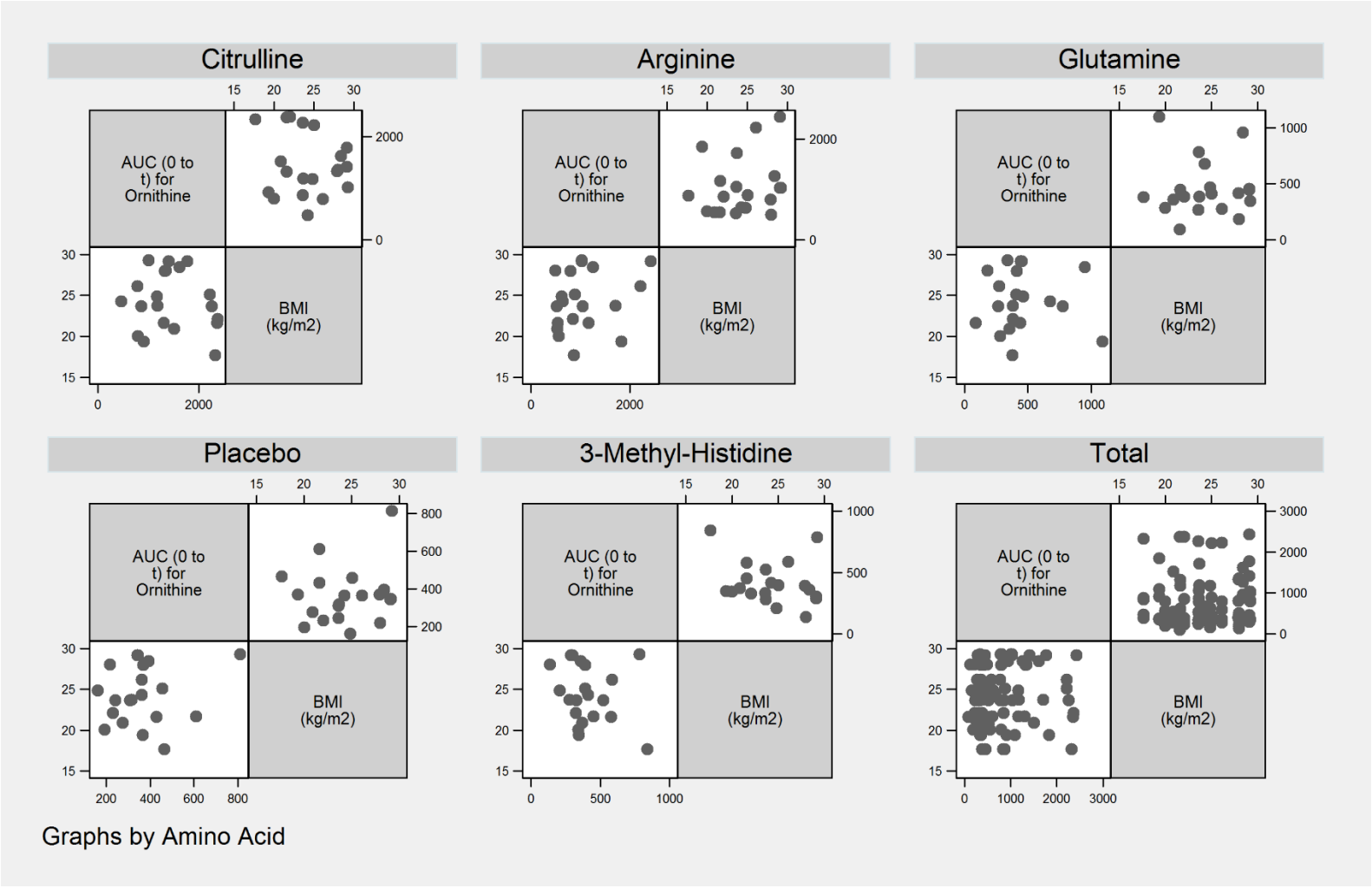


Figure C.26. Scatterplot of ornithine AUC with age by BMI and in total. No differences noted.

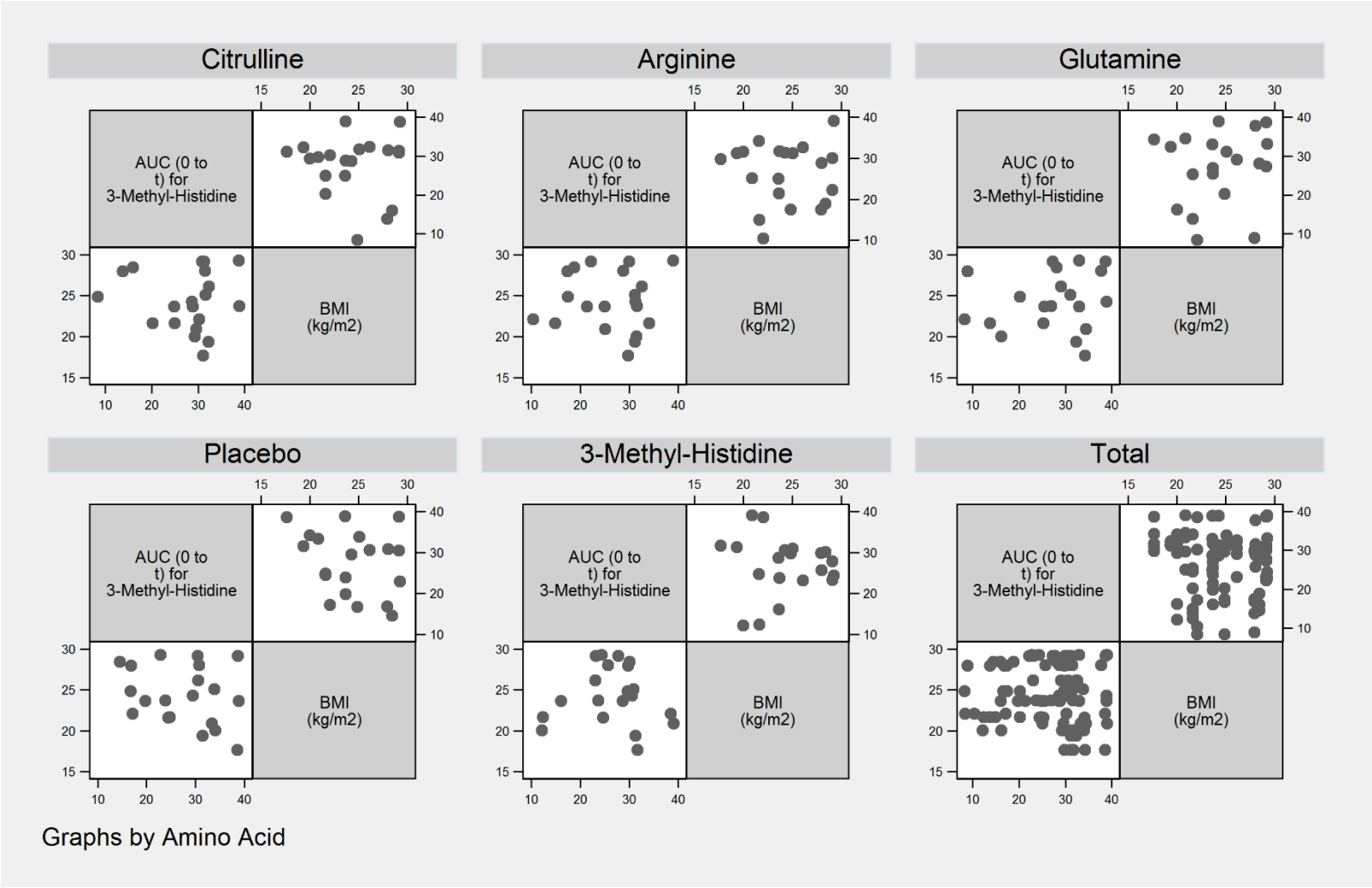


Figure C.27. Scatterplot of 3-methylhistidine AUC with BMI by treatment and in total. No differences noted.

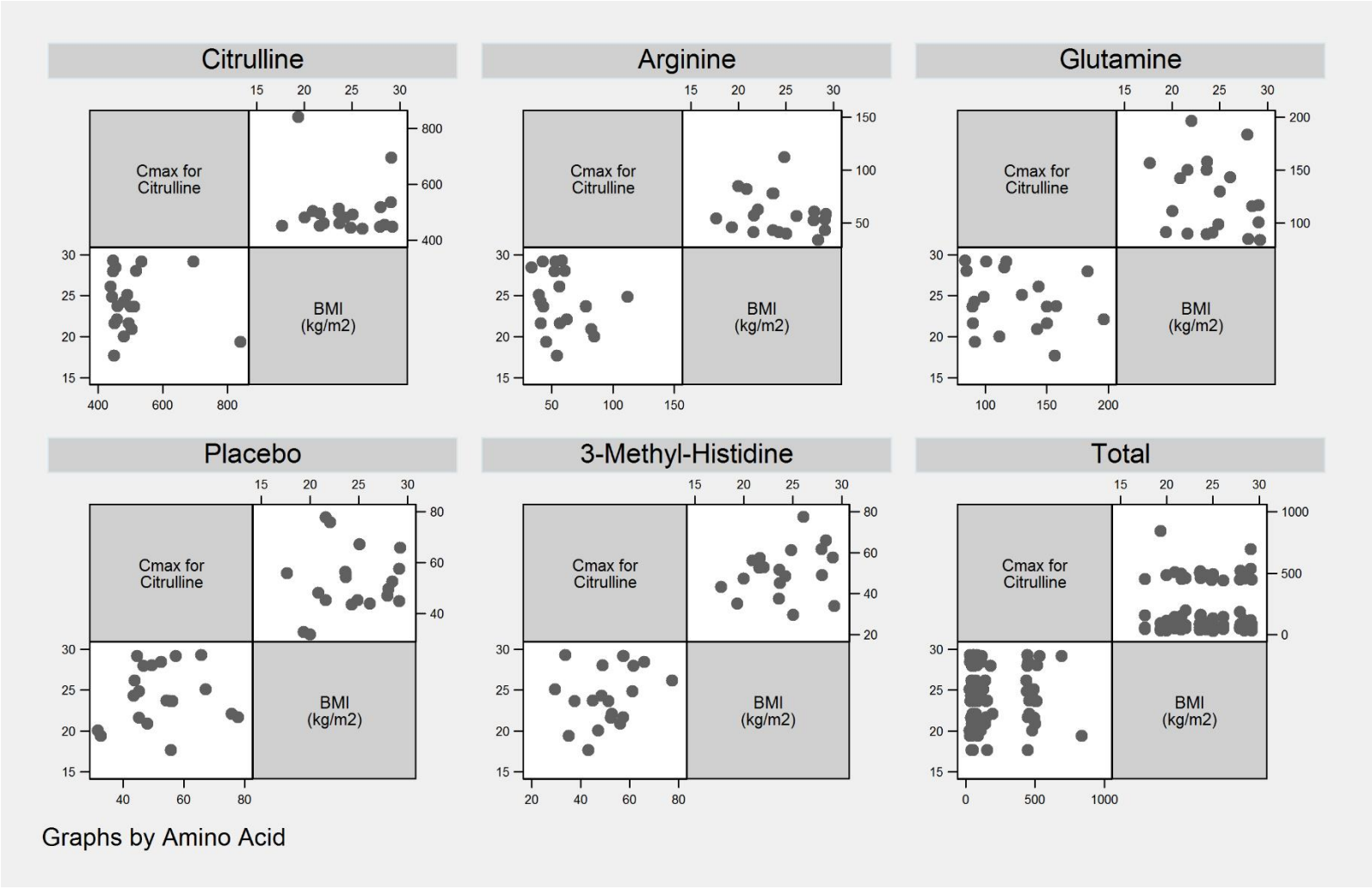


Figure C.28. Scatterplot of citrulline C_{max} with BMI by treatment and in total. No differences noted.

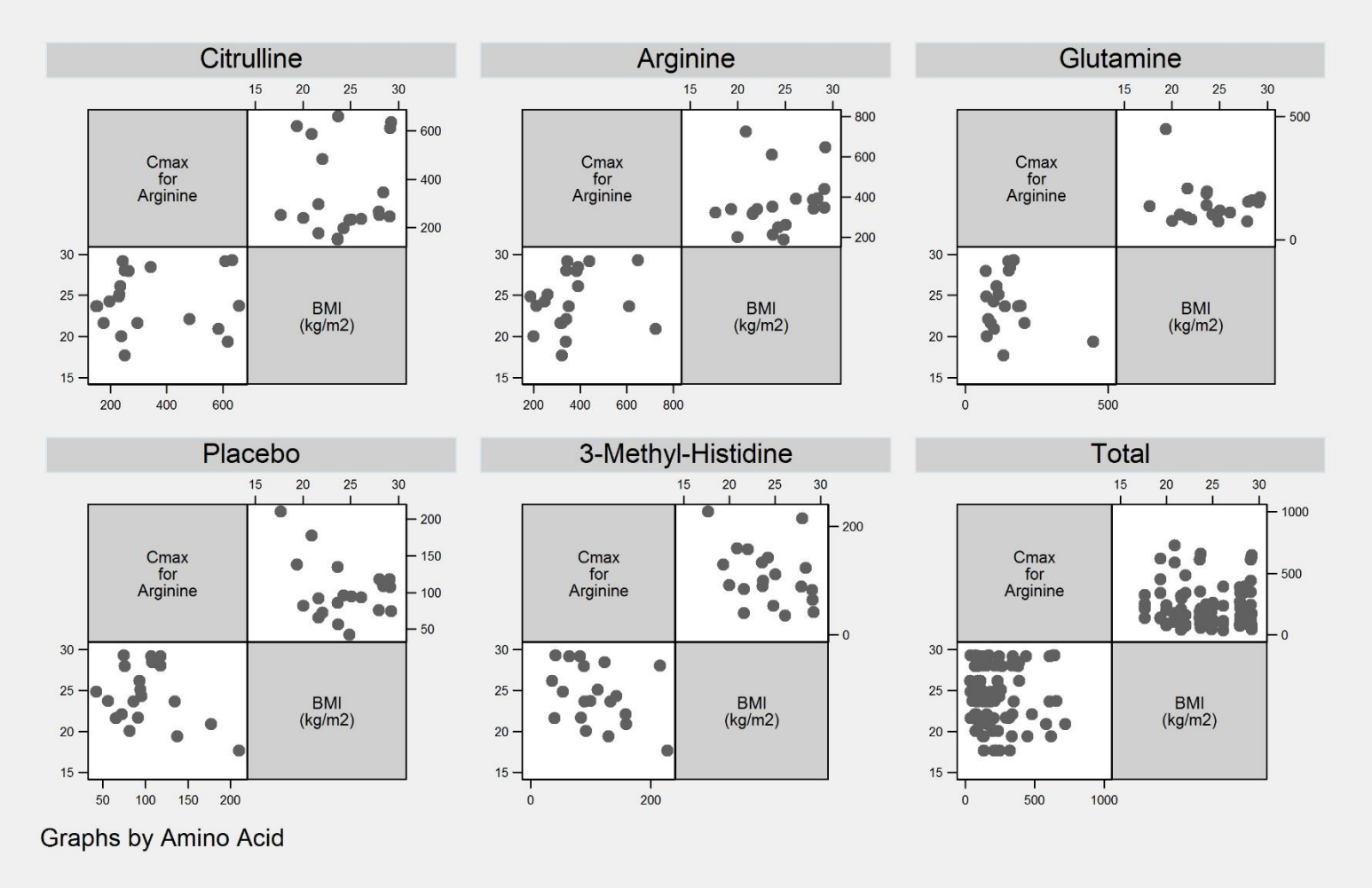


Figure C.29. Scatterplot of arginine C_{max} with BMI by treatment and in total. No differences noted.

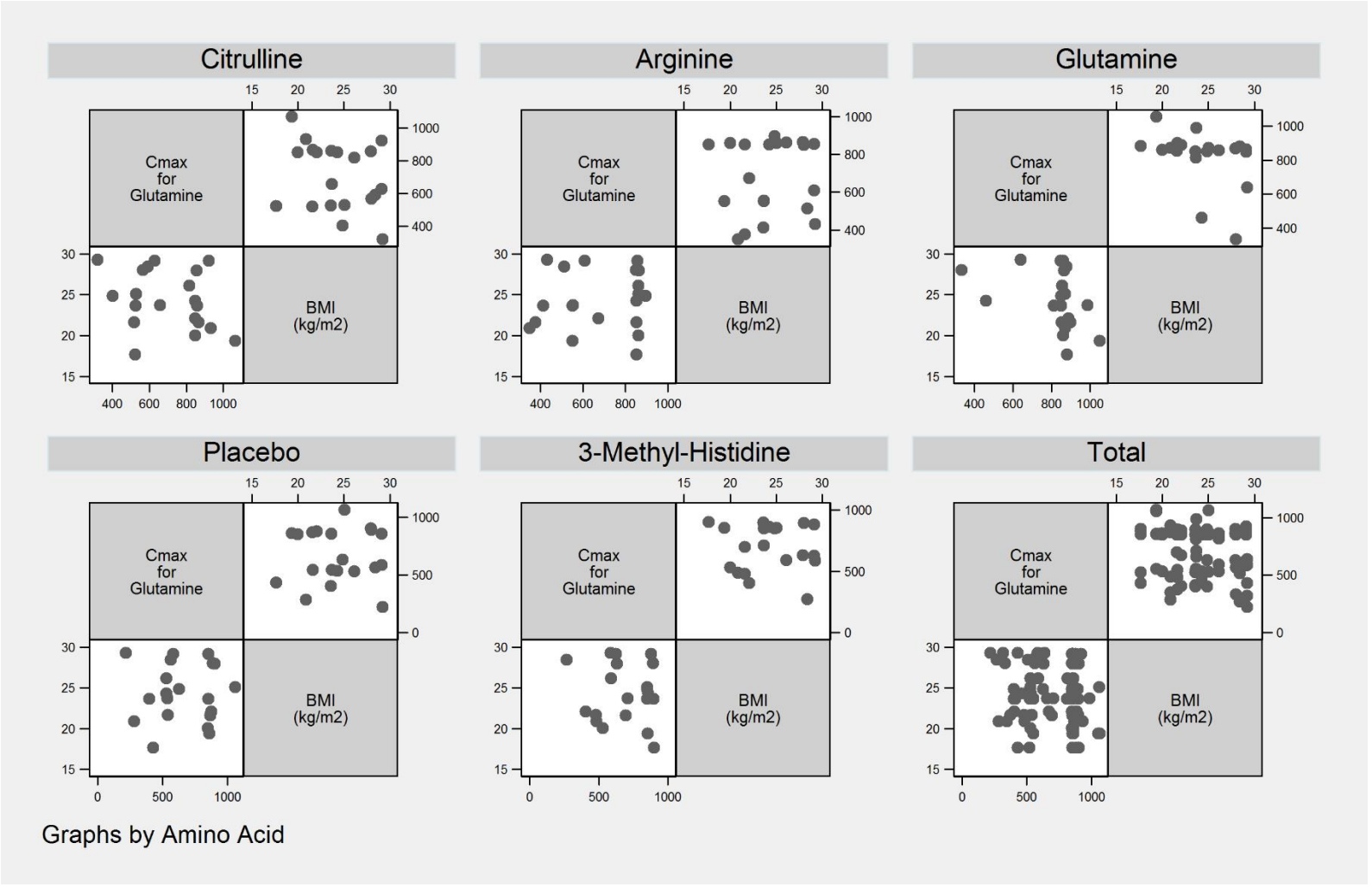


Figure C.30. Scatterplot of glutamine C_{max} with BMI by treatment and in total. No differences noted.

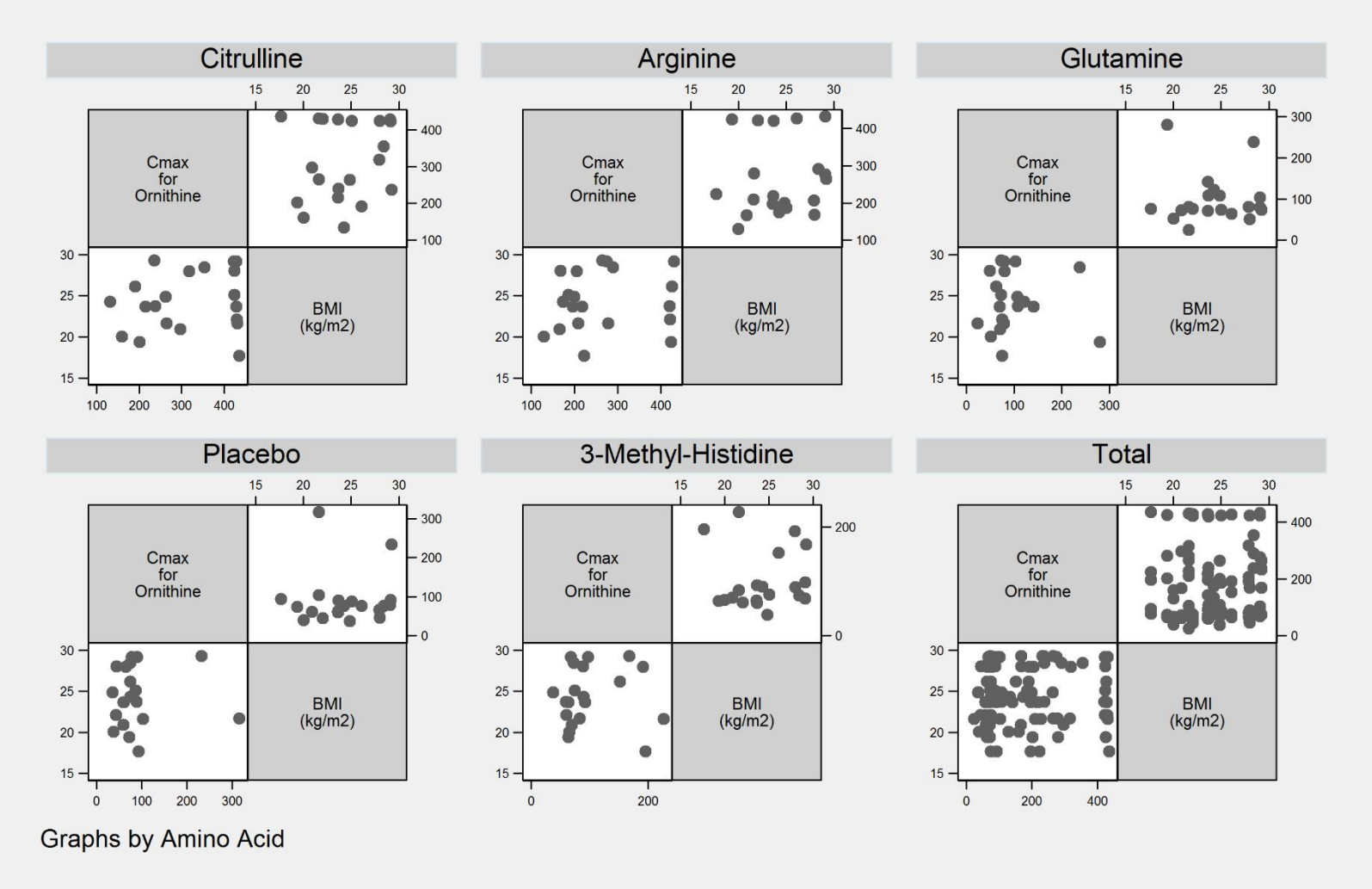


Figure C.31. Scatterplot of ornithine C_{max} with BMI by treatment and in total. No differences noted.

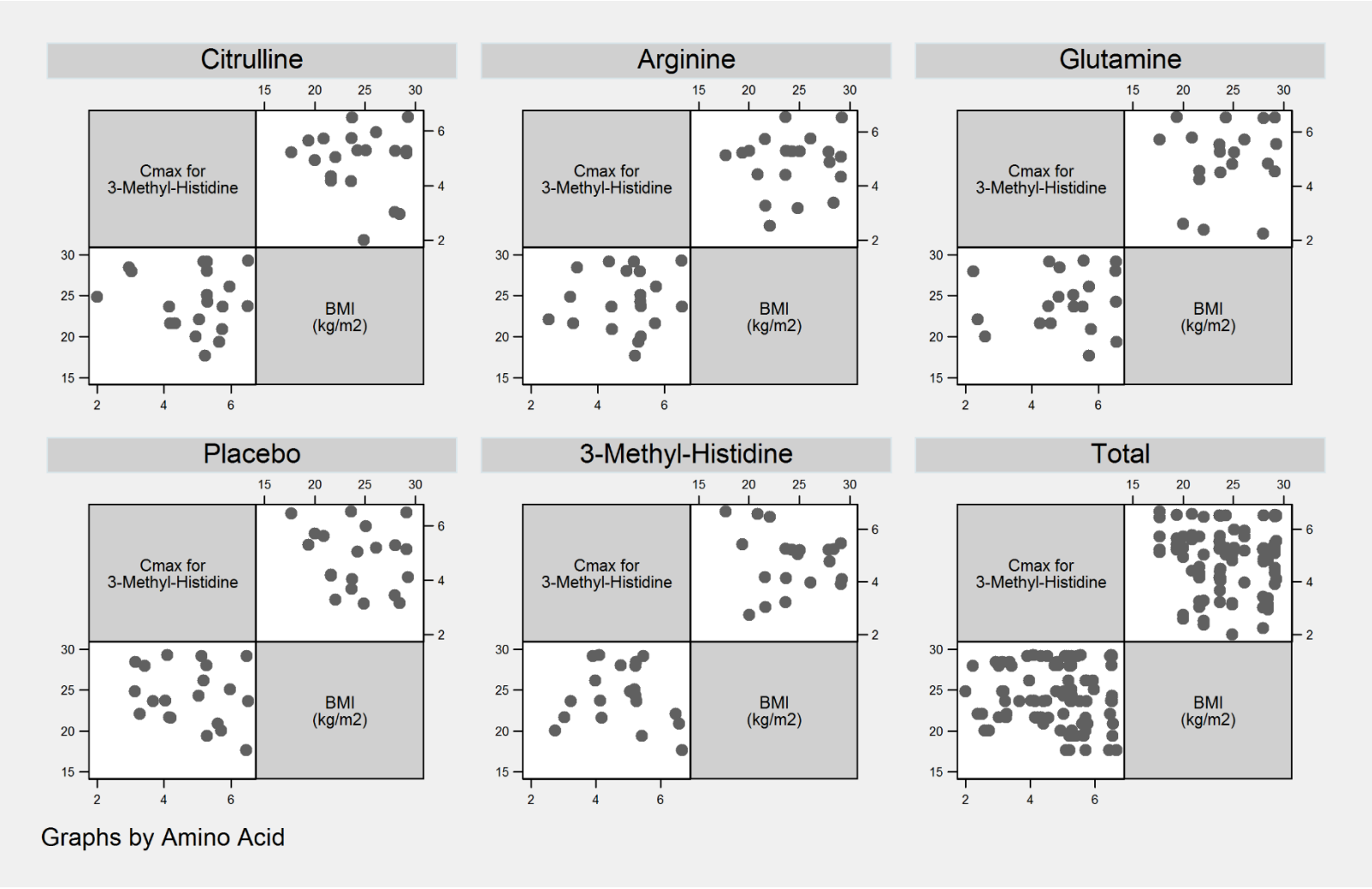


Figure C.32. Scatterplot of 3-methylhistidine C_{max} with BMI by treatment and in total. No differences noted.

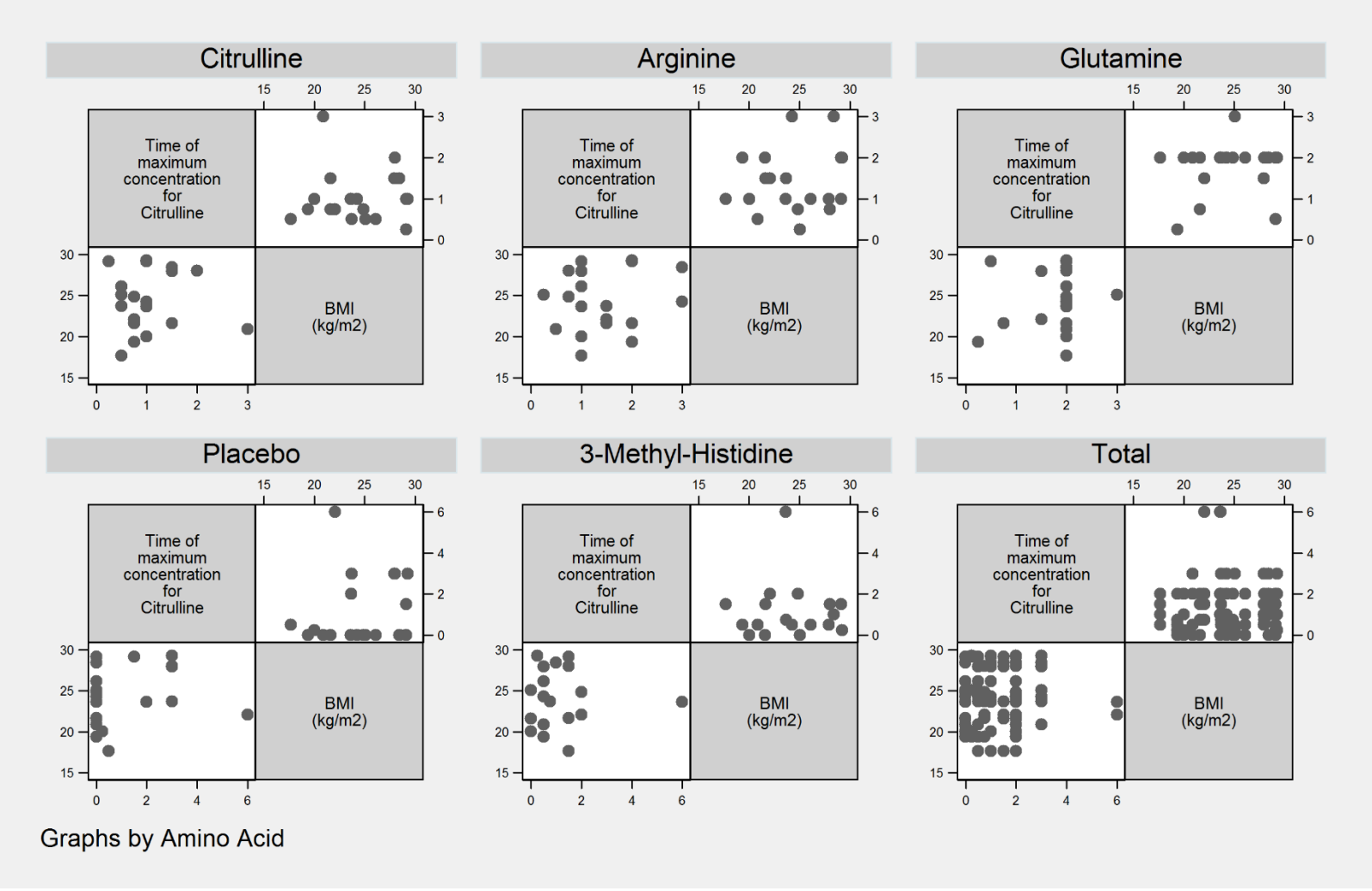


Figure C.33. Scatterplot of citrulline TOMC with BMI by treatment and in total. No differences noted.

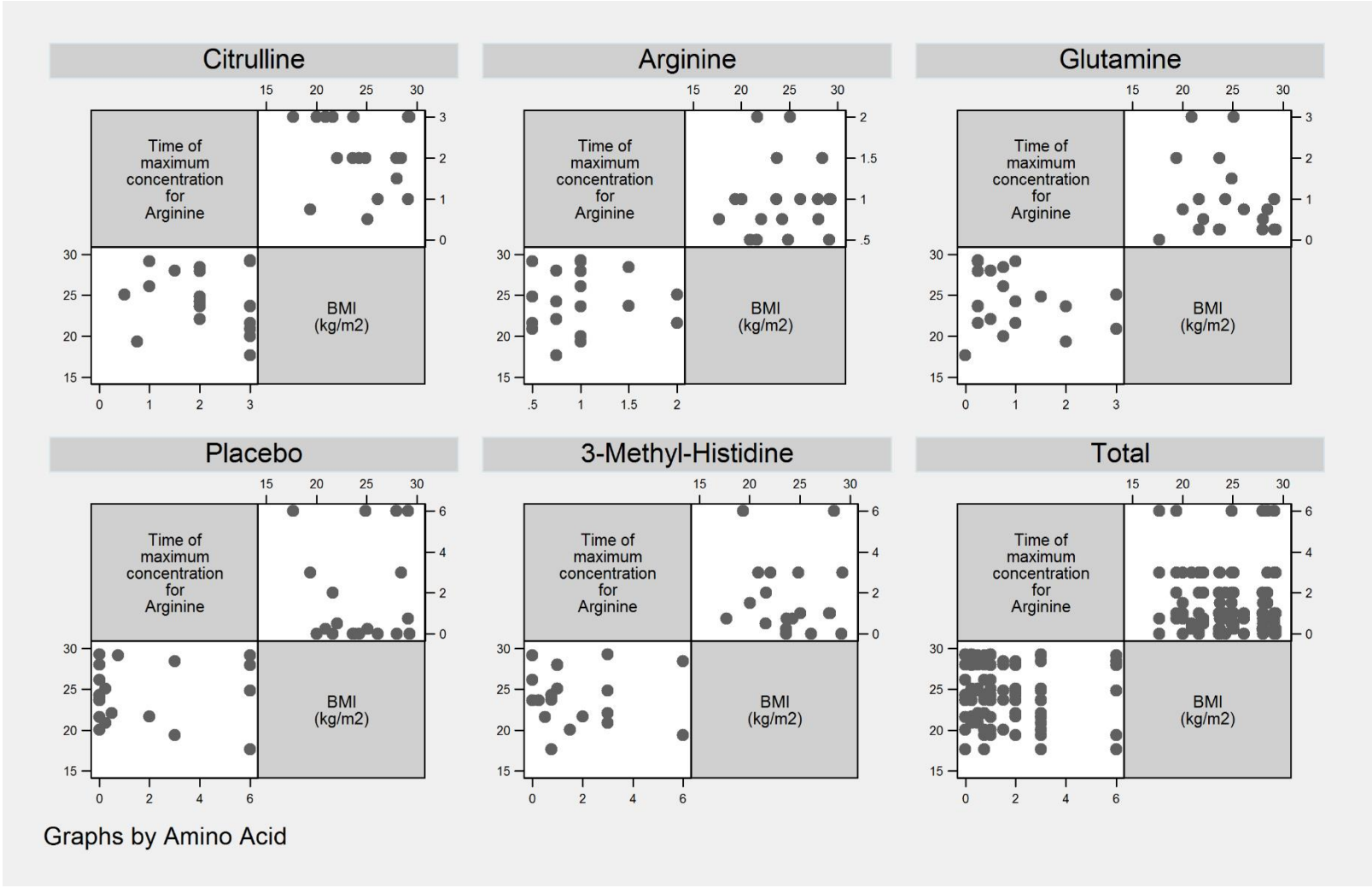


Figure C.34. Scatterplot of arginine TOMC with BMI by treatment and in total. No differences noted.

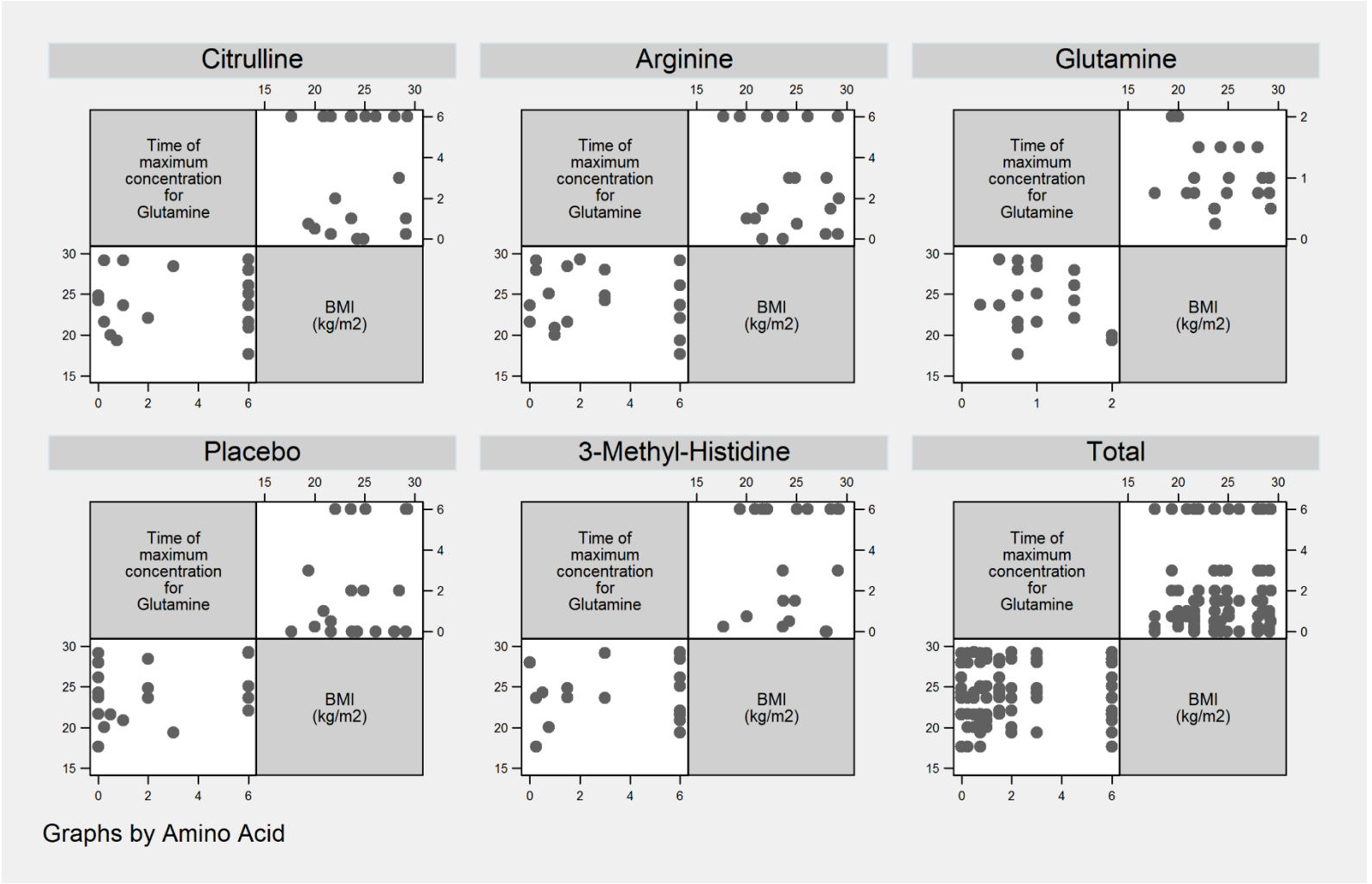


Figure C.35. Scatterplot of glutamine TOMC with BMI by treatment and in total. No differences noted.

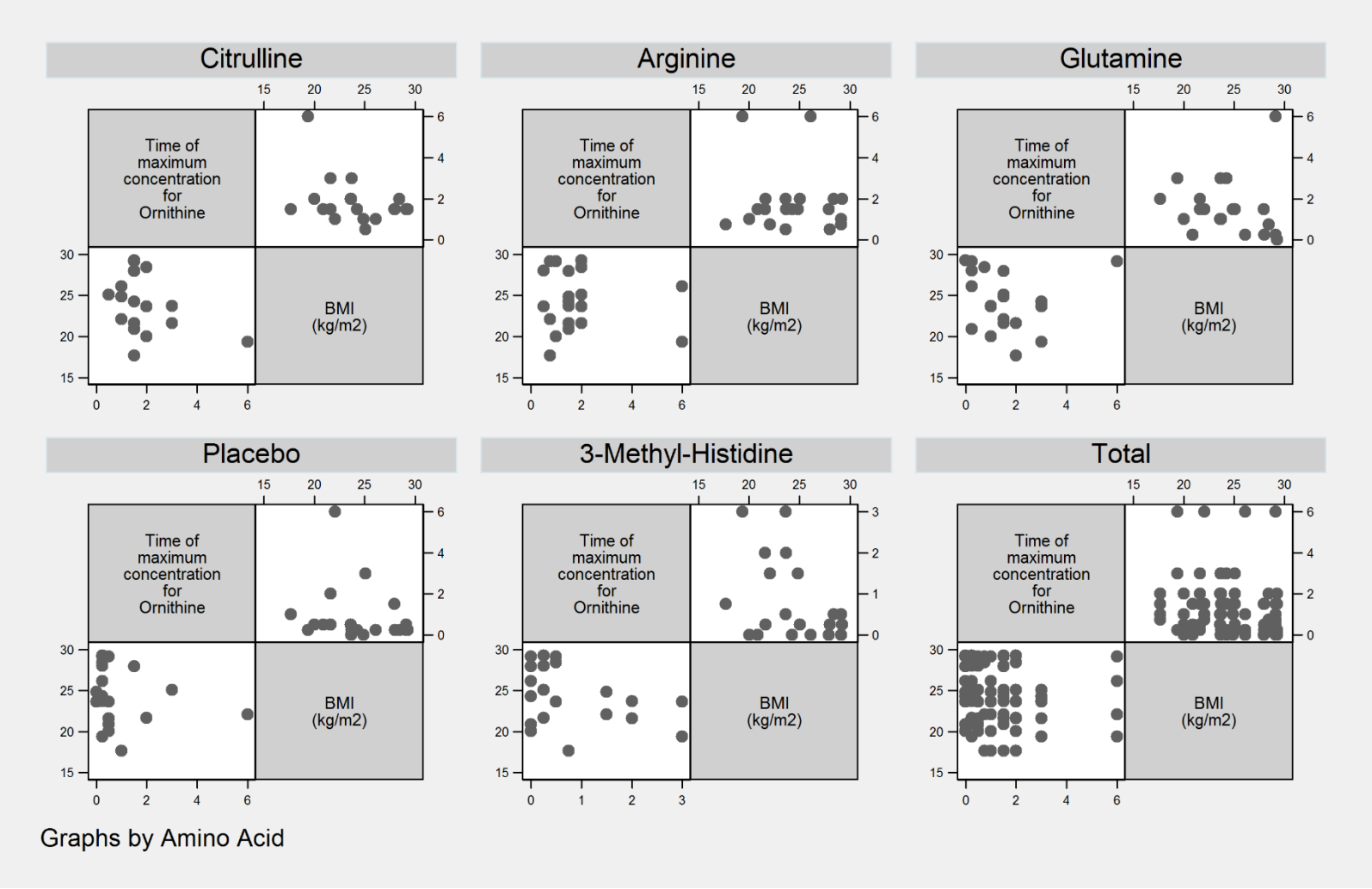


Figure C.36. Scatterplot of ornithine TOMC with BMI by treatment and in total. No differences noted.

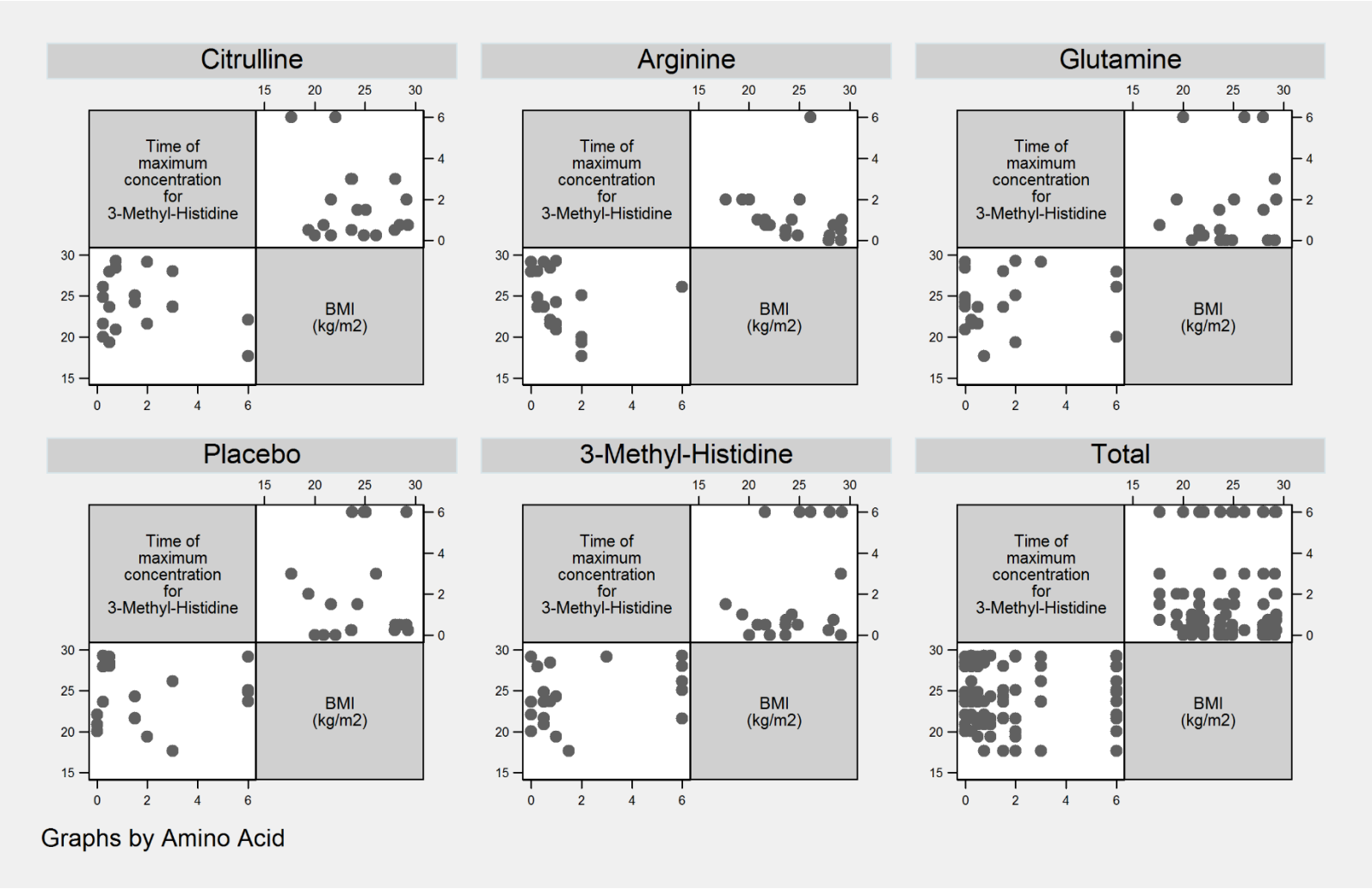


Figure C.37. Scatterplot of 3-methylhistidine TOMC with BMI by treatment and in total. No differences noted.

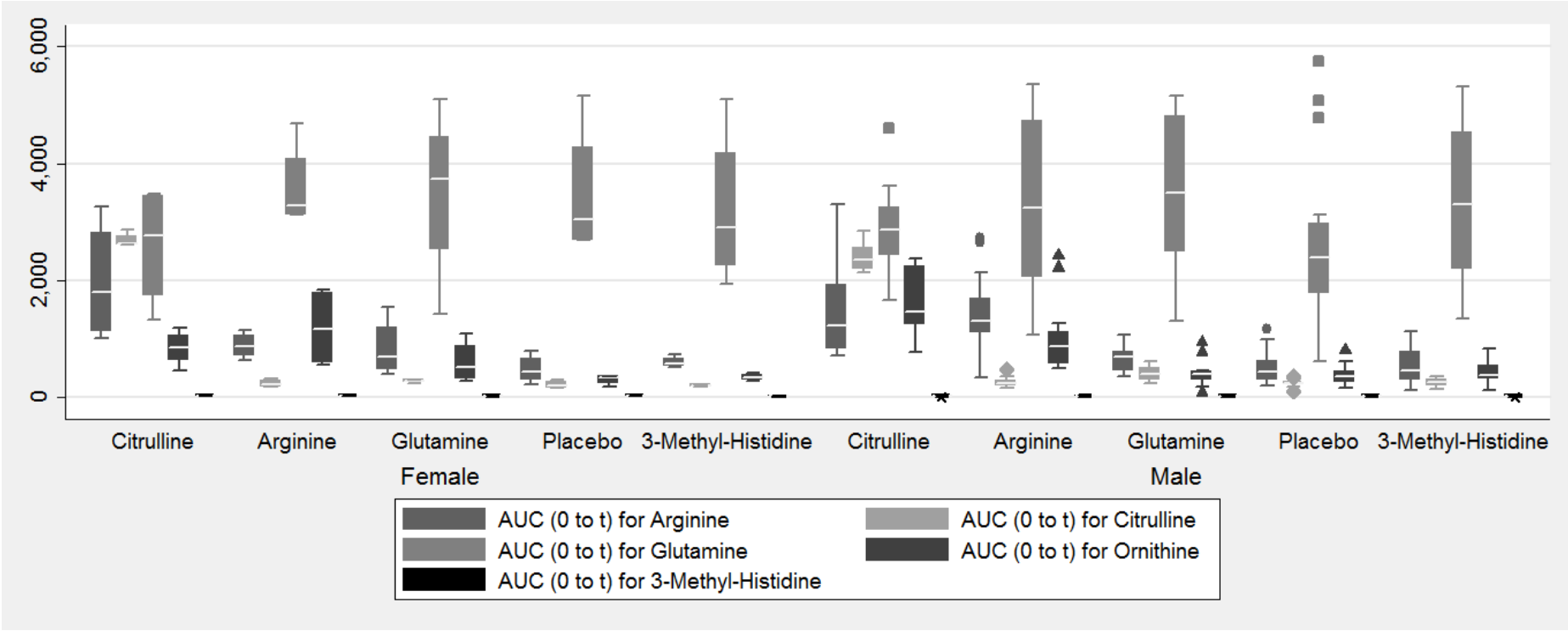


Figure C.38. Boxplot of AUC for plasma amino acids by treatment and gender. No differences noted.

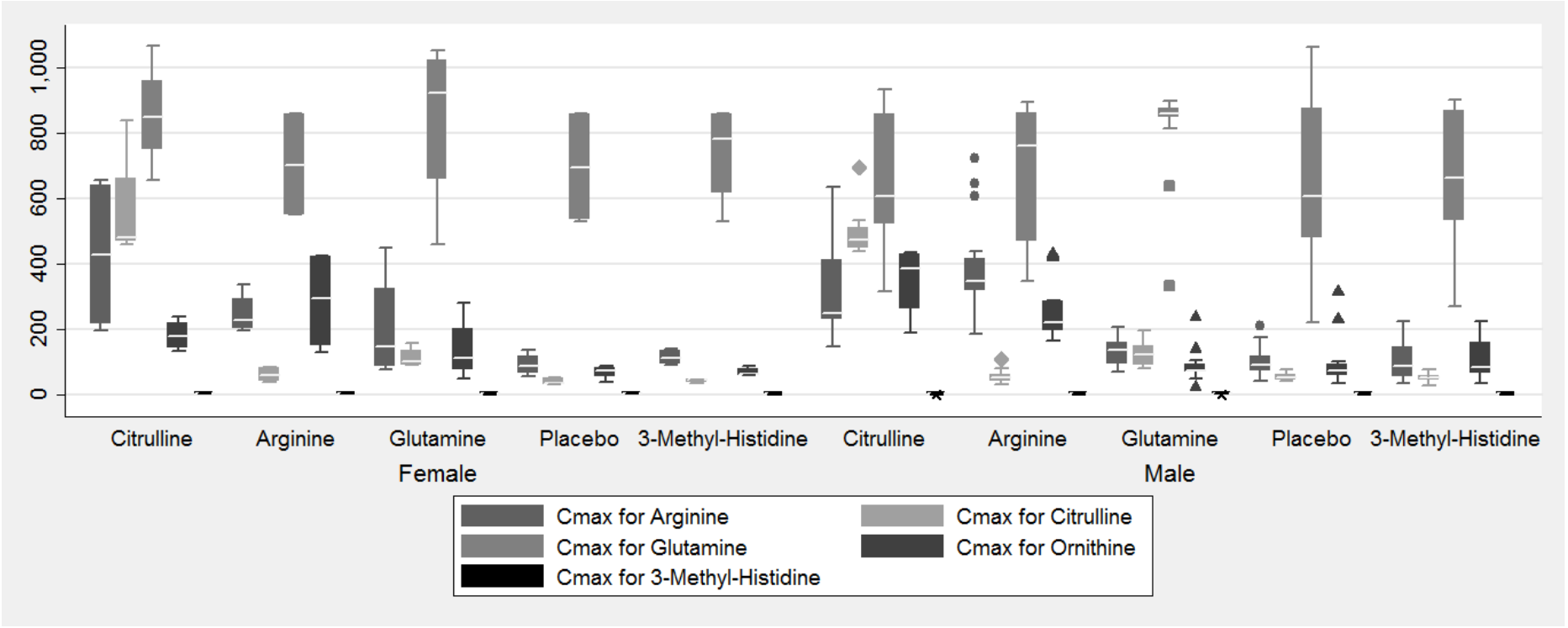


Figure C.39. Boxplot of C_{max} for plasma amino acids by treatment and gender. No differences noted.

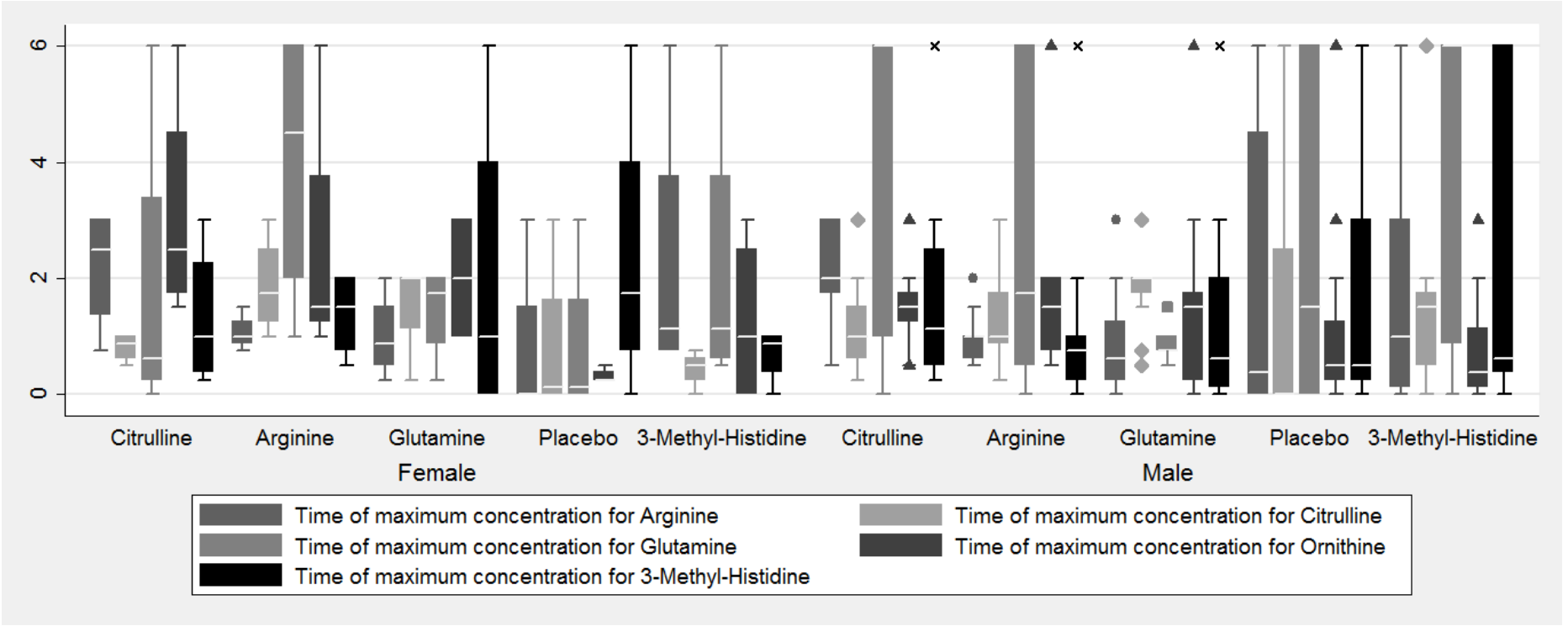


Figure C.40. Boxplot of TOMC for plasma amino acids by treatment and gender. No differences noted.

Appendix D

Other Important Bibliography on Citrulline

D.1 Other Citrulline Human Studies

These studies are simply cited here with no further explanation. They mainly involve physiological studies of citrulline administration and consequent use by the human body as well as other clinical condition not related to the intestine (Felig *et al.*, 1973; Rajantie *et al.*, 1980a; Rajantie *et al.*, 1980b; Yajima *et al.*, 1982; Largillière *et al.*, 1989; Tuchman, 1989; Castillo *et al.*, 1995; Yu *et al.*, 1995; Castillo *et al.*, 1996; Wu *et al.*, 2004; Mandel *et al.*, 2005; Moinard *et al.*, 2005; Naini *et al.*, 2005; Fujita and Yanaga, 2007; Ligthart-Melis *et al.*, 2007; Rougé *et al.*, 2007; Van De Poll *et al.*, 2007a; Van De Poll *et al.*, 2007b; Wiese *et al.*, 2008; Demacker *et al.*, 2009a; Demacker *et al.*, 2009b; Figueroa *et al.*, 2009; Sureda *et al.*, 2009; Bourdon *et al.*, 2010; Hozyasz *et al.*, 2010; Mao *et al.*, 2010; Perez-Guisado and Jakeman, 2010; Goossens *et al.*, 2011; Shin *et al.*, 2011; Squellerio *et al.*, 2011; Thibault *et al.*, 2011; Bourdon *et al.*, 2012; Wessells *et al.*, 2013; van Wijck *et al.*, 2014; Vidal-Casariego *et al.*, 2014; Blasco-Alonso *et al.*, 2015).

D.2 Reviews, Editorials, Commentaries and Letters to the Editor

There have been quite a few interesting reviews over the years regarding citrulline and metabolism, clinical significance, biochemistry; there have also been interesting reviews about many of the topics touched in the present thesis that might not have been cited but are very useful for a bibliography (Cynober, 1994; Levin, 1994; Nightingale, 1994; Shanbhogue and Molenaar, 1994; Bjarnason *et al.*, 1995; Cynober *et al.*, 1995; Rabier and Kamoun, 1995; Wu, 1998; Fürst, 2000; Kornberg, 2000; Fürst, 2001; Goodlad *et al.*, 2001; Mithieux, 2001; Nightingale, 2001; Cynober, 2002; 2003; Alteheld *et al.*, 2003; Boelens and van Leeuwen, 2003; Boirie *et al.*, 2003; Buchman *et al.*, 2003; Calder and Yaqoob, 2003; Cano, 2003; Collin and Vapaatalo, 2003; Cynober, 2003; Daignault *et al.*, 2003; Darmaun and Cynober, 2003; De Bandt, 2003; Dejong *et al.*, 2003; Dhatariya and Sreekumaran Nair, 2003; Foulks, 2003; Fürst and Stehle, 2003; Ganapathy *et al.*, 2003; Gleeson and Jeukendrup, 2003; Grimble, 2003; Heyland *et al.*, 2003; Høime Hansen, 2003; Holeček, 2003; Hue and Bertrand, 2003; Kinney, 2003; Leverve, 2003; Mackenzie

and Baracos, 2003; Mahoney and Albina, 2003; Malaisse, 2003; Meijer, 2003; Meijer and Dubbelhuis, 2003; Muscaritoli *et al.*, 2003; Neveux *et al.*, 2003; Nightingale, 2003b; Nightingale, 2003a; Nissim and Welbourne, 2003; Obled *et al.*, 2003; Oehler and Roth, 2003; Patureau Mirand *et al.*, 2003; Radrizzani *et al.*, 2003; Rassin, 2003; Sacks and Kudsk, 2003; Selvaggi *et al.*, 2003; Soeters *et al.*, 2003; vom Dahl and Häussinger, 2003; Wagenmakers, 2003; Wakabayashi, 2003; Walrand and Boirie, 2003; Wernerman, 2003; Wu and Morris, 2003; Young and Tharakan, 2003; Abumrad and Barbul, 2004; Brosnan and Brosnan, 2004; Keller *et al.*, 2004; Blijlevens, 2005; Curis *et al.*, 2005; Middleton and Jamieson, 2005; Goulet and Sauvat, 2006; Lin and Stoll, 2006; Nightingale and Woodward, 2006; Romero *et al.*, 2006; Curis *et al.*, 2007; Lutgens and Lambin, 2007; Moinard and Cynober, 2007; Schiller, 2007; Crenn, 2008; Crenn *et al.*, 2008; Cynober *et al.*, 2008; Deutz, 2008; Goulet *et al.*, 2008; Peters *et al.*, 2008a; Lankisch, 2009; Schulzke *et al.*, 2009; Tooley *et al.*, 2009; Wallis *et al.*, 2009; Wu *et al.*, 2009; Crenn and Annane, 2010; Crenn and Cynober, 2010; Cynober *et al.*, 2010; Keur *et al.*, 2010; Oliverius *et al.*, 2010; Crenn *et al.*, 2011; Fragkos and Forbes, 2011b, a; Hurt *et al.*, 2011; Ligthart-Melis and Deutz, 2011; Neu and Walker, 2011; Peters *et al.*, 2011; Peterson and Kerner, 2012; Cynober, 2013; Kaore *et al.*, 2013; Barzal *et al.*, 2014; Kaore and Kaore, 2014; Vaira, 2014; Walker, 2014; Breuillard *et al.*, 2015; Piton and Capellier, 2015).

Appendix E

Stata Code

```
**This is the analysis strategy for pharmacokinetic dataof Chapter 5
*********************************************************************
***1. Analyse amino acids by treatment by doing spaghetti plots for each
amino acid concentration and then by subject.
***   1. PLOTS   ****************************************************
***Overall command to produce and save individual graphs
***Treatments `i': Cit==1; Arg==2; Gln==3; Placebo==4; 3MH==5.
forvalues i=1/5 {
clear all
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
***spaghetti plots
***Concentration plot
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
twoway (connected `x'_Concnmolml_1 time if treatment==`i', connect(L))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_tr`i'_con_`x'.png"
***Area Plot
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
twoway (connected `x'_Area_1 time if treatment==`i', connect(L))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_tr`i'_area_`x'.png"
***Mean plot by time
***Concentration plot
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
meansdplot   `x'_Concnmolml_1   time   if   treatment==`i',   ytitle(`x'
Concentration ({&mu}mol/l))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_tr`i'_mcon_`x'.png"
***Area Plot
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
meansdplot `x'_Area_1 time if treatment==`i', ytitle(`x' Area)
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_tr`i'_marea_`x'.png"
***plots by subjects
***Concentration plot
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
twoway  (connected  `x'_Concnmolml_1  time  if  treatment==`i',  connect(L)),
by(id)
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_tr`i'_subjcon_`x'.png"
***Area Plot
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
twoway (connected `x'_Area_1 time if treatment==`i', connect(L)), by(id)
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_tr`i'_subjarea_`x'.png"
}
}
*********************************************************************
*********************************************************************
*********************************************************************
```

```
*************************************************************************
****Alternative graph by subjects, where each subject has all five amino
acids on its graph
*************************************************************************
*************************************************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
twoway (connected Citrulline_Concnmolml_1 time if treatment==1, connect(L)
msymbol(O)) ///
(connected  Citrulline_Concnmolml_1  time  if  treatment==2,  connect(L)
msymbol(D) yaxis(2)) ///
(connected  Citrulline_Concnmolml_1  time  if  treatment==3,  connect(L)
msymbol(T) yaxis(2)) ///
(connected  Citrulline_Concnmolml_1  time  if  treatment==4,  connect(L)
msymbol(S) yaxis(2)) ///
(connected  Citrulline_Concnmolml_1  time  if  treatment==5,  connect(L)
msymbol(Oh) yaxis(2)), ///
by(id, legend(position(6))) ///
legend(title("Amino  acid  ingested",  size(vsmall))  label(1  "Citrulline")
label(2 "Arginine") label(3 "Glutamine") ///
label(4 "Placebo") label(5 "3MH") rows(1) size(vsmall))
*************************************************************************
*************************************************************************
***Overall command to produce and save graphs combine in three rows, one
column*************************
*************************************************************************
*****Treatment ==1: Citrulline*****************************************
*************************************************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
twoway  (connected  `x'_Concnmolml_1  time  if  treatment==1,  connect(L)),
name(`x'_1_1) title("Spaghetti Plot")
meansdplot   `x'_Concnmolml_1   time   if   treatment==1,   ytitle("`x'
Concentration ({&mu}mol/l)") name(`x'_1_2) title ("Means Plot")
twoway (connected `x'_Concnmolml_1 time if treatment==1, connect(L)), by(id,
title("Concentrations by Subject")) name(`x'_1_3)
graph combine `x'_1_1 `x'_1_2 `x'_1_3, rows(3) cols(1) ysize(20) xsize(7)
title("`x'  Concentrations  post  ingestion  of  Citrulline",  size(large))
name(tr1_`x')
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014      analysis\\`c(current_date)'_Conc_tr1_`x'.tif",      width(1809)
height(2800)
}
*************************************************************************
*** COMBINATION OF ALL GRAPHS INTO ONE GRAPH  ***********************
*** graph combine tr1_Citrulline tr1_Arginine tr1_Glutamine tr1_Ornithine
tr1_A_3MH, rows(1) cols(5) ysize(10) xsize(20)
*****************************
*****************************
*****Treatment ==2: Arginine**********************
*************************************************************************
***********
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
twoway  (connected  `x'_Concnmolml_1  time  if  treatment==2,  connect(L)),
name(`x'_2_1) title("Spaghetti Plot")
meansdplot   `x'_Concnmolml_1   time   if   treatment==2,   ytitle("`x'
Concentration ({&mu}mol/l)") name(`x'_2_2) title ("Means Plot")
twoway (connected `x'_Concnmolml_1 time if treatment==2, connect(L)), by(id,
title("Concentrations by Subject")) name(`x'_2_3)
graph combine `x'_2_1 `x'_2_2 `x'_2_3, rows(3) cols(1) ysize(20) xsize(7)
title("`x'  Concentrations  post  ingestion  of  Arginine",  size(large))
name(tr2_`x')
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014      analysis\\`c(current_date)'_Conc_tr2_`x'.tif",      width(1809)
height(2800)
}
```

```
**************************
*************************************************************************
*** COMBINATION OF ALL GRAPHS INTO ONE GRAPH  ************************
*** graph combine tr2_Citrulline tr2_Arginine tr2_Glutamine tr2_Ornithine
tr2_A_3MH, rows(1) cols(5) ysize(10) xsize(20)
*****************************************************
*****Treatment ==3: Glutamine**********************
*************************************************************************
***********
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
twoway (connected `x'_Concnmolml_1 time if treatment==3, connect(L)),
name(`x'_3_1) title("Spaghetti Plot")
meansdplot `x'_Concnmolml_1 time if treatment==3, ytitle("`x'
Concentration ({&mu}mol/l)") name(`x'_3_2) title ("Means Plot")
twoway (connected `x'_Concnmolml_1 time if treatment==3, connect(L)), by(id,
title("Concentrations by Subject")) name(`x'_3_3)
graph combine `x'_3_1 `x'_3_2 `x'_3_3, rows(3) cols(1) ysize(20) xsize(7)
title("`x' Concentrations post ingestion of Glutamine", size(large))
name(tr3_`x')
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_Conc_tr3_`x'.tif", width(1809)
height(2800)
}
**************************
*** COMBINATION OF ALL GRAPHS INTO ONE GRAPH  ************************
*** graph combine tr3_Citrulline tr3_Arginine tr3_Glutamine tr3_Ornithine
tr3_A_3MH, rows(1) cols(5) ysize(10) xsize(20)
*************************************************************************
***********
*****Treatment ==4: Placebo**********************
*************************************************************************
***********
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
twoway (connected `x'_Concnmolml_1 time if treatment==4, connect(L)),
name(`x'_4_1) title("Spaghetti Plot")
meansdplot `x'_Concnmolml_1 time if treatment==4, ytitle("`x'
Concentration ({&mu}mol/l)") name(`x'_4_2) title ("Means Plot")
twoway (connected `x'_Concnmolml_1 time if treatment==4, connect(L)), by(id,
title("Concentrations by Subject")) name(`x'_4_3)
graph combine `x'_4_1 `x'_4_2 `x'_4_3, rows(3) cols(1) ysize(20) xsize(7)
title("`x' Concentrations post ingestion of Placebo", size(large))
name(tr4_`x')
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_Conc_tr4_`x'.tif", width(1809)
height(2800)
}
**************************
*** COMBINATION OF ALL GRAPHS INTO ONE GRAPH  ************************
*** graph combine tr4_Citrulline tr4_Arginine tr4_Glutamine tr4_Ornithine
tr4_A_3MH, rows(1) cols(5) ysize(10) xsize(20)
*****************************************************
**************************
*****Treatment ==5: 3MH**********************
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
twoway (connected `x'_Concnmolml_1 time if treatment==5, connect(L)),
name(`x'_5_1) title("Spaghetti Plot")
meansdplot `x'_Concnmolml_1 time if treatment==5, ytitle("`x'
Concentration ({&mu}mol/l)") name(`x'_5_2) title ("Means Plot")
twoway (connected `x'_Concnmolml_1 time if treatment==5, connect(L)), by(id,
title("Concentrations by Subject")) name(`x'_5_3)
graph combine `x'_5_1 `x'_5_2 `x'_5_3, rows(3) cols(1) ysize(20) xsize(7)
title("`x' Concentrations post ingestion of 3-Methyl-histidine",
size(large)) name(tr5_`x')
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_Conc_tr5_`x'.tif", width(1809)
height(2800)
```

```
}
**********************************************************************
*** COMBINATION OF ALL GRAPHS INTO ONE GRAPH  ************************
*** graph combine tr5_Citrulline tr5_Arginine tr5_Glutamine tr5_Ornithine
tr5_A_3MH, rows(1) cols(5) ysize(10) xsize(20)
**********************************************************************
**********************************************************************
*** HISTOGRAMS AND COMBINATION OF ALL GRAPHS INTO ONE GRAPH
*******  AUC  and CMAX***************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
histogram auc_`x'_Concnmolml_1, percent normal name(`x'_auc)
histogram  cmax_`x'_Concnmolml_1, percent normal name(`x'_cmax)
}
**********************************AUC**********************************
graph  combine  Citrulline_auc  Arginine_auc  Glutamine_auc  Ornithine_auc
A_3MH_auc, rows(2) cols(3) title("Histograms for AUC", size(large))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_hist_auc.tif"
**********************************************************************
**********************************CMAX**********************************
graph combine Citrulline_cmax Arginine_cmax Glutamine_cmax Ornithine_cmax
A_3MH_cmax, rows(2) cols(3) title("Histograms for C{subscript:max}
", size(large))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_hist_cmax.tif"
**********************************************************************
***************BOXPLOTS OF AUC, CMAX AND TOMC IN ONE GRAPH
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph    box    auc_Arginine_Concnmolml_1    auc_Citrulline_Concnmolml_1
auc_Glutamine_Concnmolml_1                    auc_Ornithine_Concnmolml_1
auc_A_3MH_Concnmolml_1, over(treatment) name(boxAUC)
graph    box    cmax_Arginine_Concnmolml_1    cmax_Citrulline_Concnmolml_1
cmax_Glutamine_Concnmolml_1                   cmax_Ornithine_Concnmolml_1
cmax_A_3MH_Concnmolml_1, over(treatment) name(boxCMAX)
graph    box    tomc_Arginine_Concnmolml_1    tomc_Citrulline_Concnmolml_1
tomc_Glutamine_Concnmolml_1                   tomc_Ornithine_Concnmolml_1
tomc_A_3MH_Concnmolml_1, over(treatment) name(boxTOMC)
graph combine boxAUC boxCMAX boxTOMC, rows(3) cols(1) ysize(20) xsize(7)
title("Boxplots for AUC, C{subscript:max} and TOMC", size(large))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014 analysis\\`c(current_date)'_boxplots.tif", width(1809) height(2800)
********************graphs for age/bmi/gender with AUC/CMAX/TOMC
**********************************AUC
*****************************1. Age / AUC
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph    matrix    auc_`x'_Concnmolml_1    Age,    by(treatment,    total)
diagonal(,size(small)) xlabel(,labsize(vsmall)) ylabel(,labsize(vsmall))
graph  export  "C:\Users\kfragkos\Documents\Citrulline  research\September
2014      analysis\\`c(current_date)'_age_auc_`x'.tif",      width(2800)
height(1809)
}

*****************************2. BMI / AUC**********

clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
```

```
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph matrix auc_`x'_Concnmolml_1 BMI, by(treatment, total)
diagonal(,size(small)) xlabel(,labsize(vsmall)) ylabel(,labsize(vsmall))
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_BMI_auc_`x'.tif", width(2800)
height(1809)
}
**********
*****************************3. Gender / AUC
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph box auc_Arginine_Concnmolml_1 auc_Citrulline_Concnmolml_1
auc_Glutamine_Concnmolml_1 auc_Ornithine_Concnmolml_1
auc_A_3MH_Concnmolml_1, over(treatment) over(Gender) xsize(20) ysize(8)
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_boxplots_gender_auc.tif"
************************CMAX*********************************************
**1. Age / CMAXclear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph matrix cmax_`x'_Concnmolml_1 Age, by(treatment, total)
diagonal(,size(small)) xlabel(,labsize(vsmall)) ylabel(,labsize(vsmall))
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_age_cmax_`x'.tif", width(2800)
height(1809)
}
2. BMI / CMAX*****************************************
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph matrix cmax_`x'_Concnmolml_1 BMI, by(treatment, total)
diagonal(,size(small)) xlabel(,labsize(vsmall)) ylabel(,labsize(vsmall))
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_BMI_cmax_`x'.tif", width(2800)
height(1809)
}
3. Gender / CMAX**************************************
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph box cmax_Arginine_Concnmolml_1 cmax_Citrulline_Concnmolml_1
cmax_Glutamine_Concnmolml_1 cmax_Ornithine_Concnmolml_1
cmax_A_3MH_Concnmolml_1, over(treatment) over(Gender) xsize(20) ysize(8)
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_boxplots_gender_cmax.tif"
TOMC*****************************************
1. Age / TOMC*****************************************
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph matrix tomc_`x'_Concnmolml_1 Age, by(treatment, total)
diagonal(,size(small)) xlabel(,labsize(vsmall)) ylabel(,labsize(vsmall))
```

```
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_age_tomc_`x'.tif", width(2800)
height(1809)
}
2. BMI / TOMC*****************************************
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph matrix tomc_`x'_Concnmolml_1 BMI, by(treatment, total)
diagonal(,size(small)) xlabel(,labsize(vsmall)) ylabel(,labsize(vsmall))
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_BMI_tomc_`x'.tif", width(2800)
height(1809)
}
*****************************************3. Gender / TOMCclear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
graph box tomc_Arginine_Concnmolml_1 tomc_Citrulline_Concnmolml_1
tomc_Glutamine_Concnmolml_1 tomc_Ornithine_Concnmolml_1
tomc_A_3MH_Concnmolml_1, over(treatment) over(Gender) xsize(20) ysize(8)
graph export "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\\`c(current_date)'_boxplots_gender_tomc.tif"
*************************************************************************
***   2. PKCROSS: CROSS OVER ANOVA in Concentrations and AREAS   ******
***   2.1 Cross over anova for concetration with all subjects**********
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
pkcross `x'_Concnmolml_1, treatment(treatment)
}
*************************************************************************
***   3. How to reshape dataset - PKSHAPE  ***************************
*************************************************************************
forvalues i=1/5 {
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\DS_25012015_corrected_nomissingvalues_alltreatments.dta", clear
pkcollapse time Arginine_Concnmolml_1 ///
Citrulline_Concnmolml_1 ///
Glutamine_Concnmolml_1 ///
Ornithine_Concnmolml_1 ///
A_3MH_Concnmolml_1 ///
if treatment==`i', id(id) fit(9) force keep(sequence treatment carry
period)
drop in 21
saveold "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\tr`i'_outcome_`c(current_date)'.dta"
}
*************************************************************************
*************************************************************************
***      Then we append the datasets created with the following command
*************************************************************************
*************************************************************************
clear all
use "C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\tr1_outcome_`c(current_date)'.dta", clear
append using "C:\Users\kfragkos\Documents\Citrulline research\September
2014 analysis\tr2_outcome_`c(current_date)'.dta" ///
"C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\tr3_outcome_`c(current_date)'.dta" ///
"C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\tr4_outcome_`c(current_date)'.dta" ///
"C:\Users\kfragkos\Documents\Citrulline research\September 2014
analysis\tr5_outcome_`c(current_date)'.dta"
```

```
saveold  "C:\Users\kfragkos\Documents\Citrulline  research\September  2014
analysis\completedatasetPK_`c(current_date)'.dta"
*************************************************************************
*************************************************************************
***4. PKCROSS: CROSS OVER ANOVA in AUC of AREAS and Concenrtations  ***
*************************************************************************
****SUMMARY STATISTICS   ****************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
by treatment, sort: summarize auc_* cmax_* tomc_*
***cross over anova for AUC with all subjects**************************
*************************************************************************
*************************************************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
pkcross auc_`x'_Concnmolml_1, treatment(treatment)
}
*************************************************************************
***cross over anova for CMAX with all subjects**************************
*************************************************************************
*************************************************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
pkcross cmax_`x'_Concnmolml_1, treatment(treatment)
}
*************************************************************************
*************************************************************************
**Equivalence testing: need for natural logarithm**********************
*************************************************************************
**********AUC*************************************************************
foreach x in CIT ARG GLN ORN 3MH {
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
pkcross LN_AUC_`x', treatment(treatment)
pkequiv LN_AUC_`x' treatment period sequence id, compare(1 2) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(1 3) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(1 4) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(1 5) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(2 3) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(2 4) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(2 5) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(3 4) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(3 5) anderson
pkequiv LN_AUC_`x' treatment period sequence id, compare(4 5) anderson
}
*************************************************************************
**********CMAX************************************************************
foreach x in cit arg gln orn 3mh {
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
pkcross ln_cmax_`x', treatment(treatment)
pkequiv ln_cmax_`x' treatment period sequence id, compare(1 2) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(1 3) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(1 4) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(1 5) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(2 3) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(2 4) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(2 5) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(3 4) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(3 5) anderson
pkequiv ln_cmax_`x' treatment period sequence id, compare(4 5) anderson
}
```

```
*************************************************************************
*************************************************************************
*Analysis of reshaped dataset
*Equivalence and effect
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_`c(current_date)'.dta", clear
pkcross auc_Citrulline_Concnmolml_1, treatment(treatment)
pkequiv auc_Citrulline_Concnmolml_1 treatment period period id, compare(1
2)
pkequiv LN_AUC_ARG treatment period sequence id, compare(1 2) anderson
*ANOVA
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_`c(current_date)'.dta", clear
anova auc_Citrulline_Concnmolml_1 id treatment, repeated(treatment)
pwcompare treatment, cimargins
*************************************************************************
***************Miscellaneous stuff*******************************
*************************************************************************
*************************************************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_26 Jan 2015.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
anova auc_`x'_Concnmolml_1 id treatment, repeated(treatment)
pwcompare treatment}
*************************************************************************
*****************Analysis of Outliers*****************************
*************************************************************************
clear all
use   "C:\Users\kfragkos\Documents\Citrulline   research\September   2014
analysis\completedatasetPK_`c(current_date)'.dta", clear
foreach x in Citrulline Arginine Glutamine Ornithine A_3MH {
anova auc_`x'_Concnmolml_1 id treatment, repeated(treatment)
estat hettest
predict d_`x'_anova, cooksd
list id treatment d_`x'_anova if d_`x'_anova>4/100
dfbeta i.id i.treatment
}
*************************************************************************
*************************************************************************
***Graph of DFBETA for amino acids AUC by ID**************************
*************************************************************************
*************************************************************************
***citrulline levels
scatter  _dfbeta_1  _dfbeta_2  _dfbeta_3  _dfbeta_4  _dfbeta_5  _dfbeta_6
_dfbeta_7 _dfbeta_8 _dfbeta_9 _dfbeta_10 _dfbeta_11 _dfbeta_12 _dfbeta_13
_dfbeta_14 ///
_dfbeta_15 _dfbeta_16 _dfbeta_17 _dfbeta_18 _dfbeta_19 treatment, ylabel(-
1(.5)3) yline(.20 -.20) xlabel(,val labs(small)) title("Plasma Citrulline
AUC") ///
legend(pos(2) ring(0) size(tiny) cols(4)) xtitle(,size(small)) name(gcit)
msize(small small small small small small small small small small small
small small small small small small small small)
***arginine Levels
scatter _dfbeta_24 _dfbeta_25 _dfbeta_26 _dfbeta_27 _dfbeta_28 _dfbeta_29
_dfbeta_30   _dfbeta_31   _dfbeta_32   _dfbeta_33   _dfbeta_34   _dfbeta_35
_dfbeta_36 ///
_dfbeta_37   _dfbeta_38   _dfbeta_39   _dfbeta_40   _dfbeta_41   _dfbeta_42
treatment,  ylabel(-1(.5)3)  yline(.20  -.20)  xlabel(,val  labs(small))
title("Plasma Arginine AUC") ///
legend(pos(2) ring(0) size(tiny) cols(4)) xtitle(,size(small)) name(garg)
msize(small small small small small small small small small small small
small small small small small small small small)
***Glutamine levels
scatter _dfbeta_47 _dfbeta_48 _dfbeta_49 _dfbeta_50 _dfbeta_51 _dfbeta_52
_dfbeta_53   _dfbeta_54   _dfbeta_55   _dfbeta_56   _dfbeta_57   _dfbeta_58
_dfbeta_59 ///
```

```
_dfbeta_60 _dfbeta_61 _dfbeta_62 _dfbeta_63 _dfbeta_64 _dfbeta_65
treatment, ylabel(-1(.5)3) yline(.20 -.20) xlabel(,val labs(small))
title("Plasma Glutamine AUC") ///
legend(pos(2) ring(0) size(tiny) cols(4)) xtitle(,size(small)) name(ggln)
msize(small small small small small small small small small small small
small small small small small small small small)
***Ornithine levels
scatter _dfbeta_70 _dfbeta_71 _dfbeta_72 _dfbeta_73 _dfbeta_74 _dfbeta_75
_dfbeta_76 _dfbeta_77 _dfbeta_78 _dfbeta_79 _dfbeta_80 _dfbeta_81
_dfbeta_82 ///
_dfbeta_83 _dfbeta_84 _dfbeta_85 _dfbeta_86 _dfbeta_87 _dfbeta_88
treatment, ylabel(-1(.5)3) yline(.20 -.20) xlabel(,val labs(small))
title("Plasma Ornithine AUC") ///
legend(pos(2) ring(0) size(tiny) cols(4)) xtitle(,size(small)) name(gorn)
msize(small small small small small small small small small small small
small small small small small small small small)
***3MH levels
scatter _dfbeta_93 _dfbeta_94 _dfbeta_95 _dfbeta_96 _dfbeta_97 _dfbeta_98
_dfbeta_99 _dfbeta_100 _dfbeta_101 _dfbeta_102 _dfbeta_103 _dfbeta_104
_dfbeta_105 ///
_dfbeta_106 _dfbeta_107 _dfbeta_108 _dfbeta_109 _dfbeta_110 _dfbeta_111
treatment, ylabel(-1(.5)3) yline(.20 -.20) xlabel(,val labs(small))
title("Plasma 3-Methyl-histidine AUC") ///
legend(pos(2) ring(0) size(tiny) cols(4)) xtitle(,size(small)) name(g3mh)
msize(small small small small small small small small small small small
small small small small small small small small)
***Combining Graphs
graph combine gcit garg ggln gorn g3mh, xsize(20) ysize(10) title("DFBETAs
by Treatment") imargin(vlarge) altshrink
```

References

(1882a). Hypodermic Administration of Cathartics. *American Medical Digest, 1-10*, 156.

(1882b). Selections: Hypodermic Administration of Cathartics. *Louisville Medical News, 14*(10), 117-118.

(1882c). The subcutaneous insertion of laxative remedies. *Medical Record, 22*, 604.

(1882d). *The London Medical Record, 10*, 490.

(1882e). Sub-Cutaneous Use of Laxatives. *Chicago Medical Review, 6*(12), 562-563.

(1882f). Gleanings from Exchanges: Hypodermic application of purgatives. *Medical Times and Register, 13*, 70-71.

(1883a). Progress of Medicine. *North Carolina Medical Journal, 11*(1), 38-49.

(1883b). *Pharmaceutical Journal and Transactions, Series 3, Volume 13*, 343.

(1889a). Manual of Subcutaneous Injections. *Wood's Medical and Surgical Monographs, 3*(2), 504.

(1889b). Notes and Comments: Case of Colocynth poisoning. *Medical and Surgical Reporter, 60*(16), 498.

(1891). *Meyer Druggist*, 358.

(1893a). Suppositoria. *Proceedings of the American Pharmaceutical Association at the Annual Meeting Scio, Ohio; 1893 vol 41, p 450, 41*, 450.

(1893b). Local application per rectum of aloin, cathartic acid, colocynthin, and citrullin as laxatives. *Therapeutic Gazette, 17*, 703-704.

(1893c). *Bulletin of Pharmacy, 7*.

(1893d). Referate. *Therapeutische Monatshefte, 7*(Januar), 34.

(1893e). Therapeutic Notes: Glycerin suppositories, with aloin, citrullin, and colocynthin. *Medical Bulletin, 15*(8), 310.

(1894). Glycerin and Citrullin Suppositories. *Medical Age, 12*(4), 115.

(1907). Merck's 1907 Index. 3rd edn. Merck, New York

(1909). Queries and Minor Notes. *Journal of the American Medical Association, 53*(4), 310. doi:10.1001/jama.1909.02550040062014

(2003). American Gastroenterological Association medical position statement: Short bowel syndrome and intestinal transplantation. *Gastroenterology, 124*(4), 1105-1110. doi:10.1053/gast.2003.50139

Abeyasekera, S., & Curnow, R. N. (1984). The Desirability of Adjusting for Residual Effects in a Crossover Design. *Biometrics, 40*(4), 1071-1078. doi:10.2307/2531157

Abumrad, N. N., & Barbul, A. (2004). The use of arginine in clinical practice. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 595-612). London: CRC Press. doi:10.1201/9780203010266.ch35

Ackermann, D. (1931). Über den biologischen Abbau des Arginins zu Citrullin. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie, 203*(1-2), 66-69. doi:10.1515/bchm2.1931.203.1-2.66

Alekseeva, E. V., & Sal'nikov, P. S. (2014). [Glutamine, glutamate and citrulline concentration in blood plasma in patients in critical condition (pilot study results)]. *Patologicheskaia Fiziologiia i Eksperimental'naia Terapiia*(2), 45-51.

Allan, P., & Lal, S. (2018). Intestinal failure: a review. *F1000Research, 7*, 85. doi:10.12688/f1000research.12493.1

Alteheld, B., Stehle, P., & Fürst, P. (2003). Measurement of amino acid concentrations in biological fluids and tissues using reversed-phase HPLC-based methods. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 29-44). London: CRC Press. doi:10.1201/9780203010266.ch2

Amiot, A., Messing, B., Corcos, O., Panis, Y., & Joly, F. (2013). Determinants of home parenteral nutrition dependence and survival of 268 patients with non-malignant

short bowel syndrome. *Clinical Nutrition, 32*(3), 368-374. doi:10.1016/j.clnu.2012.08.007

Anderson, S., & Hauck, W. W. (2007). A new procedure for testing equivalence in comparative bioavailability and other clinical trials. *Communications in Statistics - Theory and Methods, 12*(23), 2663-2692. doi:10.1080/03610928308828634

Armstrong, A. M., Campbell, G. R., Gannon, C., Kirk, S. J., & Gardiner, K. R. (2000). Oral administration of inducible nitric oxide synthase inhibitors reduces nitric oxide synthesis but has no effect on the severity of experimental colitis. *Scandinavian Journal of Gastroenterology, 35*(8), 832-838. doi:10.1080/003655200750023200

Avitzur, Y., & Miserachs, M. (2018). Quality of life on long-term parenteral nutrition: can it be an indication for intestinal transplantation? *Current Opinion in Organ Transplantation, 23*(2), 199-206. doi:10.1097/MOT.0000000000000503

Bacchieri, A., & Della Cioppa, G. (2007). Fundamentals of clinical research : bridging medicine, statistics, and operations. Statistics for Biology and Health. Springer, Milano. doi:10.1007/978-88-470-0492-4

Bachmann, K., Galeazzi, R., Millet, A., & Burger, A. G. (1984). Plasma levels of 3-methylhistidine after ingestion of the pure amino acid or of muscular proteins measured by radioimmunoassay. *Metabolism, 33*(2), 107-110. doi:10.1016/0026-0495(84)90120-3

Backman, L., Hallberg, D., & Kallner, A. (1975). Amino acid pattern in plasma before and after jejuno-ileal shunt operation for obesity. *Scandinavian Journal of Gastroenterology, 10*(8), 811-816.

Bahri, S., Curis, E., El Wafi, F.-Z., Aussel, C., Chaumeil, J.-C., Cynober, L., & Zerrouk, N. (2008). Mechanisms and kinetics of citrulline uptake in a model of human intestinal epithelial cells. *Clinical Nutrition, 27*(6), 872-880. doi:10.1016/j.clnu.2008.08.003

Bailey, R. A. (2008). Design of comparative experiments. Cambridge University Press, Cambridge. doi:10.1017/CBO9780511611483

Bailly-Botuha, C., Colomb, V., Thioulouse, E., Berthe, M.-C., Garcette, K., Dubern, B., Goulet, O., Couderc, R., & Girardet, J.-P. (2009). Plasma citrulline concentration reflects enterocyte mass in children with short bowel syndrome. *Pediatric Research, 65*(5), 559-563. doi:10.1203/PDR.0b013e31819986da

Bandler, S. W. (1915). Medical Gynecology. 3rd edn. Saunders, Philadelphia

Barzal, J. A., Szczylik, C., Rzepecki, P., Jaworska, M., & Anuszewska, E. (2014). Plasma citrulline level as a biomarker for cancer therapy-induced small bowel mucosal damage. *Acta Biochimica Polonica, 61*(4), 615-631.

Basso, M. S., Capriati, T., Goffredo, B. M., Panetta, F., & Diamanti, A. (2014). Citrulline as marker of atrophy in celiac disease. *Internal and Emergency Medicine, 9*(6), 705-707. doi:10.1007/s11739-014-1074-7

Bate, S. T., & Jones, B. (2006). The construction of nearly balanced and nearly strongly balanced uniform cross-over designs. *Journal of Statistical Planning and Inference, 136*(9), 3248-3267. doi:10.1016/j.jspi.2004.11.012

Batista, M. A., Nicoli, J. R., Martins Fdos, S., Machado, J. A., Arantes, R. M., Quirino, I. E., Correia, M. I., & Cardoso, V. N. (2012). Pretreatment with citrulline improves gut barrier after intestinal obstruction in mice. *Journal of Parenteral and Enteral Nutrition, 36*(1), 69-76. doi:10.1177/0148607111414024

Baum, H. (1893). Ist Citrullin ein Abführmittel für Haustiere? *Archiv für Wissenschftl und Praktische Tierheilkunde, 19*, 414-433.

Baxter, J. P., Fayers, P. M., & McKinlay, A. W. (2010). The clinical and psychometric validation of a questionnaire to assess the quality of life of adult patients treated with long-term parenteral nutrition. *Journal of Parenteral and Enteral Nutrition, 34*(2), 131-142. doi:10.1177/0148607109348612

Beaufrere, A. M., Neveux, N., Patureau Mirand, P., Buffiere, C., Marceau, G., Sapin, V., Cynober, L., & Meydinal-Denis, D. (2014). Long-term intermittent glutamine supplementation repairs intestinal damage (structure and functional mass) with

advanced age: assessment with plasma citrulline in a rodent model. *Journal of Nutrition, Health & Aging*, *18*(9), 814-819. doi:10.1007/s12603-014-0468-6

Becker, R. M., Wu, G., Galanko, J. A., Chen, W., Maynor, A. R., Bose, C. L., & Rhoads, J. M. (2000). Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. *Journal of Pediatrics*, *137*(6), 785-793. doi:10.1067/mpd.2000.109145

Begg, C. B., & Mazumdar, M. (1994). Operating Characteristics of a Rank Correlation Test for Publication Bias. *Biometrics*, *50*(4), 1088-1101. doi:10.2307/2533446

Bensaci, J., Curis, E., Nicolis, I., de Bandt, J. P., & Bénazeth, S. (2012). An in silico model of enterocytic glutamine to citrulline conversion pathway. *Amino Acids*, *43*(4), 1727-1737. doi:10.1007/s00726-012-1257-3

Berghöfer, P., Fragkos, K. C., Baxter, J. P., Forbes, A., Joly, F., Heinze, H., Loth, S., Pertkiewicz, M., Messing, B., & Jeppesen, P. B. (2013). Development and validation of the disease-specific Short Bowel Syndrome-Quality of Life (SBS-QoL™) scale. *Clinical Nutrition*, *32*(5), 789-796. doi:10.1016/j.clnu.2012.12.001

Berkeveld, M., Langendijk, P., Verheijden, J. H., Taverne, M. A., van Nes, A., van Haard, P., & Koets, A. P. (2008). Citrulline and intestinal fatty acid-binding protein: longitudinal markers of postweaning small intestinal function in pigs? *Journal of Animal Science*, *86*(12), 3440-3449. doi:10.2527/jas.2008-1167

Berkeveld, M., Langendijk, P., Soede, N. M., Kemp, B., Taverne, M. A., Verheijden, J. H., Kuijken, N., & Koets, A. P. (2009). Improving adaptation to weaning: effect of intermittent suckling regimens on piglet feed intake, growth, and gut characteristics. *Journal of Animal Science*, *87*(10), 3156-3166. doi:10.2527/jas.2008-1764

Bernini, P., Bertini, I., Calabro, A., la Marca, G., Lami, G., Luchinat, C., Renzi, D., & Tenori, L. (2011). Are patients with potential celiac disease really potential? The answer of metabonomics. *Journal of Proteome Research*, *10*(2), 714-721. doi:10.1021/pr100896s

Berrino, L., Hey, C., Rossi, F., & Racké, K. (1995). Stimulation-induced 3H-L-citrulline accumulation in isolated longitudinal muscle myenteric plexus preparations of rat small intestine: a measure to characterize nitrergic neurons. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *352*(5), 506-511. doi:10.1007/BF00169384

Bjarnason, I., MacPherson, A., & Hollander, D. (1995). Intestinal permeability: An overview. *Gastroenterology*, *108*(5), 1566-1581. doi:10.1016/0016-5085(95)90708-4

Bjornvad, C. R., Thymann, T., Deutz, N. E., Burrin, D. G., Jensen, S. K., Jensen, B. B., Molbak, L., Boye, M., Larsson, L. I., Schmidt, M., Michaelsen, K. F., & Sangild, P. T. (2008). Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs on parenteral nutrition. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *295*(5), G1092-1103. doi:10.1152/ajpgi.00414.2007

Blachier, F., Darcy-Vrillon, B., Sener, A., Duee, P. H., & Malaisse, W. J. (1991a). Arginine metabolism in rat enterocytes. *Biochimica et Biophysica Acta*, *1092*(3), 304-310. doi:10.1016/S0167-4889(97)90005-7

Blachier, F., M'Rabet-Touil, H., Darcy-Vrillon, B., Posho, L., & Duee, P. H. (1991b). Stimulation by D-glucose of the direct conversion of arginine to citrulline in enterocytes isolated from pig jejunum. *Biochemical and Biophysical Research Communications*, *177*(3), 1171-1177. doi:10.1016/0006-291X(91)90663-R

Blachier, F., M'Rabet-Touil, H., Posho, L., Darcy-Vrillon, B., & Duee, P. H. (1993). Intestinal arginine metabolism during development. Evidence for de novo synthesis of L-arginine in newborn pig enterocytes. *European Journal of Biochemistry*, *216*(1), 109-117. doi:10.1111/j.1432-1033.1993.tb18122.x

Blasco-Alonso, J., SánchezYáñez, P., Rosa Camacho, V., Camacho Alonso, J. M., Yahyaoui Macías, R., Gil-Gómez, R., & Milano Manso, G. (2015). La cinética de

la citrulina y la arginina y su valor como factor pronóstico en pacientes pediátricos críticamente enfermos. *Anales de Pediatría*. doi:10.1016/j.anpedi.2014.10.032

Blasco Alonso, J., Serrano Nieto, J., Navas Lopez, V. M., Barco Galvez, A., Vicioso, I., Carazo Gallego, B., Ortiz Perez, P., & Sierra Salinas, C. (2011). [Plasma citrulline as a marker of loss of enterocitary mass in coeliac disease in childhood]. *Nutrición Hospitalaria*, *26*(4), 807-813. doi:10.1590/S0212-16112011000400021

Blijlevens, N. M., Donnelly, J. P., & DePauw, B. E. (2005a). Inflammatory response to mucosal barrier injury after myeloablative therapy in allogeneic stem cell transplant recipients. *Bone Marrow Transplantation*, *36*(8), 703-707. doi:10.1038/sj.bmt.1705118

Blijlevens, N. M., Donnelly, J. P., Naber, A. H., Schattenberg, A. V., & DePauw, B. E. (2005b). A randomised, double-blinded, placebo-controlled, pilot study of parenteral glutamine for allogeneic stem cell transplant patients. *Supportive Care in Cancer*, *13*(10), 790-796. doi:10.1007/s00520-005-0790-y

Blijlevens, N. M. A., Lutgens, L. C. H. W., Schattenberg, A. V. M. B., & Donnelly, J. P. (2004). Citrulline: A potentially simple quantitative marker of intestinal epithelial damage following myeloablative therapy. *Bone Marrow Transplantation*, *34*(3), 193-196. doi:10.1038/sj.bmt.1704563

Blijlevens, N. M. A. (2005). Implications of treatment-induced mucosal barrier injury. *Current Opinion in Oncology*, *17*(6), 605-610. doi:10.1097/01.cco.0000183672.15133.ab

Bloomfield, F. H., van Zijl, P. L., Bauer, M. K., & Harding, J. E. (2002). Effects of intrauterine growth restriction and intraamniotic insulin-like growth factor-I treatment on blood and amniotic fluid concentrations and on fetal gut uptake of amino acids in late-gestation ovine fetuses. *Journal of Pediatric Gastroenterology and Nutrition*, *35*(3), 287-297. doi:10.1097/00005176-200209000-00010

Boas, I. (1904). Diseases of the intestines (trans: S. Basch). 2nd edn. Appleton, New York and London

Boelens, P. G., & van Leeuwen, P. A. M. (2003). Glutamine-supplemented diets in enteral nutrition. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 577-594). London: CRC Press. doi:10.1201/9780203010266.ch34

Boelens, P. G., van Leeuwen, P. A. M., Dejong, C. H. C., & Deutz, N. E. P. (2005). Intestinal renal metabolism of L-citrulline and L-arginine following enteral or parenteral infusion of L-alanyl-L-[2,^{15}N]glutamine or L-[2,^{15}N]glutamine in mice. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *289*(4), G679-G685. doi:10.1152/ajpgi.00026.2005

Boirie, Y., Walrand, S., & Beaufrère, B. (2003). Control of amino acid metabolism by lipids, ketone bodies, and glucose substrates. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 241-252). London: CRC Press. doi:10.1201/9780203010266.ch15

Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2008). Introduction to meta-analysis. John Wiley & Sons, Chichester, U.K.

Bourdon, A., Rougé, C., Legrand, A., Des Robert, C., Piloquet, H., Vodovar, M., Voyer, M., Rozé, J. C., & Darmaun, D. (2010). PP416 Urinary citrulline in very low birth weight infants receiving intravenous parenteral nutrition. *Clinical Nutrition Supplements*, *5*(2), 186-186. doi:10.1016/S1744-1161(10)70491-9

Bourdon, A., Rouge, C., Legrand, A., Des Robert, C., Piloquet, H., Vodovar, M., Voyer, M., Roze, J. C., & Darmaun, D. (2012). Urinary citrulline in very low birth weight preterm infants receiving intravenous nutrition. *British Journal of Nutrition*, *108*(7), 1150-1154. doi:10.1017/S0007114511006660

Boutry, C., Matsumoto, H., Bos, C., Moinard, C., Cynober, L., Yin, Y., Tomé, D., & Blachier, F. (2012). Decreased glutamate, glutamine and citrulline concentrations in plasma and muscle in endotoxemia cannot be reversed by glutamate or glutamine supplementation: a primary intestinal defect? *Amino Acids*, *43*(4), 1485-1498. doi:10.1007/s00726-012-1221-2

Bowden, J., Tierney, J. F., Copas, A. J., & Burdett, S. (2011). Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Medical Research Methodology*, *11*, 41. doi:10.1186/1471-2288-11-41

Brady, D., Horn, S., Yakkundi, S., McGarry, C. K., Hounsell, A. R., Prise, K. M., & O'Sullivan, J. M. (2015). PO-0739: Plasma citrulline is a potential biomarker for small bowel toxicity following radiotherapy for prostate cancer. *Radiotherapy and Oncology*, *115*(Supplement 1), S366. doi:10.1016/S0167-8140(15)40731-5

Braithwaite, J. O. (1893). *Yearbook of Pharmacy*, 220.

Breuillard, C., Cynober, L., & Moinard, C. (2015). Citrulline and nitrogen homeostasis: an overview. *Amino Acids*, *47*(4), 685-691. doi:10.1007/s00726-015-1932-2

Breusch, T. S., & Pagan, A. R. (1979). A Simple Test for Heteroscedasticity and Random Coefficient Variation. *Econometrica*, *47*(5), 1287-1294. doi:10.2307/1911963

Brosnan, M. E., & Brosnan, J. T. (2004). Renal arginine metabolism. *Journal of Nutrition*, *134*(10 Supplement), 2791S-2795S; discussion 2796S-2797S. doi:10.1093/jn/134.10.2791S

Bruce, J. M. (1884). Materia Medica and Therapeutics: an introduction to the rational treatment of disease. Cassell & Co., London

Bruins, M. J., Luiking, Y. C., Soeters, P. B., Lamers, W. H., Akkermans, L. M., & Deutz, N. E. (2004). Effects of long-term intravenous and intragastric L-arginine intervention on jejunal motility and visceral nitric oxide production in the hyperdynamic compensated endotoxaemic pig. *Neurogastroenterology and Motility*, *16*(6), 819-828. doi:10.1111/j.1365-2982.2004.00579.x

Buchman, A. L., Scolapio, J., & Fryer, J. (2003). AGA technical review on Short bowel syndrome and intestinal transplantation. *Gastroenterology*, *124*(4), 1111-1134. doi:10.1053/gast.2003.50139a

Buchman, A. L., Katz, S., Fang, J. C., Bernstein, C. N., & Abou-Assi, S. G. (2010). Teduglutide, a novel mucosally active analog of glucagon-like peptide-2 (GLP-2) for the treatment of moderate to severe Crohn's disease. *Inflammatory Bowel Diseases*, *16*(6), 962-973. doi:10.1002/ibd.21117

Cakmaz, R., Buyukasik, O., Kahramansoy, N., Erkol, H., Col, C., Boran, C., & Bugdayci, G. (2013). A combination of plasma DAO and citrulline levels as a potential marker for acute mesenteric ischemia. *Libyan Journal of Medicine*, *8*, 1-6. doi:10.3402/ljm.v8i0.20596

Calder, P. C., & Yaqoob, P. (2003). Amino acids and immune function. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 305-320). London: CRC Press. doi:10.1201/9780203010266.ch19

Cano, N. (2003). Branched-chain amino and keto acids in renal failure. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 557-576). London: CRC Press. doi:10.1201/9780203010266.ch33

Carlsson, E., Bosaeus, I., & Nordgren, S. (2003a). What Concerns Subjects with Inflammatory Bowel Disease and an Ileostomy? *Scandinavian Journal of Gastroenterology*, *38*(9), 978-984. doi:10.1080/00365520310004687

Carlsson, E., Bosaeus, I., & Nordgren, S. (2003b). Quality of life and concerns in patients with short bowel syndrome. *Clinical Nutrition*, *22*(5), 445-452. doi:10.1016/S0261-5614(03)00042-6

Carswell, K. A., Vincent, R. P., Belgaumkar, A. P., Sherwood, R. A., Amiel, S. A., Patel, A. G., & le Roux, C. W. (2014). The effect of bariatric surgery on intestinal absorption and transit time. *Obesity Surgery*, *24*(5), 796-805. doi:10.1007/s11695-013-1166-x

Castillo, L., Sanchez, M., Vogt, J., Chapman, T. E., Derojas-Walker, T. C., Tannenbaum, S. R., Ajami, A. M., & Young, V. R. (1995). Plasma arginine, citrulline, and ornithine kinetics in adults, with observations on nitric oxide synthesis. *American Journal of Physiology - Endocrinology and Metabolism*, *268*(2 Pt 1), E360-E367. doi:10.1152/ajpendo.1995.268.2.E360

Castillo, L., Beaumier, L., Ajami, A. M., & Young, V. R. (1996). Whole body nitric oxide synthesis in healthy men determined from [15N]arginine-to-[15N]citrulline labeling. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(21), 11460-11465. doi:10.1073/pnas.93.21.11460

Celik, I. H., Demirel, G., Canpolat, F. E., & Dilmen, U. (2013). Reduced plasma citrulline levels in low birth weight infants with necrotizing enterocolitis. *Journal of Clinical Laboratory Analysis*, *27*(4), 328-332. doi:10.1002/jcla.21607

Chakder, S., & Rattan, S. (1997). L-arginine deficiency causes suppression of nonadrenergic noncholinergic nerve-mediated smooth muscle relaxation: role of L-citrulline recycling. *Journal of Pharmacology and Experimental Therapeutics*, *282*(1), 378-384.

Chapman, J. C., Liu, Y., Zhu, L., & Rhoads, J. M. (2012). Arginine and Citrulline Protect Intestinal Cell Monolayer Tight Junctions from Hypoxia-Induced Injury. *Pediatric Research*. doi:10.1038/pr.2012.137

Chaturvedi, M., Mali, P. C., & Ansari, A. S. (2003). Induction of reversible antifertility with a crude ethanol extract of *Citrullus colocynthis* Schrad fruit in male rats. *Pharmacology*, *68*(1), 38-48. doi:10.1159/000068727

Chen, K., Nezu, R., Sando, K., Haque, S. M., Iiboshi, Y., Masunari, A., Yoshida, H., Kamata, S., Takagi, Y., & Okada, A. (1996). Influence of glutamine-supplemented parenteral nutrition on intestinal amino acid metabolism in rats after small bowel resection. *Surgery Today*, *26*(8), 618-623. doi:10.1007/BF00311667

Chow, S.-C., & Liu, J.-p. (2008). Design and Analysis of Bioavailability and Bioequivalence Studies. Chapman & Hall/CRC Biostatistics Series, 3rd edn. Chapman and Hall/CRC Press, Boca Raton, FL. doi:10.1201/9781420011678

Cochran, W. G., & Cox, G. M. (1957). Experimental designs. 2nd edn. Wiley; London: Chapman & Hall, New York

Cohen, J. (1988). Statistical power analysis for the behavioral sciences. 2nd edn. L. Erlbaum Associates, Hillsdale, N.J.

Collin, M., & Vapaatalo, H. (2003). L-arginine-enriched diets in cardiovascular diseases. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 729-738). London: CRC Press. doi:10.1201/9780203010266.secc

Cook, R. D. (1977). Detection of Influential Observation in Linear Regression. *Technometrics*, *19*(1), 15-18. doi:10.2307/1268249

Cook, R. D. (1979). Influential Observations in Linear Regression. *Journal of the American Statistical Association*, *74*(365), 169-174. doi:10.1080/01621459.1979.10481634

Cook, R. D., & Weisberg, S. (1983). Diagnostics for heteroscedasticity in regression. *Biometrika*, *70*(1), 1-10. doi:10.1093/biomet/70.1.1

Cooper, H. M., Hedges, L. V., & Valentine, J. C. (2009). The handbook of research synthesis and meta-analysis. 2nd edn. Russell Sage Foundation, New York

Cotogni, P., De Carli, L., Passera, R., Amerio, M. L., Agnello, E., Fadda, M., Ossola, M., Monge, T., De Francesco, A., & Bozzetti, F. (2017). Longitudinal study of quality of life in advanced cancer patients on home parenteral nutrition. *Cancer Medicine*, *6*(7), 1799-1806. doi:10.1002/cam4.1111

Cox, D. R. (1958). Planning of Experiments. John Wiley & Sons; London: Chapman & Hall, New York

Cox, D. R., & Reid, N. (2000). The theory of the design of experiments. Chapman & Hall/CRC, Boca Raton

Crenn, P., Coudray-Lucas, C., Cynober, L., & Messing, B. (1998). Post-absorptive plasma citrulline concentration: A marker of intestinal failure in humans. *Transplantation Proceedings*, *30*(6), 2528. doi:10.1016/S0041-1345(98)00711-8

Crenn, P., Coudray-Lucas, C., Thuillier, F., Cynober, L., & Messing, B. (2000). Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. *Gastroenterology*, *119*(6), 1496-1505. doi:10.1053/gast.2000.20227

Crenn, P., Vahedi, K., Lavergne-Slove, A., Cynober, L., Matuchansky, C., & Messing, B. (2003). Plasma citrulline: A marker of enterocyte mass in villous atrophy-associated small bowel disease. *Gastroenterology*, *124*(5), 1210-1219. doi:10.1016/S0016-5085(03)00170-7

Crenn, P. (2008). Citrulline et métabolisme protéique (Citrulline and protein metabolism). *Nutrition Clinique et Métabolisme*, *22*(2), 75-79. doi:10.1016/j.nupar.2008.04.004

Crenn, P., Messing, B., & Cynober, L. (2008). Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. *Clinical Nutrition*, *27*(3), 328-339. doi:10.1016/j.clnu.2008.02.005

Crenn, P., De Truchis, P., Neveux, N., Galpérine, T., Cynober, L., & Melchior, J. C. (2009a). Plasma citrulline is a biomarker of enterocyte mass and an indicator of parenteral nutrition in HIV-infected patients. *American Journal of Clinical Nutrition*, *90*(3), 587-594. doi:10.3945/ajcn.2009.27448

Crenn, P., de Truchis, P., Neveux, N., Perronne, C., Cynober, L., & Melchior, J. C. (2009b). La citrullinémie, un marqueur de l'évolution et du pronostic au cours du suivi des entéropathies. *Gastroentérologie Clinique et Biologique*, *33*(3(Supplement 1)), A204. doi:10.1016/S0399-8320(09)73003-9

Crenn, P., & Annane, D. (2010). Mesure de la fonctionnalité intestinale en réanimation par la détermination de la citrullinémie. *Réanimation*, *19*(5), 393-398. doi:10.1016/j.reaurg.2010.04.005

Crenn, P., & Cynober, L. (2010). Effect of intestinal resections on arginine metabolism: practical implications for nutrition support. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*(1), 65-69. doi:10.1097/MCO.0b013e328333c1a8

Crenn, P., Neveux, N., Chevret, S., Jaffray, P., Cynober, L., Melchior, J. C., & Annane, D. (2010). Plasma Citrulline Kinetic and Its Relation with Glutamine, Arginine, TNF[alpha] and IL10 in ICU Septic Shock Patients With Multiple Organ Failure. *Clinical Nutrition Supplements*, *5*(2), 214-215. doi:10.1016/S1744-1161(10)70562-7

Crenn, P., Hanachi, M., Neveux, N., & Cynober, L. (2011). [Circulating citrulline levels: a biomarker for intestinal functionality assessment]. *Annales de Biologie Clinique*, *69*(5), 513-521. doi:10.1684/abc.2011.0609

Culbreth, D. M. R. (1917). A Manual of Materia Medica and Pharmacology. 6th edn. Lea & Febiger, Philadelphia

Curis, E., Nicolis, I., Moinard, C., Osowska, S., Zerrouk, N., Bénazeth, S., & Cynober, L. (2005). Almost all about citrulline in mammals. *Amino Acids*, *29*(3), 177-205. doi:10.1007/s00726-005-0235-4

Curis, E., Crenn, P., & Cynober, L. (2007). Citrulline and the gut. *Current Opinion in Clinical Nutrition and Metabolic Care*, *10*(5), 620-626. doi:10.1097/MCO.0b013e32829fb38d

Cynober, L. (1994). Can arginine and ornithine support gut functions? *Gut*, *35*(1 Supplement), S42-S45. doi:10.1136/gut.35.1_suppl.s42

Cynober, L., Le Boucher, J., & Vasson, M. P. (1995). Arginine metabolism in mammals. *Journal of Nutritional Biochemistry*, *6*(8), 402-413. doi:10.1016/0955-2863(95)00066-9

Cynober, L., De Bandt, J.-P., & Moinard, C. (2008). Le temps des acides aminés est-il revenu? (Has the time of amino acids come back?). *Nutrition Clinique et Métabolisme*, *22*(4), 135-141. doi:10.1016/j.nupar.2008.10.002

Cynober, L., Moinard, C., & De Bandt, J. P. (2010). The 2009 ESPEN Sir David Cuthbertson. Citrulline: a new major signaling molecule or just another player in the pharmaconutrition game? *Clinical Nutrition*, *29*(5), 545-551. doi:10.1016/j.clnu.2010.07.006

Cynober, L. (2013). Citrulline: just a biomarker or a conditionally essential amino acid and a pharmaconutrient in critically ill patients? *Critical Care*, *17*(2), 122. doi:10.1186/cc12534

Cynober, L. A. (2002). Plasma amino acid levels with a note on membrane transport: Characteristics, regulation, and metabolic significance. *Nutrition*, *18*(9), 761-766. doi:10.1016/S0899-9007(02)00780-3

Cynober, L. A. (2003). Ornithine α-ketoglutarate. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 633-648). London: CRC Press. doi:10.1201/9780203010266.ch37

Daignault, N. M., Griffith, D. P., Nattakom, T. V., & Ziegler, T. R. (2003). Amino acid support in patients with catabolic illness. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 649-666). London: CRC Press. doi:10.1201/9780203010266.ch38

Darmaun, D., & Cynober, L. (2003). Approaches to studying amino acid metabolism. In: L. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 45-62). London: CRC Press. doi:10.1201/9780203010266.ch3

David, A. I., Gaynor, J. J., Zis, P. P., Conanan, L., Goldsmith, L., Esquenazi, V., Selvaggi, G., Weppler, D., Nishida, S., Moon, J., Madariaga, J. R., Ruiz, P., Kato, T., Levi, D. M., Kleiner, G., Tryphonopoulos, P., & Tzakis, A. G. (2006). An Association of Lower Serum Citrulline Levels Within 30 Days of Acute Rejection in Patients Following Small Intestine Transplantation. *Transplantation Proceedings*, *38*(6), 1731-1732. doi:10.1016/j.transproceed.2006.05.016

David, A. I., Selvaggi, G., Ruiz, P., Gaynor, J. J., Tryphonopoulos, P., Kleiner, G. I., Moon, J. I., Nishida, S., Pappas, P. A., Conanan, L., Weppler, D., Esquenazi, V., Levi, D. M., Kato, T., & Tzakis, A. G. (2007). Blood citrulline level is an exclusionary marker for significant acute rejection after intestinal transplantation. *Transplantation*, *84*(9), 1077-1081. doi:10.1097/01.tp.0000287186.04342.82

David, A. I., Szutan, L. A., Gaynor, J., Ruiz, P., Selvaggi, G., Tryphonopoulos, P., Kleiner, G., Moon, J. I., Nishida, S., Weppler, D., Squenazi, V., Conanan, L., Levi, D. M., Kato, T., & Tzakis, A. G. (2008). [Critical value of citrulline for complications of intestinal transplant graft]. *Revista da Associação Médica Brasileira*, *54*(5), 426-429. doi:10.1590/S0104-42302008000500016

Davis, P. K., & Wu, G. (1998). Compartmentation and kinetics of urea cycle enzymes in porcine enterocytes. *Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology*, *119*(3), 527-537. doi:10.1016/S0305-0491(98)00014-5

De Bandt, J.-P. (2003). Insulin and the regulation of amino acid catabolism and protein turnover. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 185-200). London: CRC Press. doi:10.1201/9780203010266.secb

de Jonge, W. J., Dingemanse, M. A., de Boer, P. A. J., Lamers, W. H., & Moorman, A. F. M. (1998). Arginine-metabolizing enzymes in the developing rat small intestine. *Pediatric Research*, *43*(4), 442-451. doi:10.1203/00006450-199804000-00002

de Winter, J. C. F., Dodou, D., & Wieringa, P. A. (2009). Exploratory Factor Analysis With Small Sample Sizes. *Multivariate Behavioral Research*, *44*(2), 147-181. doi:10.1080/00273170902794206

Dehon, C., Gassner, M., & Verardi, V. (2009). Beware of 'Good' Outliers and Overoptimistic Conclusions. *Oxford Bulletin of Economics and Statistics*, *71*(3), 437-452. doi:10.1111/j.1468-0084.2009.00543.x

Dejong, C. H. C., Olde Damink, S. W. M., Jalan, R., Deutz, N. E. P., & Soeters, P. B. (2003). Requirements and Supply. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 419-430). London: CRC Press. doi:10.1201/9780203010266.ch26

Dekaney, C. M., Wu, G., & Jaeger, L. A. (2001). Ornithine aminotransferase messenger RNA expression and enzymatic activity in fetal porcine intestine. *Pediatric Research*, *50*(1), 104-109. doi:10.1203/00006450-200107000-00019

Dekaney, C. M., Wu, G., & Jaeger, L. A. (2003). Gene expression and activity of enzymes in the arginine biosynthetic pathway in porcine fetal small intestine. *Pediatric Research*, *53*(2), 274-280. doi:10.1203/01.PDR.0000047518.24941.76

Demacker, P. N. M., Beijers, A. M., van Daal, H., Donnelly, J. P., Blijlevens, N. M. A., & van den Ouweland, J. M. W. (2009a). Plasma citrulline measurement using UPLC tandem mass-spectrometry to determine small intestinal enterocyte pathology. *Journal of Chromatography B*, *877*(4), 387-392. doi:10.1016/j.jchromb.2008.12.041

Demacker, P. N. M., Beijers, A. M., van Daal, H., Donnelly, J. P., Blijlevens, N. M. A., & van den Ouweland, J. M. W. (2009b). Rapid plasma-citrulline measurement by UPLC-MS/MS to determine enterocyte-mass reduction. *Nederlands Tijdschrift voor Klinische Chemie en Laboratoriumgeneeskunde*, *34*(4), 221-223.

Derikx, J. P., Blijlevens, N. M., Donnelly, J. P., Fujii, H., Kanda, T., van Bijnen, A. A., Heineman, E., & Buurman, W. A. (2009). Loss of enterocyte mass is accompanied by diminished turnover of enterocytes after myeloablative therapy in haematopoietic stem-cell transplant recipients. *Annals of Oncology*, *20*(2), 337-342. doi:10.1093/annonc/mdn579

DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials*, *7*(3), 177-188. doi:10.1016/0197-2456(86)90046-2

Deutz, N. E. P. (2008). The 2007 ESPEN Sir David Cuthbertson Lecture: amino acids between and within organs. The glutamate-glutamine-citrulline-arginine pathway. *Clinical Nutrition*, *27*(3), 321-327. doi:10.1016/j.clnu.2008.03.010

Dhatariya, K., & Sreekumaran Nair, K. (2003). Diabetes mellitus. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 355-374). London: CRC Press. doi:10.1201/9780203010266.ch22

Diamanti, A., Noto, C., Gandullia, P., Morini, F., Panetta, F., Gambarara, M., Lezo, A., Goffredo, B., Daniele, A., & Torre, G. (2010). PP6 Plasma citrulline and bowel adaptation in children with short bowel syndrome. *Digestive and Liver Disease*, *42*(Supplement 5), S331-S332. doi:10.1016/S1590-8658(10)60568-3

Diamanti, A., Knafelz, D., Panetta, F., Bracci, F., Gambarara, M., Papadatou, B., Daniele, A., Goffredo, B. M., Pezzi, S., & Torre, G. (2011a). Plasma citrulline as surrogate marker of intestinal inflammation in pediatric and adolescent with Crohn's disease: preliminary report. *International Journal of Colorectal Disease*, *26*(11), 1445-1451. doi:10.1007/s00384-011-1255-z

Diamanti, A., Panetta, F., Gandullia, P., Morini, F., Noto, C., Torre, G., Lezo, A., Goffredo, B., Daniele, A., & Gambarara, M. (2011b). Plasma citrulline as marker of bowel adaptation in children with short bowel syndrome. *Langenbeck's Archives of Surgery*, *396*(7), 1041-1046. doi:10.1007/s00423-011-0813-8

Dillon, E. L., Knabe, D. A., & Wu, G. (1999). Lactate inhibits citrulline and arginine synthesis from proline in pig enterocytes. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *276*(5), G1079-G1086. doi:10.1152/ajpgi.1999.276.5.G1079

Driscoll, W. C. (1996). Robustness of the ANOVA and Tukey-Kramer statistical tests. *Computers & Industrial Engineering*, *31*(1-2), 265-268. doi:10.1016/0360-8352(96)00127-1

Dugan, M. E., Knabe, D. A., & Wu, G. (1995). The induction of citrulline synthesis from glutamine in enterocytes of weaned pigs is not due primarily to age or change in diet. *Journal of Nutrition*, *125*(9), 2388-2393. doi:10.1093/jn/125.9.2388

Edmonds, M. S., Lowry, K. R., & Baker, D. H. (1987). Urea cycle metabolism: effects of supplemental ornithine or citrulline on performance, tissue amino acid concentrations and enzymatic activity in young pigs fed arginine-deficient diets. *Journal of Animal Science*, *65*(3), 706-716. doi:10.2527/jas1987.653706x

Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *British Medical Journal*, *315*(7109), 629-634. doi:10.1136/bmj.315.7109.629

El Khadem, H., & Abdel Rahman, M. M. A. (1963). Constituents of the Fruit of Citrullus colocynthis. *Journal of the Chemical Society, (Pt 4)*, 4991-4993. doi:10.1039/JR9630004979

Elkhatib, I., & Buchman, A. L. (2012). Plasma citrulline concentration as a marker for disease activity in patients with Crohn's disease. *Journal of Clinical Gastroenterology*, *46*(4), 308-310. doi:10.1097/MCG.0b013e31822b80e0

EMEA. (1997). Guideline for Good Clinical Practice. ICH Topic E 6. European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit, London, UK

Englund, A., Rogvi, R. á., Melgaard, L., & Greisen, G. (2014). Citrulline concentration in routinely collected neonatal dried blood spots cannot be used to predict necrotising enterocolitis. *Acta Paediatrica*, *103*(11), 1143-1147. doi:10.1111/apa.12750

Fang, Z., Huang, F., Luo, J., Wei, H., Ma, L., Jiang, S., & Peng, J. (2010). Effects of dl-2-hydroxy-4-methylthiobutyrate on the first-pass intestinal metabolism of dietary methionine and its extra-intestinal availability. *British Journal of Nutrition*, *103*(5), 643-651. doi:10.1017/S0007114509992169

Faraone, S. V. (2008). Interpreting estimates of treatment effects: implications for managed care. *Pharmacy and Therapeutics*, *33*(12), 700-711.

Faure, C., Raynaud-Simon, A., Ferry, A., Dauge, V., Cynober, L., Aussel, C., & Moinard, C. (2012). Leucine and citrulline modulate muscle function in malnourished aged rats. *Amino Acids*, *42*(4), 1425-1433. doi:10.1007/s00726-011-0841-2

Fearon, W. R. (1939). The carbamido diacetyl reaction: a test or citrulline. *Biochemical Journal*, *33(Pt 6)*, 902-907. doi:10.1042/bj0330902

Felig, P., Wahren, J., & Räf, L. (1973). Evidence of inter-organ amino-acid transport by blood cells in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *70*(6), 1775-1779. doi:10.1073/pnas.70.6.1775

Figueroa, A., Trivino, J. A., Sanchez-Gonzalez, M. A., & Vicil, F. (2009). Oral L-Citrulline Supplementation Attenuates Blood Pressure Response to Cold Pressor Test in Young Men. *American Journal of Hypertension*, *23*(1), 12-16. doi:10.1038/ajh.2009.195

Fijlstra, M., Rings, E. H., Verkade, H. J., van Dijk, T. H., Kamps, W. A., & Tissing, W. J. (2011). Lactose maldigestion during methotrexate-induced gastrointestinal mucositis in a rat model. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *300*(2), G283-G291. doi:10.1152/ajpgi.00462.2010

Fijlstra, M., Rings, E. H., van Dijk, T. H., Plosch, T., Verkade, H. J., & Tissing, W. J. (2013). Continuous enteral administration can overcome the limited capacity to absorb glucose in rats with methotrexate-induced gastrointestinal mucositis. *Supportive Care in Cancer*, *21*(3), 863-871. doi:10.1007/s00520-012-1597-2

Fishbein, T. M. (2009). Intestinal Transplantation. *New England Journal of Medicine*, *361*(10), 998-1008. doi:10.1056/NEJMra0804605

Fisher, R. A. (1935). The design of experiment. Oliver and Boyd, [S.l.]

Fisher, R. A., & Yates, F. (1963). Statistical tables for biological, agricultural and medical research. 6th edn. Oliver and Boyd, Edinburgh

Fitzgibbons, S., Ching, Y. A., Valim, C., Zhou, J., Iglesias, J., Duggan, C., & Jaksic, T. (2009). Relationship between serum citrulline levels and progression to parenteral nutrition independence in children with short bowel syndrome. *Journal of Pediatric Surgery*, *44*(5), 928-932. doi:10.1016/j.jpedsurg.2009.01.034

Flynn, N. E., & Wu, G. (1996). An important role for endogenous synthesis of arginine in maintaining arginine homeostasis in neonatal pigs. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *271*(5), R1149-R1155. doi:10.1152/ajpregu.1996.271.5.R1149

Flynn, N. E., & Wu, G. (1997). Enhanced metabolism of arginine and glutamine in enterocytes of cortisol-treated pigs. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *272*(3), G474-G480. doi:10.1152/ajpgi.1997.272.3.G474

Foulks, C. J. (2003). Amino acid solutions for acute renal failure. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 705-716). London: CRC Press. doi:10.1201/9780203010266.ch41

Fragkos, K. C., & Forbes, A. (2011a). Was citrulline first a laxative substance? The truth about modern citrulline and its isolation. *Journal of Japanese History of Medicine, 57*(3), 275-292.

Fragkos, K. C., & Forbes, A. (2011b). Are Citrulline Plasma Levels a Marker of Small Bowel Length and Absorptive Capacity? A Meta-Analysis. Paper presented at the UCL Biomedical Sciences Conference, 28/2-1/3 London, UK.

Fragkos, K. C., Tsagris, M., & Frangos, C. C. (2014). Publication Bias in Meta-Analysis: Confidence Intervals for Rosenthal's Fail-Safe Number. *International Scholarly Research Notices, 2014*, 1-17. doi:10.1155/2014/825383

Fragkos, K. C., Tsagris, M., & Frangos, C. C. (2016). Exploring the distribution for the estimator of Rosenthal's 'fail-safe' number of unpublished studies in meta-analysis. *Communications in Statistics - Theory and Methods*, *46*(11), 5672-5684. doi:10.1080/03610926.2015.1109664

French, J. M. (1882). Selections: Hypodermic Administration of Cathartics. *Cincinnati Lancet-Clinic*, *48*, 136.

Fu, Q., Berbee, M., Boerma, M., Wang, J., Schmid, H. A., & Hauer-Jensen, M. (2009). The somatostatin analog SOM230 (pasireotide) ameliorates injury of the intestinal mucosa and increases survival after total-body irradiation by inhibiting exocrine pancreatic secretion. *Radiation Research, 171*(6), 698-707. doi:10.1667/RR1685.1

Fujita, T., & Yanaga, K. (2007). Association between glutamine extraction and release of citrulline and glycine by the human small intestine. *Life Sciences, 80*(20), 1846-1850. doi:10.1016/j.lfs.2007.02.017

Fürst, P. (2000). Conditionally indispensable amino acids (glutamine, cyst(e)ine, tyrosine, arginine, ornithine, taurine) in enteral feeding and the dipeptide concept. *Nestlé Nutrition Workshop Series Clinical & Performance Programme*, *3*, 199-219. doi:10.1159/000061808

Fürst, P. (2001). New developments in glutamine delivery. *Journal of Nutrition, 131*(9 Suppl), 2562S-2568S. doi:10.1093/jn/131.9.2562S

Fürst, P., & Stehle, P. (2003). Glutamine and glutamine-containing dipeptides. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 613-632). London: CRC Press. doi:10.1201/9780203010266.ch36

Ganapathy, V., Inoue, K., Prasad, P. D., & Ganapathy, M. (2003). Cellular uptake of amino acids. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 63-82). London: CRC Press. doi:10.1201/9780203010266.ch4

Gates, C. E. (1995). What Really is Experimental Error in Block Designs? *American Statistician, 49*(4), 362-363. doi:10.1080/00031305.1995.10476183

Geng, M., Li, T., Kong, X., Song, X., Chu, W., Huang, R., Yin, Y., & Wu, G. (2011). Reduced expression of intestinal N-acetylglutamate synthase in suckling piglets: a novel molecular mechanism for arginine as a nutritionally essential amino acid for neonates. *Amino Acids*, *40*(5), 1513-1522. doi:10.1007/s00726-010-0761-6

Giesbrecht, F. G., & Gumpertz, M. L. (2004). Planning, Construction, and Statistical Analysis of Comparative Experiments. Wiley Series in Probability and Statistics. John Wiley & Sons, Hoboken, NJ. doi:10.1002/0471476471

Gijsman, H. J., Cohen, A. F., & Van Gerven, J. M. A. (2004). The Application of the Principles of Clinical Drug Development to Pharmacological Challenge Tests of the Serotonergic System. *Journal of Psychopharmacology*, *18*(1), 7-13. doi:10.1177/0269881104040205

Gilroy, R., O'Keefe, S. J., McGraw, N., Chu, H., Jeejeebhoy, K. N., & Messing, B. (2009). Citrulline: A Potential Predictor of Reductions in Parenteral Nutrition Achieved in Chronic Parenteral Nutrition Dependant Patients with Short Bowel Syndrome

(SBS) Treated with Teduglutide. *Gastroenterology*, *136*(5(Supplement 1)), A139-A140. doi:10.1016/S0016-5085(09)60627-2

Gleeson, M., & Jeukendrup, A. E. (2003). Amino acid requirements in sport. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 497-518). London: CRC Press. doi:10.1201/9780203010266.ch30

Goldfain, D., Lavergne, A., Galian, A., Chauveinc, L., & Prudhomme, F. (1989). Peculiar acute toxic colitis after ingestion of colocynth: a clinicopathological study of three cases. *Gut*, *30*(10), 1412-1418. doi:10.1136/gut.30.10.1412

Gondolesi, G., Fishbein, T., Chehade, M., Tschernia, A., Magid, M., Kaufman, S., Raymond, K., Sansaricq, C., & LeLeiko, N. (2002). Serum citrulline is a potential marker for rejection of intestinal allografts. *Transplantation Proceedings*, *34*(3), 918-920. doi:10.1016/S0041-1345(02)02669-6

Gondolesi, G., Ghirardo, S., Raymond, K., Hoppenhauer, L., Surillo, D., Rumbo, C., Fishbein, T., Sansaricq, C., & Sauter, B. (2006). The value of plasma citrulline to predict mucosal injury in intestinal allografts. *American Journal of Transplantation*, *6*(11), 2786-2790. doi:10.1111/j.1600-6143.2006.01513.x

Gondolesi, G. E., Kaufman, S. S., Sansaricq, C., Magid, M. S., Raymond, K., Iledan, L. P., Tao, Y., Florman, S. S., LeLeiko, N. S., & Fishbein, T. M. (2004). Defining Normal Plasma Citrulline in Intestinal Transplant Recipients. *American Journal of Transplantation*, *4*(3), 414-418. doi:10.1111/j.1600-6143.2004.00338.x

Gong, J.-f., Zhu, W.-m., Li, N., Liu, F.-n., Tan, L., Luo, N., & Li, J.-s. (2005). Serum citrulline is a simple quantitative marker for small intestinal enterocytes mass and absorption function in short bowel patients. *Journal of Surgical Research*, *127*(2), 177-182. doi:10.1016/j.jss.2005.04.004

Gong, J.-f., Zhu, W.-m., Li, N., Liu, F.-n., Tan, L., Luo, N., & Li, J.-s. (2007). [Serum citrulline: a potential marker for intestinal epithelial mass and absorption capacity in short bowel syndrome patients]. *Chinese Journal of Gastrointestinal Surgery*, *10*(4), 333-337. doi:10.3760/cma.j.issn.1671-0274.2007.04.009

Gong, J.-f., Zhu, W.-m., Yu, W.-k., Li, N., & Li, J.-s. (2009). Role of enteral nutrition in adult short bowel syndrome undergoing intestinal rehabilitation: the long-term outcome. *Asia Pacific Journal of Clinical Nutrition*, *18*(2), 155-163.

Goodlad, R. A., Nightingale, J. M. D., & Playford, R. J. (2001). Intestinal adaptation. *Intestinal Failure*, 243-262.

Goossens, L., Bouvry, M., Vanhaesebrouck, P., Wuyts, B., Van Maele, G., & Robberecht, E. (2011). Citrulline levels in a paediatric age group: Does measurement on dried blood spots have additional value? *Clinica Chimica Acta*, *412*(7-8), 661-664. doi:10.1016/j.cca.2010.11.021

Gosselin, K. B., Feldman, H. A., Sonis, A. L., Bechard, L. J., Kellogg, M. D., Gura, K., Venick, R., Gordon, C. M., Guinan, E. C., & Duggan, C. (2014). Serum citrulline as a biomarker of gastrointestinal function during hematopoietic cell transplantation in children. *Journal of Pediatric Gastroenterology and Nutrition*, *58*(6), 709-714. doi:10.1097/mpg.0000000000000335

Goulet, O., & Sauvat, F. (2006). Short bowel syndrome and intestinal transplantation in children. *Current Opinion in Clinical Nutrition and Metabolic Care*, *9*(3), 304-313. doi:10.1097/01.mco.0000222116.68912.fc

Goulet, O., Fusaro, F., Lacaille, F., & Sauvat, F. (2008). Permanent intestinal failure. *Indian Pediatrics*, *45*(9), 753-763.

Grider, J. R., & Jin, J.-G. (1993). Vasoactive intestinal peptide release and L-citrulline production from isolated ganglia of the myenteric plexus: evidence for regulation of vasoactive intestinal peptide release by nitric oxide. *Neuroscience*, *54*(2), 521-526. doi:10.1016/0306-4522(93)90271-G

Grimaldi, D., Guivarch, E., Neveux, N., Fichet, J., Pene, F., Marx, J. S., Chiche, J. D., Cynober, L., Mira, J. P., & Cariou, A. (2013). Markers of intestinal injury are associated with endotoxemia in successfully resuscitated patients. *Resuscitation*, *84*(1), 60-65. doi:10.1016/j.resuscitation.2012.06.010

Grimble, G. K. (2003). Quantitative and qualitative aspects of nitrogen supply in enteral nutrition in relation to free amino acids and peptides. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 529-556). London: CRC Press. doi:10.1201/9780203010266.ch32

Guihot, G., Blachier, F., Colomb, V., Morel, M.-T., Raynal, P., Corriol, O., Ricour, C., & Duée, P.-H. (1997). Effect of an elemental vs a complex diet on L-citrulline production from L-arginine in rat isolated enterocytes. *Journal of Parenteral and Enteral Nutrition*, *21*(6), 316-323. doi:10.1177/0148607197021006316

Gutierrez, I. M., Fisher, J. G., Ben-Ishay, O., Jones, B. A., Kang, K. H., Hull, M. A., Shillingford, N., Zurakowski, D., Modi, B. P., & Jaksic, T. (2014). Citrulline levels following proximal versus distal small bowel resection. *Journal of Pediatric Surgery*, *49*(5), 741-744. doi:10.1016/j.jpedsurg.2014.02.056

Haarlem. Koloniaal museum. [from old catalog], Ritsema, I. C., Sack, J., & Greshoff, M. (1905). Index phytochemicus. Uitgave van het laboratorium gedrukt en in den boekhandel verkrijgbaar bij J. H. de Bussey, Amsterdam,

Hader, R. J. (1973). An Improper Method of Randomization in Experimental Design. *American Statistician*, *27*(2), 82-84. doi:10.1080/00031305.1973.10478990

Harbord, R. M., Deeks, J. J., Egger, M., Whiting, P., & Sterne, J. A. C. (2007). A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics*, *8*(2), 239-251. doi:10.1093/biostatistics/kxl004

Hatam, N. A. R., Whiting, D. A., & Yousif, N. J. (1989). Cucurbitacin glycosides from Citrullus colocynthis. *Phytochemistry*, *28*(4), 1268-1271. doi:10.1016/0031-9422(89)80230-4

Hatam, N. A. R., Whiting, D. A., & Yousif, N. J. (1990). Lipids and Sterols of Citrullus colocynthis. *International Journal of Crude Drug Research*, *28*(3), 183-184. doi:10.3109/13880209009082809

Herbers, A. H., Blijlevens, N. M., Donnelly, J. P., & de Witte, T. J. (2008). Bacteraemia coincides with low citrulline concentrations after high-dose melphalan in autologous HSCT recipients. *Bone Marrow Transplantation*, *42*(5), 345-349. doi:10.1038/bmt.2008.170

Herbers, A. H., Feuth, T., Donnelly, J. P., & Blijlevens, N. M. (2010). Citrulline-based assessment score: first choice for measuring and monitoring intestinal failure after high-dose chemotherapy. *Annals of Oncology*, *21*(8), 1706-1711. doi:10.1093/annonc/mdp596

Herzfeld, A., & Raper, S. M. (1976). Enzymes of ornithine metabolism in adult and developing rat intestine. *Biochimica et Biophysica Acta*, *428*(3), 600-610. doi:10.1016/0304-4165(76)90188-4

Heyland, D. K., Dhaliwal, R., Dover, J. W., Gramlich, L., & Dodek, P. (2003). Canadian clinical practice guidelines for nutrition support in mechanically ventilated, critically ill adult patients. *Journal of Parenteral and Enteral Nutrition*, *27*(5), 355-373. doi:10.1177/0148607103027005355

Hibi, T., Nishida, S., Garcia, J., Tryphonopoulos, P., Tekin, A., Selvaggi, G., Weppler, D., Levi, D. M., Ruiz, P., & Tzakis, A. G. (2012). Citrulline level is a potent indicator of acute rejection in the long term following pediatric intestinal/multivisceral transplantation. *American Journal of Transplantation*, *12*(Supplement 4), S27-32. doi:10.1111/j.1600-6143.2012.04155.x

Higgins, J. P. T., & Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine*, *21*(11), 1539-1558. doi:10.1002/sim.1186

Higgins, J. P. T., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *British Medical Journal*, *327*(7414), 557-560. doi:10.1136/bmj.327.7414.557

Higgins, J. P. T., Green, S., & Cochrane Collaboration. (2008). Cochrane handbook for systematic reviews of interventions. Cochrane book series. Wiley-Blackwell, Chichester, England ; Hoboken, NJ

Hiller, A. (1882). Ueber die subcutane Anwendung von Abführmitteln. *Zeitschrift für Klinische Medicin*, *4*(4), 481-497.

Hills, M., & Armitage, P. (1979). The two-period cross-over clinical trial. *British Journal of Clinical Pharmacology*, *8*(1), 7-20. doi:10.1111/j.1365-2125.1979.tb05903.x

Hoenig, L. J., & Boyle, J. D. (1988). The life and death of Ismar Boas. *Journal of Clinical Gastroenterology*, *10*(1), 16-24. doi:10.1097/00004836-198802000-00006

Høime Hansen, S. (2003). Taurine homeostasis and its importance for physiological functions. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 739-748). London: CRC Press. doi:10.1201/9780203010266.ch44

Holeček, M. (2003). Contribution of amino acids to ketogenesis. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 97-110). London: CRC Press. doi:10.1201/9780203010266.ch6

Hollander, N. (1927). Beiträge zur Frage der pharmakologischen Eigenschaften des Koloquints. *Skandinavisches Archiv Für Physiologie*, *50*(1), 155-196. doi:10.1111/j.1748-1716.1927.tb00212.x

Hozyasz, K. K., Szaflarska-Popławska, A., Ołtarzewski, M., Mazur, J., Müller, L., Jabłońska, E., & Milanowski, A. (2006). [Whole blood citrulline levels in patients with coeliac disease]. *Polski Merkuriusz Lekarski*, *20*(116), 173-175.

Hozyasz, K. K., Oltarzewski, M., & Lugowska, I. (2010). Whole blood citrulline concentrations in newborns with non-syndromic oral clefts - a preliminary report. *Asia Pacific Journal of Clinical Nutrition*, *19*(2), 217-222.

Hue, L., & Bertrand, L. (2003). Anabolic effects and signaling pathways triggered by amino acids in the liver. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 291-304). London: CRC Press. doi:10.1201/9780203010266.ch18

Huedo-Medina, T. B., Sanchez-Meca, J., Marin-Martinez, F., & Botella, J. (2006). Assessing heterogeneity in meta-analysis: Q statistic or I2 index? *Psychological Methods*, *11*(2), 193-206. doi:10.1037/1082-989X.11.2.193

Huisman-de Waal, G., Schoonhoven, L., Jansen, J., Wanten, G., & van Achterberg, T. (2007). The impact of home parenteral nutrition on daily life-a review. *Clinical Nutrition*, *26*(3), 275-288. doi:10.1016/j.clnu.2006.10.002

Hurt, R. T., Frazier, T. H., McClave, S. A., & Cave, M. C. (2011). Pharmaconutrition for the obese, critically ill patient. *Journal of Parenteral and Enteral Nutrition*, *35*(5 Supplement), 60S-72S. doi:10.1177/0148607111413775

Hurwitz, R., & Kretchmer, N. (1986). Development of arginine-synthesizing enzymes in mouse intestine. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *251*(1), G103-G110. doi:10.1152/ajpgi.1986.251.1.G103

Impellizzeri, G., Mangiafico, S., Oriente, G., Piattelli, M., Sciuto, S., Fattorusso, E., Magno, S., Santacroce, C., & Sica, D. (1975). Amino acids and low-molecular-weight carbohydrates of some marine red algae. *Phytochemistry*, *14*(7), 1549-1557. doi:10.1016/0031-9422(75)85349-0

Ioannou, H. P., Fotoulaki, M., Pavlitou, A., Efstratiou, I., & Augoustides-Savvopoulou, P. (2011). Plasma citrulline levels in paediatric patients with celiac disease and the effect of a gluten-free diet. *European Journal of Gastroenterology and Hepatology*, *23*(3), 245-249. doi:10.1097/MEG.0b013e3283438ad7

Ioannou, H. P., Diamanti, E., Piretzi, K., Drossou-Agakidou, V., & Augoustides-Savvopoulou, P. (2012). Plasma citrulline levels in preterm neonates with necrotizing enterocolitis. *Early Human Development*, *88*(7), 563-566. doi:10.1016/j.earlhumdev.2011.11.008

Jakobsson, S., Ahlberg, K., Taft, C., & Ekman, T. (2010). Exploring a link between fatigue and intestinal injury during pelvic radiotherapy. *Oncologist*, *15*(9), 1009-1015. doi:10.1634/theoncologist.2010-0097

Jalowiec, A., Murphy, S. P., & Powers, M. J. (1984). Psychometric assessment of the Jalowiec Coping Scale. *Nursing Research*, *33*(3), 157-161. doi:10.1097/00006199-198405000-00008

Jansen. (1889). Ueber Coloquinthen-Vergiftung. *Therapeutische Monatshefte*, *3*(1), 39-41.

Jeevanandam, M., Ramias, L., & Schiller, W. R. (1991). Altered plasma free amino acid levels in obese traumatized man. *Metabolism*, *40*(4), 385-390. doi:10.1016/0026-0495(91)90149-q

Jenicek, M. (1989). Meta-analysis in medicine Where we are and where we want to go. *Journal of Clinical Epidemiology*, *42*(1), 35-44. doi:10.1016/0895-4356(89)90023-1

Jeppesen, P. B., Langholz, E., & Mortensen, P. B. (1999). Quality of life in patients receiving home parenteral nutrition. *Gut*, *44*(6), 844-852.

Jeppesen, P. B., Tappenden, K. A., Gilroy, R., O'Keefe, S. J., Seidner, D. L., McGraw, N., Chu, H., & Messing, B. (2009). 897 Teduglutide, a Novel GLP-2 Analogue, Decreases Fecal Wet Weight, Sodium and Potassium Excretion in Short Bowel Syndrome (SBS) Patients Dependent On Parenteral Nutrition (PN). *Gastroenterology*, *136*(5(Supplement 1)), A139. doi:10.1016/S0016-5085(09)60626-0

Jeppesen, P. B., Gilroy, R., Pertkiewicz, M., Allard, J. P., Messing, B., & O'Keefe, S. J. (2011). Randomised placebo-controlled trial of teduglutide in reducing parenteral nutrition and/or intravenous fluid requirements in patients with short bowel syndrome. *Gut*, *60*(7), 902-914. doi:10.1136/gut.2010.218271

Jeppesen, P. B., Pertkiewicz, M., Messing, B., Iyer, K., Seidner, D. L., O'keefe, S. J. D., Forbes, A., Heinze, H., & Joelsson, B. (2012). Teduglutide reduces need for parenteral support among patients with short bowel syndrome with intestinal failure. *Gastroenterology*, *143*(6), 1473-1481 e1473. doi:10.1053/j.gastro.2012.09.007

Jeppesen, P. B., Pertkiewicz, M., Forbes, A., Pironi, L., Gabe, S. M., Joly, F., Messing, B., Loth, S., Youssef, N. N., Heinze, H., & Berghofer, P. (2013). Quality of life in patients with short bowel syndrome treated with the new glucagon-like peptide-2 analogue teduglutide--analyses from a randomised, placebo-controlled study. *Clinical Nutrition*, *32*(5), 713-721. doi:10.1016/j.clnu.2013.03.016

John-Baptiste, A., Huang, W., Kindt, E., Wu, A., Vitsky, A., Scott, W., Gross, C., Yang, A. H., Schaiff, W. T., & Ramaiah, S. K. (2012). Evaluation of potential gastrointestinal biomarkers in a PAK4 inhibitor-treated preclinical toxicity model to address unmonitorable gastrointestinal toxicity. *Toxicologic Pathololog*y, *40*(3), 482-490. doi:10.1177/0192623311432289

Jones, B., & Kenward, M. G. (2003). Design and analysis of cross-over trials. 2nd edn. Chapman & Hall/CRC, Boca Raton

Kábrt, J., Hyánek, J., Šťastná, S., & Pospíšilová, E. (2003). Plasma Citrulline Concentration as a Marker of Small Intestine Failure. *Biomedical Papers*, *147*(Supplement 1), 75.

Kalaitzakis, E., Carlsson, E., Josefsson, A., & Bosaeus, I. (2008). Quality of life in short-bowel syndrome: impact of fatigue and gastrointestinal symptoms. *Scandinavian Journal of Gastroenterology*, *43*(9), 1057-1065. doi:10.1080/00365520802078325

Kao, C., Hsu, J., Bandi, V., & Jahoor, F. (2013). Alterations in glutamine metabolism and its conversion to citrulline in sepsis. *American Journal of Physiology - Endocrinology and Metabolism*, *304*(12), E1359-1364. doi:10.1152/ajpendo.00628.2012

Kaore, S. N., Amane, H. S., & Kaore, N. M. (2013). Citrulline: pharmacological perspectives and its role as an emerging biomarker in future. *Fundamental & Clinical Pharmacology*, *27*(1), 35-50. doi:10.1111/j.1472-8206.2012.01059.x

Kaore, S. N., & Kaore, N. M. (2014). Chapter 53 - Citrulline: Pharmacological perspectives and role as a biomarker in diseases and toxicities. In: R. C. Gupta (Ed.), *Biomarkers in Toxicology* (pp. 883-905). Boston: Academic Press. doi:10.1016/B978-0-12-404630-6.00053-1

Karlik, J. B., Kesavan, A., Nieder, M. L., Hawks, R., Jin, Z., Bhatia, M., & Ladas, E. J. (2014). Plasma citrulline as a biomarker for enterocyte integrity in pediatric blood and BMT. *Bone Marrow Transplantation*, *49*(3), 449-450. doi:10.1038/bmt.2013.165

Keller, J., Panter, H., & Layer, P. (2004). Management of the short bowel syndrome after extensive small bowel resection. *Best Practice & Research Clinical Gastroenterology*, *18*(5), 977-992. doi:10.1016/j.bpg.2004.05.002

Kerem, M., Salman, B., Pasaoglu, H., Bedirli, A., Alper, M., Katircioglu, H., Atici, T., Percin, E. F., & Ofluoglu, E. (2008). Effects of microalgae Chlorella species crude extracts on intestinal adaptation in experimental short bowel syndrome. *World Journal of Gastroenterology*, *14*(28), 4512-4517. doi:10.3748/wjg.14.4512

Kershner, R. P., & Federer, W. T. (1981). Two-Treatment Crossover Designs for Estimating a Variety of Effects. *Journal of the American Statistical Association*, *76*(375), 612-619. doi:10.1080/01621459.1981.10477693

Keur, M. B., Beishuizen, A., & van Bodegraven, A. A. (2010). Diagnosing malabsorption in the intensive care unit. *F1000 Medicine Reports*, *2*, 7. doi:10.3410/m2-7

Khan, M. A., Miserachs, M., Hofmeister, B., Nespor, C., Castillo, R., & Kerner, J. A. (2011). S79 - Degree of Parenteral Nutrition Support Correlates with Serum Citrulline Levels in Short Bowel Syndrome. *Journal of Parenteral and Enteral Nutrition*, *35*(1), 62. doi:10.1177/0148607110395865

Kinney, J. M. (2003). Introduction. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 1-16). London: CRC Press. doi:10.1201/9780203010266.ch0

Koga, Y., & Ohtake, R. (1914). [Study report on the constituents of squeezed watermelon]. *Tokyo Kagaku Kaishi [Journal of the Tokyo Chemical Society]*, *35*(5), 519-528. doi:10.1246/nikkashi1880.35.519

Köhler, E. S., Sankaranarayanan, S., van Ginneken, C. J., van Dijk, P., Vermeulen, J. L. M., Ruijter, J. M., Lamers, W. H., & Bruder, E. (2008). The human neonatal small intestine has the potential for arginine synthesis; developmental changes in the expression of arginine-synthesizing and -catabolizing enzymes. *BMC Developmental Biology*, *8*(11), 107. doi:10.1186/1471-213X-8-107

Kohlstock, P. (1892). Ueber subcutane und rectale Anwendung von Abführmitteln. *Charité-Annalen*, *17*, 283-294.

Kong, W., Wang, J., Ping, X., Shen, J., Ni, X., Liu, F., Yu, B., & Li, Y. (2015). Biomarkers for assessing mucosal barrier dysfunction induced by chemotherapy: Identifying a rapid and simple biomarker. *Clinical Laboratory*, *61*(3-4), 371-378. doi:10.7754/Clin.Lab.2014.140712

Kornberg, H. (2000). Krebs and his trinity of cycles. *Nature Reviews Molecular Cell Biology*, *1*(3), 225-228. doi:10.1038/35043073

Krebs, H. A., & Henseleit, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie*, *210*(1-2), 33-66. doi:10.1515/bchm2.1932.210.1-2.33

Lankisch, P. G. (2009). Secretion and absorption (methods and functions). *Best Practice & Research Clinical Gastroenterology*, *23*(3), 325-335. doi:10.1016/j.bpg.2009.03.001

Lardy, H., Mouille, B., Thomas, M., Darcy-Vrillon, B., Vaugelade, P., Blachier, F., Bernard, F., Cherbuy, C., Robert, V., Corriol, O., Ricour, C., Goulet, O., Duee, P. H., & Colomb, V. (2004). Enterocyte metabolism during early adaptation after extensive intestinal resection in a rat model. *Surgery*, *135*(6), 649-656. doi:10.1016/j.surg.2003.11.013

Largillière, C., Houssin, D., Gottrand, F., Mathey, C., Checoury, A., Alagille, D., & Farriaux, J. P. (1989). Liver transplantation for ornithine transcarbamylase deficiency in a girl. *Journal of Pediatrics*, *115*(3), 415-417. doi:10.1016/S0022-3476(89)80843-1

Laska, E., Meisner, M., & Kushner, H. B. (1983). Optimal Crossover Designs in the Presence of Carryover Effects. *Biometrics*, *39*(4), 1087-1091. doi:10.2307/2531343

Laska, E. M., & Meisner, M. (1985). A Variational Approach to Optimal Two-Treatment Crossover Designs: Application to Carryover-Effect Models. *Journal of the*

American Statistical Association, *80*(391), 704-710. doi:10.1080/01621459.1985.10478172

Lee, C. H., Hsiao, C. C., Hung, C. Y., Chang, Y. J., & Lo, H. C. (2012). Long-term enteral arginine supplementation in rats with intestinal ischemia and reperfusion. *Journal of Surgical Research*, *175*(1), 67-75. doi:10.1016/j.jss.2011.02.003

Lee, E. H., Ko, J. S., & Seo, J. K. (2013). Correlations of plasma citrulline levels with clinical and endoscopic score and blood markers according to small bowel involvement in pediatric Crohn disease. *Journal of Pediatric Gastroenterology and Nutrition*, *57*(5), 570-575. doi:10.1097/MPG.0b013e31829e264e

Lescher, F. H. (1884). Recent materia medica: notes on their origin and therapeutics. Churchill, London

Leverve, X. M. (2003). Amino acid metabolism and gluconeogenesis. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn). London: CRC Press. doi:10.1201/9780203010266.pt2

Levin, R. J. (1994). Digestion and absorption of carbohydrates—from molecules and membranes to humans. *American Journal of Clinical Nutrition*, *59*(3 Supplement), 690S-698S. doi:10.1093/ajcn/59.3.690S

Leyden, E. v., & Klemperer, F. (1907). Die Deutsche Klinik am Eingange des zwanzigsten Jahrhunderts in akademischen Vorlesungen. Urban & Schwarzenberg, Berlin; Wien

Ligthart-Melis, G. C., van de Poll, M. C. G., Dejong, C. H. C., Boelens, P. G., Deutz, N. E. P., & van Leeuwen, P. A. M. (2007). The route of administration (enteral or parenteral) affects the conversion of isotopically labeled L-[2-15N]glutamine into citrulline and arginine in humans. *Journal of Parenteral and Enteral Nutrition*, *31*(5), 343-348; discussion 349-350. doi:10.1177/0148607107031005343

Ligthart-Melis, G. C., & Deutz, N. E. P. (2011). Is glutamine still an important precursor of citrulline? *American Journal of Physiology - Endocrinology and Metabolism*, *301*(2), E264-266. doi:10.1152/ajpendo.00223.2011

Lin, P. W., & Stoll, B. J. (2006). Necrotising enterocolitis. *Lancet*, *368*(9543), 1271-1283. doi:10.1016/S0140-6736(06)69525-1

Lipsey, M. W., & Wilson, D. B. (2001). Practical meta-analysis. Applied social research methods series; vol 49. Sage Publications, Inc, Thousand Oaks, CA, US

Liu, F.-N., Tan, L., Luo, N., Jiang, F., Li, Y.-X., & Li, Y.-S. (2004). Determination of serum citrulline levels in normal Chinese people with HPLC. *Parenteral & Enteral Nutrition*, *11*(2), 116-117. doi:10.3969/j.issn.1007-810X.2004.02.016

Ljungqvist, O., & Pichard, C. (2008). Inspired by the pioneers: the lesson of the Judy Taylor story. *Clinical Nutrition*, *27*(3), 319-320. doi:10.1016/j.clnu.2008.04.008

Lloyd, A., Kerr, C., Breheny, K., Brazier, J., Ortiz, A., & Borg, E. (2014). Economic evaluation in short bowel syndrome (SBS): an algorithm to estimate utility scores for a patient-reported SBS-specific quality of life scale (SBS-QoL). *Quality of Life Research*, *23*(2), 449-458. doi:10.1007/s11136-013-0516-4

Long, C. L., Haverberg, L. N., Young, V. R., Kinney, J. M., Munro, H. N., & Geiger, J. W. (1975). Metabolism of 3-methylhistidine in man. *Metabolism*, *24*(8), 929-935. doi:10.1016/0026-0495(75)90084-0

Luiking, Y. C., Poeze, M., Ramsay, G., & Deutz, N. E. P. (2009). Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production. *American Journal of Clinical Nutrition*, *89*(1), 142-152. doi:10.3945/ajcn.2007.25765

Lundy, J., Chung, K. K., Cancio, L. C., White, C. E., & Ziegler, T. R. (2012). Observations on serial plasma citrulline concentrations in a patient with intestinal ischemia and full-thickness necrosis after severe thermal injury. *Journal of Burn Care & Research*, *33*(6), e316-318. doi:10.1097/BCR.0b013e31824ff9a5

Luo, M., Fernández-Estívariz, C., Manatunga, A. K., Bazargan, N., Gu, L. H., Jones, D. P., Klapproth, J. M., Sitaraman, S. V., Leader, L. M., Galloway, J. R., & Ziegler, T. R. (2007). Are plasma citrulline and glutamine biomarkers of intestinal absorptive

function in patients with short bowel syndrome? *Journal of Parenteral and Enteral Nutrition*, *31*(1), 1-7. doi:10.1177/014860710703100101

Lutgens, L., Granzier, M., Beets-Tan, R., De Ruysscher, D., Berger, M., Cleutjens, J., von Meyenfeldt, M., Deutz, N., & Lambin, P. (2002). 638 Poster Plasma citrulline levels correlate with CT scan based small bowel volume parameters. Results of a pilot study in ten patients. *Radiotherapy and Oncology*, *64*(Supplement 1), S196. doi:10.1016/S0167-8140(02)82946-2

Lutgens, L., & Lambin, P. (2007). Biomarkers for radiation-induced small bowel epithelial damage: An emerging role for plasma citrulline. *World Journal of Gastroenterology*, *13*(22), 3033-3042. doi:10.3748/wjg.v13.i22.3033

Lutgens, L. C. H. W., Deutz, N. E. P., Gueulette, J., Cleutjens, J. P. M., Berger, M. P. F., Wouters, B. G., von Meyenfeldt, M. F., & Lambin, P. (2003). Citrulline: A physiologic marker enabling quantitation and monitoring of epithelial radiation-induced small bowel damage. *International Journal of Radiation Oncology, Biology, Physics*, *57*(4), 1067-1074. doi:10.1016/S0360-3016(03)00781-8

Lutgens, L. C. H. W., Deutz, N., Granzier-Peeters, M., Beets-Tan, R., De Ruysscher, D., Gueulette, J., Cleutjens, J., Berger, M., Wouters, B., von Meyenfeldt, M., & Lambin, P. (2004). Plasma citrulline concentration: a surrogate end point for radiation-induced mucosal atrophy of the small bowel. A feasibility study in 23 patients. *International Journal of Radiation Oncology, Biology, Physics*, *60*(1), 275-285. doi:10.1016/j.ijrobp.2004.02.052

Lutgens, L. C. H. W., Blijlevens, N. M. A., Deutz, N. E. P., Donnelly, J. P., Lambin, P., & de Pauw, B. E. (2005). Monitoring myeloablative therapy-induced small bowel toxicity by serum citrulline concentration: a comparison with sugar permeability tests. *Cancer*, *103*(1), 191-199. doi:10.1002/cncr.20733

Mackenzie, M., & Baracos, V. E. (2003). Cancer-associated cachexia. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 339-354). London: CRC Press. doi:10.1201/9780203010266.pt3

Mahoney, E. J., & Albina, J. E. (2003). Nitric oxide. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 211-240). London: CRC Press. doi:10.1201/9780203010266.ch14

Malaisse, W. J. (2003). Amino acid-mediated insulin secretion. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 321-338). London: CRC Press. doi:10.1201/9780203010266.ch20

Malo, C. (1994). Free amino acid levels in serum and small intestine during the post-natal development of normal and sparse-fur mutant mice. *Comparative Biochemistry and Physiology - Part A: Physiology*, *109*(4), 1049-1057. doi:10.1016/0300-9629(94)90254-2

Mandel, H., Levy, N., Izkovitch, S., & Korman, S. H. (2005). Elevated plasma citrulline and arginine due to consumption of Citrullus vulgaris (watermelon). *Journal of Inherited Metabolic Disease*, *28*(4), 467-472. doi:10.1007/s10545-005-0467-1

Mao, H.-m., Wei, W., Xiong, W.-j., Lu, Y., Chen, B.-g., & Liu, Z. (2010). Simultaneous determination of l-citrulline and l-arginine in plasma by high performance liquid chromatography. *Clinical Biochemistry*, *43*(13-14), 1141-1147. doi:10.1016/j.clinbiochem.2010.05.017

Marini, J. C., Didelija, I. C., Castillo, L., & Lee, B. (2010). Plasma arginine and ornithine are the main citrulline precursors in mice infused with arginine-free diets. *Journal of Nutrition*, *140*(8), 1432-1437. doi:10.3945/jn.110.125377

Marini, J. C., Keller, B., Didelija, I. C., Castillo, L., & Lee, B. (2011). Enteral arginase II provides ornithine for citrulline synthesis. *American Journal of Physiology - Endocrinology and Metabolism*, *300*(1), E188-194. doi:10.1152/ajpendo.00413.2010

Marini, J. C. (2012). Arginine and ornithine are the main precursors for citrulline synthesis in mice. *Journal of Nutrition*, *142*(3), 572-580. doi:10.3945/jn.111.153825

Marini, J. C., Stoll, B., Didelija, I. C., & Burrin, D. G. (2012). De novo synthesis is the main source of ornithine for citrulline production in neonatal pigs. *American*

Journal of Physiology - Endocrinology and Metabolism, *303*(11), E1348-1353. doi:10.1152/ajpendo.00399.2012

Marmot, M., Allen, J., Bell, R., Bloomer, E., Goldblatt, P., Consortium for the European Review of Social Determinants of, H., & the Health, D. (2012). WHO European review of social determinants of health and the health divide. *Lancet*, *380*(9846), 1011-1029. doi:10.1016/S0140-6736(12)61228-8

Matthews, J. N. S. (1988). Recent Developments in Crossover Designs. *International Statistical Review / Revue Internationale de Statistique*, *56*(2), 117-127. doi:10.2307/1403636

Meijer, A. J. (2003). Ureagenesis and ammoniagenesis. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 111-122). London: CRC Press. doi:10.1201/9780203010266.ch7

Meijer, A. J., & Dubbelhuis, P. E. (2003). Amino acid signaling and the control of protein metabolism. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 253-274). London: CRC Press. doi:10.1201/9780203010266.ch16

Miceli, E., Poggi, N., Missanelli, A., Bianchi, P., Moratti, R., & Corazza, G. R. (2008). Is serum citrulline measurement clinically useful in coeliac disease? . *Internal and Emergency Medicine*, *3*(3), 233-236. doi:10.1007/s11739-008-0155-x

Middleton, R. (1893). Extracts from German periodicals: Purgative agents. *American Veterinary Review*, *17*(June), 138-139.

Middleton, S. J., & Jamieson, N. V. (2005). The current status of small bowel transplantation in the UK and internationally. *Gut*, *54*(11), 1650-1657. doi:10.1136/gut.2004.062612

Mithieux, G. (2001). New data and concepts on glutamine and glucose metabolism in the gut. *Current Opinion in Clinical Nutrition and Metabolic Care*, *4*(4), 267-271. doi:10.1097/00075197-200107000-00004

Mittal, N., Kanwar, S. S., & Sanyal, S. N. (2008). Effect of nonsteroidal anti-inflammatory drugs and the procarcinogen 1,2-dimethylhydrazine on the antioxidant defense system. *International Journal of Toxicology*, *27*(2), 169-174. doi:10.1080/10915810801977880

Moerk, F. X. (1893). Gleanings from the German journals: Medicated glycerin suppositories. *American Journal of Pharmacy*, *65*(May), 225.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Journal of Clinical Epidemiology*, *62*(10), 1006-1012. doi:10.1016/j.jclinepi.2009.06.005

Moinard, C., Nicolis, I., Neveux, N., Le Guern, M. E., Darquy, S., Benazeth, S., & Cynober, L. (2005). L'étude citrudose: Pharmacocinétique de la citrulline après administration chez le volontaire sain. *Nutrition Clinique et Métabolisme*, *19*(Supplement 1), S59. doi:10.1016/j.nupar.2005.10.005

Moinard, C., & Cynober, L. (2007). Citrulline: A new player in the control of nitrogen homeostasis. *Journal of Nutrition*, *137*(6), 1621S-1625S. doi:10.1093/jn/137.6.1621S

Moinard, C., Nicolis, I., Neveux, N., Darquy, S., Bénazeth, S., & Cynober, L. (2008). Dose-ranging effects of citrulline administration on plasma amino acids and hormonal patterns in healthy subjects: the Citrudose pharmacokinetic study. *British Journal of Nutrition*, *99*(4), 855-862. doi:10.1017/S0007114507841110

Montgomery, D. C. (2009). Design and analysis of experiments. 7th ed., international ed. edn. John Wiley & Sons, Hoboken

Morse, W. H. (1882). New therapeutical agents. Davis, Detroit

Müller, W. A., Cüppers, H. J., Zimmermann-Telschow, H., Micheli, H., Wyss, T., Renold, A. E., & Berger, M. (1983). Amino acids and lipoproteins in plasma of duodenopancreatectomized patients: effects of glucagon in physiological amounts. *European Journal of Clinical Investigation*, *13*(2), 141-149. doi:10.1111/j.1365-2362.1983.tb00079.x

Muscaritoli, M., Rossi Fanelli, F., Meguid, M. M., & Campos, A. C. L. (2003). Amino acid requirement in cancer. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 689-704). London: CRC Press. doi:10.1201/9780203010266.ch40

Nadalin, S., Biglarnia, A. R., Testa, G., Koppara, T. R., Schaffer, R., Johnson, C., Toetsch, M., Broelsch, C. E., & Malago, M. (2007). Role and significance of plasma citrulline in the early phase after small bowel transplantation in pigs. *Transplant International*, *20*(5), 425-431. doi:10.1111/j.1432-2277.2007.00460.x

Naghibi, M., Smith, T. R., & Elia, M. (2015). A systematic review with meta-analysis of survival, quality of life and cost-effectiveness of home parenteral nutrition in patients with inoperable malignant bowel obstruction. *Clinical Nutrition*, *34*(5), 825-837. doi:10.1016/j.clnu.2014.09.010

Naimi, R. M., Madsen, K. B., Askov-Hansen, C., Brandt, C. F., Hartmann, B., Holst, J. J., Mortensen, P. B., & Jeppesen, P. B. (2013). A dose-equivalent comparison of the effects of continuous subcutaneous glucagon-like peptide 2 (GLP-2) infusions versus meal related GLP-2 injections in the treatment of short bowel syndrome (SBS) patients. *Regulatory Peptides*, *184*, 47-53. doi:10.1016/j.regpep.2013.03.023

Naini, A., Kaufmann, P., Shanske, S., Engelstad, K., De Vivo, D. C., & Schon, E. A. (2005). Hypocitrullinemia in patients with MELAS: an insight into the "MELAS paradox". *Journal of the Neurological Sciences*, *229-230*, 187-193. doi:10.1016/j.jns.2004.11.026

Nakamura, H., Kawamata, Y., Kuwahara, T., Torii, K., & Sakai, R. (2013). Nitrogen in dietary glutamate is utilized exclusively for the synthesis of amino acids in the rat intestine. *American Journal of Physiology - Endocrinology and Metabolism*, *304*(1), E100-108. doi:10.1152/ajpendo.00331.2012

Neu, J., & Walker, W. A. (2011). Necrotizing Enterocolitis. *New England Journal of Medicine*, *364*(3), 255-264. doi:10.1056/NEJMra1005408

Neveux, N., David, P., & Cynober, L. (2003). Measurement of amino acid concentrations in biological fluids and tissues using ion exchange chromatography. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 17-28). Boca Raton: CRC Press. doi:10.1201/9780203010266.pt1

Nightingale, J. (ed) (2001) Intestinal Failure. Greenwich, London

Nightingale, J., & Woodward, J. M. (2006). Guidelines for management of patients with a short bowel. *Gut*, *55*(Suppl 4), iv1-iv12. doi:10.1136/gut.2006.091108

Nightingale, J. M. D., Lennard-Jones, J. E., Gertner, D. J., Wood, S. R., & Bartram, C. I. (1992). Colonic preservation reduces need for parenteral therapy, increases incidence of renal stones, but does not change high prevalence of gall stones in patients with a short bowel. *Gut*, *33*(11), 1493-1497. doi:10.1136/gut.33.11.1493

Nightingale, J. M. D. (1994). The Sir David Cuthbertson Medal Lecture. Clinical problems of a short bowel and their treatment. *Proceedings of the Nutrition Society*, *53*(2), 373-391. doi:10.1079/PNS19940043

Nightingale, J. M. D. (2003a). Hepatobiliary, renal and bone complications of intestinal failure. *Bailliere's Best Practice and Research in Clinical Gastroenterology*, *17*(6), 907-929. doi:10.1016/S1521-6918(03)00108-2

Nightingale, J. M. D. (2003b). The medical management of intestinal failure: Methods to reduce the severity. *Proceedings of the Nutrition Society*, *62*(3), 703-710. doi:
10.1079/PNS2003283

Nion-Larmurier, I., Seksik, P., Sebbagh Humbert, V., Cardenas, D., Pernet, P., & Cosnes, J. (2007). La citrullinémie préopératoire est un marqueur du potentiel fonctionnel du grêle court après rétablissement de la continuité. *Gastroentérologie Clinique et Biologique*, *31*, A40.

Nissim, I., & Welbourne, T. (2003). Acidosis and amino acid metabolism. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical*

Nutrition (2nd edn, pp. 375-388). London: CRC Press. doi:10.1201/9780203010266.ch23

Noordally, S. O., Sohawon, S., Semlali, H., Michely, D., Devriendt, J., & Gottignies, P. (2012). Is There a Correlation Between Circulating Levels of Citrulline and Intestinal Dysfunction in the Critically Ill? *Nutrition in Clinical Practice*, *27*(4), 527-532. doi:10.1177/0884533612449360

Noto, C., Diamanti, A., Basso, M., Gambarara, M., Bracci, F., Papadatou, B., Ferretti, F., Panetta, F., & Castro, M. (2008a). O048 Clinical Role of Plasma Citrulline in Predicting Enteral Tolerance in Children with Small Bowel Syndrome. *Clinical Nutrition Supplements*, *3*(Supplement 1), 22-23. doi:10.1016/S1744-1161(08)70050-4

Noto, C., Diamanti, A., Basso, M. S., Gambarara, M., Bracci, F., Papadatou, B., Ferretti, F., Panetta, F., & Castro, M. (2008b). Clinical role of plasma citrulline in predicting enteral tolerance in children with small bowel syndrome. *Digestive and Liver Disease*, *40*(10), A62-A63. doi:10.1016/j.dld.2008.07.210

Obled, C., Papet, I., & Breuillé, D. (2003). Sulfur-containing amino acids and glutathione in diseases. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 667-688). London: CRC Press. doi:10.1201/9780203010266.ch39

Oehler, R., & Roth, E. (2003). Glutamine metabolism. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 169-184). London: CRC Press. doi:10.1201/9780203010266.ch11

Olieman, J. F., Penning, C., Poley, M. J., Utens, E. M., Hop, W. C., & Tibboel, D. (2012). Impact of infantile short bowel syndrome on long-term health-related quality of life: a cross-sectional study. *Journal of Pediatric Surgery*, *47*(7), 1309-1316. doi:10.1016/j.jpedsurg.2012.01.005

Oliverius, M., Kudla, M., Balaz, P., & Valsamis, A. (2010). [Plasma citrulline concentration--a reliable noninvasive marker of functional enterocyte mass]. *Casopis Lekaru Ceskych*, *149*(4), 160-162.

Onal, C., Kotek, A., Unal, B., Arslan, G., Yavuz, A., Topkan, E., & Yavuz, M. (2011). Plasma citrulline levels predict intestinal toxicity in patients treated with pelvic radiotherapy. *Acta Oncologica*, *50*(8), 1167-1174. doi:10.3109/0284186x.2011.584557

Osowska, S., Moinard, C., Neveux, N., Loï, C., & Cynober, L. (2004). Citrulline increases arginine pools and restores nitrogen balance after massive intestinal resection. *Gut*, *53*(12), 1781-1786. doi:10.1136/gut.2004.042317

Osowska, S., Duchemann, T., Walrand, S., Paillard, A., Boirie, Y., Cynober, L., & Moinard, C. (2006). Citrulline modulates muscle protein metabolism in old malnourished rats. *American Journal of Physiology - Endocrinology and Metabolism*, *291*(3), E582-E586. doi:10.1152/ajpendo.00398.2005

Osowska, S., Neveux, N., Nakib, S., Lasserre, V., Cynober, L., & Moinard, C. (2008). Impairment of arginine metabolism in rats after massive intestinal resection: effect of parenteral nutrition supplemented with citrulline compared with arginine. *Clinical Science*, *115*(5), 159-166. doi:10.1042/CS20070451

Ouelaa, W., Jegatheesan, P., M'Bouyou-Boungou, J., Vicente, C., Nakib, S., Nubret, E., & De Bandt, J. P. (2017). Citrulline decreases hepatic endotoxin-induced injury in fructose-induced non-alcoholic liver disease: an ex vivo study in the isolated perfused rat liver. *British Journal of Nutrition*, *117*(11), 1487-1494. doi:10.1017/S0007114517001453

Pan, L., Wang, X., Li, W., Li, N., & Li, J. (2010). The intestinal fatty acid binding protein diagnosing gut dysfunction in acute pancreatitis: a pilot study. *Pancreas*, *39*(5), 633-638. doi:10.1097/MPA.0b013e3181c79654

Panetta, F., Diamanti, A., Bracci, F., Gambarara, M., Daniele, A., Goffredo, B., Knafelz, D., Papadatou, B., Noto, C., & Torre, G. (2010). CO10 Postabsorbtive plasma citrulline: A surrogate marker of intestinal inflammation in children and

adolescents with Crohn's disease. *Digestive and Liver Disease*, *42*(Supplement 5), S325-S326. doi:10.1016/S1590-8658(10)60553-1

Papadia, C., Sherwood, R. A., Kalantzis, T., Volta, U., Fiorini, E., & Forbes, A. (2006a). Plasma Citrulline Concentration: A Simple, Sensitive, and Non-Invasive Method to Monitor Small Bowel Absorptive Function in Patients with Crohn's Disease. *Gut*, *55*(Supplement 2), A11.

Papadia, C., Sherwood, R. A., Kalantzis, T., Wallis, K., Volta, U., Fiorini, E., & Forbes, A. (2006b). Plasma Citrulline Concentration Is a Reliable Marker of Small Bowel Absorptive Capacity in Crohn's Disease and Is Independent of Intestinal Inflammation. *Gastroenterology*, *130*(4(Supplement 2)), A-611. doi:10.1016/S0016-5085(06)60008-5

Papadia, C., Sherwood, R. A., Kalantzis, C., Wallis, K., Volta, U., Fiorini, E., & Forbes, A. (2007). Plasma citrulline concentration: A reliable marker of small bowel absorptive capacity independent of intestinal inflammation. *American Journal of Gastroenterology*, *102*(7), 1474-1482. doi:10.1111/j.1572-0241.2007.01239.x

Papadia, C., Dhaliwal, W., Kelly, P., Corazza, G. R., Franze, A., & Di Sabatino, A. (2009a). Plasma Citrulline as a Quantitative Biomarker of HIV-Associated Duodenal Mucosal Damage in a Tropical Enteropathy Population. *Digestive and Liver Disease*, *41*(Supplement 1), S65-S66. doi:10.1016/S1590-8658(09)60170-5

Papadia, C., Kelly, P., Corazza, G. R., Franze, A., Forbes, A., & Di Sabatino, A. (2009b). Plasma Citrulline As Quantitative Biomarker of HIV Associate Villous Atrophy in a Tropical Enteropathy Population. *Gastroenterology*, *136*(5(Supplement 1)), A323. doi:10.1016/S0016-5085(09)61480-3

Papadia, C., Kelly, P., Caini, S., Corazza, G. R., Shawa, T., Franze, A., Forbes, A., & Di Sabatino, A. (2010). Plasma citrulline as a quantitative biomarker of HIV-associated villous atrophy in a tropical enteropathy population. *Clinical Nutrition*, *29*(6), 795-800. doi:10.1016/j.clnu.2010.04.008

Pappas, P. A., Saudubray, J. M., Tzakis, A. G., Rabier, D., Carreno, M. R., Gomez-Marin, O., Huijing, F., Gelman, B., Levi, D. M., Nery, J. R., Kato, T., Mittal, N., Nishida, S., Thompson, J. F., & Ruiz, P. (2001). Serum citrulline and rejection in small bowel transplantation: A preliminary report. *Transplantation*, *72*(7), 1212-1216. doi:10.1097/00007890-200110150-00005

Pappas, P. A., Saudubray, J. M., Tzakis, A. G., Rabier, D., Carreno, M. R., & Gomez-Marin, O. (2002). Serum citrulline as a marker of acute cellular rejection for intestinal transplantation. *Transplantation Proceedings*, *34*(3), 915-917. doi:10.1016/s0041-1345(02)02668-4

Pappas, P. A., G Tzakis, A., Gaynor, J. J., Carreno, M. R., Ruiz, P., Huijing, F., Kleiner, G., Rabier, D., Kato, T., Levi, D. M., Nishida, S., Gelman, B., Thompson, J. F., Mittal, N., & Saudubray, J.-M. (2004a). An analysis of the association between serum citrulline and acute rejection among 26 recipients of intestinal transplant. *American Journal of Transplantation*, *4*(7), 1124-1132. doi:10.1111/j.1600-6143.2004.00469.x

Pappas, P. A., Tzakis, A. G., Saudubray, J.-M., Gaynor, J. J., Carreno, M. R., Huijing, F., Kleiner, G., Rabier, D., Kato, T., Levi, D. M., Nishida, S., Gelman, B., Thompson, J. F., Mittal, N., & Ruiz, P. (2004b). Trends in serum citrulline and acute rejection among recipients of small bowel transplants. *Transplantation Proceedings*, *36*(2), 345-347. doi:10.1016/j.transproceed.2003.12.007

Parekh, N. R., Natowicz, M., Lopez, R., Seidner, D. L., Su, L., & Steiger, E. (2008). Plasma Citrulline Is a Marker of Home Parenteral Nutrition Dependence in Patients with Short Bowel Syndrome. *Clinical Nutrition Supplements*, *3*(Supplement 1), 71-72. doi:10.1016/S1744-1161(08)70163-7

Patterson, H. D., & Lucas, H. L. (1962). Change-over designs. North Carolina Agricultural Experiment Station and US Dept. of Agriculture,

Patureau Mirand, P., Mosoni, L., & Rémond, D. (2003). Amino acid requirements in the elderly. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids*

in Clinical Nutrition (2nd edn, pp. 483-496). London: CRC Press. doi:10.1201/9780203010266.ch29

Perez-Guisado, J., & Jakeman, P. M. (2010). Citrulline malate enhances athletic anaerobic performance and relieves muscle soreness. *Journal of Strength and Conditioning Research*, *24*(5), 1215-1222. doi:10.1519/jsc.0b013e3181cb28e0

Peters, J. H., Wierdsma, N. J., Teerlink, T., van Leeuwen, P. A., Mulder, C. J., & van Bodegraven, A. A. (2007a). Sensitivity And Specificity Of Fasting Plasma Citrulline Concentration To Assess Enterocyte Dysfunction. *Journal of Parenteral and Enteral Nutrition*, *31*(2), S55. doi:10.1177/0148607107031002S1

Peters, J. H., Wierdsma, N. J., Teerlink, T., van Leeuwen, P. A., Mulder, C. J., & van Bodegraven, A. A. (2007b). Citrulline Stimulation Test To Assess Enterocyte Metabolic Function Is Feasible: Reference Values Of A New Test. *Journal of Parenteral and Enteral Nutrition*, *31*(2), S8-S9. doi:10.1177/01486071070310O2S1

Peters, J. H., Mulder, C. J., & van Bodegraven, A. A. (2008a). Fasting plasma citrulline concentrations do not reflect intestinal absorption capacity. *Journal of Parenteral and Enteral Nutrition*, *32*(3), 288; author reply 289. doi:10.1177/0148607108316186

Peters, J. H., Beishuizen, A., Keur, M. B., Dobrowolski, L., Wierdsma, N. J., & van Bodegraven, A. A. (2011). Assessment of small bowel function in critical illness: potential role of citrulline metabolism. *Journal of Intensive Care Medicine*, *26*(2), 105-110. doi:10.1177/0885066610387998

Peters, J. H. C., Wierdsma, N. J., Teerlink, T., van Leeuwen, P. A. M., Mulder, C. J. J., & van Bodegraven, A. A. (2007c). Poor diagnostic accuracy of a single fasting plasma citrulline concentration to assess intestinal energy absorption capacity. *American Journal of Gastroenterology*, *102*(12), 2814-2819. doi:10.1111/j.1572-0241.2007.01513.x

Peters, J. H. C., Wierdsma, N. J., Teerlink, T., van Leeuwen, P. A. M., Mulder, C. J. J., & van Bodegraven, A. A. (2008b). The citrulline generation test: proposal for a new enterocyte function test. *Alimentary Pharmacology & Therapeutics*, *27*(12), 1300-1310. doi:10.1111/j.1365-2036.2008.03678.x

Peters, J. H. C., Dobrowolski, L. C., Wierdsma, N. J., Teerlink, T., Girbes, A. R. J., Mulder, C. J. J., Beishuizen, B., & van Bodegraven, A. A. (2009). The citrulline generation test in stable ICU patients: optimizing a new enterocyte function test. *European Journal of Gastroenterology and Hepatology*, *21*(3), A62. doi:10.1097/MEG.0b013e3283297513

Peterson, J., & Kerner, J. A., Jr. (2012). New advances in the management of children with intestinal failure. *Journal of Parenteral and Enteral Nutrition*, *36*(1 Supplement), 36S-42S. doi:10.1177/0148607111422069

Picot, D., Garin, L., Trivin, F., Kossovsky, M. P., Darmaun, D., & Thibault, R. (2010). Plasma citrulline is a marker of absorptive small bowel length in patients with transient enterostomy and acute intestinal failure. *Clinical Nutrition*, *29*(2), 235-242. doi:10.1016/j.clnu.2009.08.010

Pinkhof, H. (1894). Therapeutische nieuwsberichten. *Nederlands Tijdschrift voor Geneeskunde*, *38*, 1009-1012.

Pinto Costa, B., Serodio, M., Simoes, M., Verissimo, C., Castro Sousa, F., & Grazina, M. (2013). Citrullinemia stimulation test in the evaluation of the intestinal function. *Nutrición Hospitalaria*, *28*(1), 202-210. doi:10.3305/nh.2013.28.1.6243

Pironi, L., Lauro, A., Spinucci, G., Guidetti, M., Piazzi, S., D'Errico, A., & Pinna, A. (2005). Plasma citrulline in short bowel syndrome and intestinal transplantation. *Clinical Nutrition*, *24*(4), 630. doi:10.1016/j.clnu.2005.05.012

Pironi, L., Guidetti, M., Agostini, F., Pazzeschi, C., & Petitto, R. (2011). PP040-SUN Translating plasma citrulline concentration in clinical practice. *Clinical Nutrition Supplements*, *6*(1), 38. doi:10.1016/S1744-1161(11)70094-1

Pironi, L., Guidetti, M., Agostini, F., Soverini, V., Pazzeschi, C., & Petitto, R. (2012). OC.10.4 Translating the assessment of plasma citrulline concentration in clinical

practice. *Digestive and Liver Disease*, *44*(Supplement 2), S77. doi:10.1016/S1590-8658(12)60207-2

Pironi, L., Arends, J., Baxter, J., Bozzetti, F., Pelaez, R. B., Cuerda, C., Forbes, A., Gabe, S., Gillanders, L., Holst, M., Jeppesen, P. B., Joly, F., Kelly, D., Klek, S., Irtun, O., Olde Damink, S. W., Panisic, M., Rasmussen, H. H., Staun, M., Szczepanek, K., Van Gossum, A., Wanten, G., Schneider, S. M., Shaffer, J., Home Artificial Nutrition, Chronic Intestinal Failure, & Acute Intestinal Failure Special Interest Groups of ESPEN. (2015). ESPEN endorsed recommendations. Definition and classification of intestinal failure in adults. *Clinical Nutrition, 34*(2), 171-180. doi:10.1016/j.clnu.2014.08.017

Pironi, L., Arends, J., Bozzetti, F., Cuerda, C., Gillanders, L., Jeppesen, P. B., Joly, F., Kelly, D., Lal, S., Staun, M., Szczepanek, K., Van Gossum, A., Wanten, G., Schneider, S. M., Home Artificial Nutrition, & Chronic Intestinal Failure Special Interest Group of ESPEN. (2016). ESPEN guidelines on chronic intestinal failure in adults. *Clinical Nutrition, 35*(2), 247-307. doi:10.1016/j.clnu.2016.01.020

Pironi, L., Konrad, D., Brandt, C., Joly, F., Wanten, G., Agostini, F., Chambrier, C., Aimasso, U., Zeraschi, S., Kelly, D., Szczepanek, K., Jukes, A., Di Caro, S., Theilla, M., Kunecki, M., Daniels, J., Serlie, M., Poullenot, F., Wu, J., Cooper, S. C., Rasmussen, H. H., Compher, C., Seguy, D., Crivelli, A., Pagano, M. C., Hughes, S. J., Guglielmi, F. W., Kozjek, N. R., Schneider, S. M., Gillanders, L., Ellegard, L., Thibault, R., Matras, P., Zmarzly, A., Matysiak, K., Van Gossum, A., Forbes, A., Wyer, N., Taus, M., Virgili, N. M., O'Callaghan, M., Chapman, B., Osland, E., Cuerda, C., Sahin, P., Jones, L., Lee, A. D. W., Bertasi, V., Orlandoni, P., Izbeki, F., Spaggiari, C., Diez, M. B., Doitchinova-Simeonova, M., Garde, C., Serralde-Zuniga, A. E., Olveira, G., Krznaric, Z., Czako, L., Kekstas, G., Sanz-Paris, A., Jauregui, E. P., Murillo, A. Z., Schafer, E., Arends, J., Suarez-Llanos, J. P., Shaffer, J., & Lal, S. (2018). Clinical classification of adult patients with chronic intestinal failure due to benign disease: An international multicenter cross-sectional survey. *Clinical Nutrition, 37*(2), 728-738. doi:10.1016/j.clnu.2017.04.013

Pita, A. M., Wakabayashi, Y., Fernandez-Bustos, M. A., Virgili, N., Riudor, E., Soler, J., & Farriol, M. (2003). Plasma urea-cycle-related amino acids, ammonium levels, and urinary orotic acid excretion in short-bowel patients managed with an oral diet. *Clinical Nutrition*, *22*(1), 93-98. doi:10.1054/clnu.2002.0606

Pita, A. M., Fernandez-Bustos, A., Rodes, M., Arranz, J. A., Fisac, C., Virgili, N., Soler, J., & Wakabayashi, Y. (2004). Orotic aciduria and plasma urea cycle-related amino acid alterations in short bowel syndrome, evoked by an arginine-free diet. *Journal of Parenteral and Enteral Nutrition, 28*(5), 315-323. doi:10.1177/0148607104028005315

Piton, G., Manzon, C., Monnet, E., Cypriani, B., Barbot, O., Navellou, J.-C., Carbonnel, F., & Capellier, G. (2010). Plasma citrulline kinetics and prognostic value in critically ill patients. *Intensive Care Medicine, 36*(4), 702-706. doi:10.1007/s00134-010-1751-6

Piton, G., Belon, F., Cypriani, B., Regnard, J., Puyraveau, M., Manzon, C., Navellou, J.-C., & Capellier, G. (2013). Enterocyte damage in critically ill patients is associated with shock condition and 28-day mortality. *Critical Care Medicine*, *41*(9), 2169-2176. doi:10.1097/CCM.0b013e31828c26b5

Piton, G., Belin, N., Barrot, L., Belon, F., Cypriani, B., Navellou, J.-C., & Capellier, G. (2015a). Enterocyte Damage: A Piece in the Puzzle of Post Cardiac Arrest Syndrome. *Shock*, *44*(5), 438-444. doi:10.1097/shk.0000000000000440

Piton, G., & Capellier, G. (2015). Plasma citrulline in the critically ill: intriguing biomarker, cautious interpretation. *Critical Care, 19*, 204. doi:10.1186/s13054-015-0881-1

Piton, G., Cypriani, B., Regnard, J., Patry, C., Puyraveau, M., & Capellier, G. (2015b). Catecholamine use is associated with enterocyte damage in critically ill patients. *Shock*, *43*(5), 437-442. doi:10.1097/shk.0000000000000327

Plauth, M., Raible, A., Vieillard-Baron, D., Bauder-Gross, D., & Hartmann, F. (1999a). Is glutamine essential for the maintenance of intestinal function? A study in the

isolated perfused rat small intestine. *International Journal of Colorectal Disease*, *14*(2), 86-94. doi:10.1007/s003840050191

Plauth, M., Schneider, B. H., Raible, A., & Hartmann, F. (1999b). Effects of vascular or luminal administration and of simultaneous glucose availability on glutamine utilization by isolated rat small intestine. *International Journal of Colorectal Disease*, *14*(2), 95-100. doi:10.1007/s003840050192

Poole, A., Deane, A., Summers, M., Fletcher, J., & Chapman, M. (2015). The relationship between fasting plasma citrulline concentration and small intestinal function in the critically ill. *Critical Care*, *19*(1), 1-8. doi:10.1186/s13054-014-0725-4

Power, F. B., & Moore, C. W. (1910). The constituents of colocynth. *Journal of the Chemical Society, Transactions*, *97(Pt 1)*, 99-110. doi:10.1039/CT9109700099

Puiman, P. J., Stoll, B., van Goudoever, J. B., & Burrin, D. G. (2011). Enteral arginine does not increase superior mesenteric arterial blood flow but induces mucosal growth in neonatal pigs. *Journal of Nutrition*, *141*(1), 63-70. doi:10.3945/jn.110.131888

Rabier, D., & Kamoun, P. (1995). Metabolism of citrulline in man. *Amino Acids*, *9*(4), 299-316. doi:10.1007/BF00807268

Radrizzani, D., Iapichino, G., & Cynober, L. A. (2003). Quantitative and qualitative amino acid intake by the parenteral route. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 519-528). London: CRC Press. doi:10.1201/9780203010266.pt5

Raijman, L. (1974). Citrulline synthesis in rat tissues and liver content of carbamoyl phosphate and ornithine. *Biochemical Journal*, *138*(2), 225-232. doi:10.1042/bj1380225

Rajantie, J., Simell, O., & Perheentupa, J. (1980a). Intestinal absorption in lysinuric protein intolerance: impaired for diamino acids, normal for citrulline. *Gut*, *21*(6), 519-524. doi:10.1136/gut.21.6.519

Rajantie, J., Simell, O., Rapola, J., & Perheentupa, J. (1980b). Lysinuric protein intolerance: a two-year trial of dietary supplementation therapy with citrulline and lysine. *Journal of Pediatrics*, *97*(6), 927-932. doi:10.1016/s0022-3476(80)80422-7

Raphael, B. P., Nurko, S., Jiang, H., Hart, K., Kamin, D. S., Jaksic, T., & Duggan, C. (2011). Cisapride improves enteral tolerance in pediatric short-bowel syndrome with dysmotility. *Journal of Pediatric Gastroenterology and Nutrition*, *52*(5), 590-594. doi:10.1097/MPG.0b013e3181fe2d7a

Rassin, D. K. (2003). Neonatal requirements for amino acids. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 471-482). London: CRC Press. doi:10.1201/9780203010266.ch28

Rattan, S., & Chakder, S. (1997). L-citrulline recycling in opossum internal anal sphincter relaxation by nonadrenergic, noncholinergic nerve stimulation. *Gastroenterology*, *112*(4), 1250-1259. doi:10.1016/S0016-5085(97)70137-9

Rhoads, J. M., Plunkett, E., Galanko, J., Lichtman, S., Taylor, L., Maynor, A., Weiner, T., Freeman, K., Guarisco, J. L., & Wu, G. Y. (2005). Serum citrulline levels correlate with enteral tolerance and bowel length in infants with short bowel syndrome. *Journal of Pediatrics*, *146*(4), 542-547. doi:10.1016/j.jpeds.2004.12.027

Romero, M. J., Platt, D. H., Caldwell, R. B., & Caldwell, R. W. (2006). Therapeutic use of citrulline in cardiovascular disease. *Cardiovascular Drug Reviews*, *24*(3-4), 275-290. doi:10.1111/j.1527-3466.2006.00275.x

Rosenthal, R. (1979). The file drawer problem and tolerance for null results. *Psychological Bulletin*, *86*(3), 638-641. doi:10.1037/0033-2909.86.3.638

Rougé, C., Des Robert, C., Robins, A., Le Bacquer, O., Volteau, C., De La Cochetière, M.-F., & Darmaun, D. (2007). Manipulation of citrulline availability in humans. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *293*(5), G1061-G1067. doi:10.1152/ajpgi.00289.2007

Ruiz, P., Tryphonopoulos, P., Island, E., Selvaggi, G., Nishida, S., Moon, J., Berlanga, A., Defranc, T., Levi, D., Tekin, A., & Tzakis, A. G. (2010). Citrulline evaluation in

bowel transplantation. *Transplantation Proceedings*, *42*(1), 54-56. doi:10.1016/j.transproceed.2009.12.029

Sacks, G. S., & Kudsk, K. A. (2003). Amino acids to support gut function and morphology. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 717-728). London: CRC Press. doi:10.1201/9780203010266.ch42

Sandstrom, P., Gasslander, T., Sundqvist, T., Franke, J., & Svanvik, J. (2003). Depletion of serum L-arginine in patients with acute pancreatitis. *Pancreas*, *27*(3), 261-266. doi:10.1097/00006676-200310000-00012

Sandstrom, P., Trulsson, L., Gasslander, T., Sundqvist, T., von Dobeln, U., & Svanvik, J. (2008). Serum amino acid profile in patients with acute pancreatitis. *Amino Acids*, *35*(1), 225-231. doi:10.1007/s00726-007-0557-5

Santarpia, L., Catanzano, F., Ruoppolo, M., Alfonsi, L., Vitale, D. F., Pecce, R., Pasanisi, F., Contaldo, F., & Salvatore, F. (2008). Citrulline Blood Levels as Indicators of Residual Intestinal Absorption in Patients with Short Bowel Syndrome. *Annals of Nutrition & Metabolism*, *53*(2), 137-142. doi:10.1159/000170888

Schiller, L. R. (2007). The core curriculum in gastroenterology: training the gastroenterologists of the future. *American Journal of Gastroenterology*, *102*(5), 919-920. doi:10.1111/j.1572-0241.2007.01287.x

Schmider, E., Ziegler, M., Danay, E., Beyer, L., & Bühner, M. (2010). Is It Really Robust? *Methodology*, *6*(4), 147-151. doi:10.1027/1614-2241/a000016

Schuirmann, D. J. (1987). A comparison of the Two One-Sided Tests Procedure and the Power Approach for assessing the equivalence of average bioavailability. *Journal of Pharmacokinetics and Biopharmaceutics*, *15*(6), 657-680. doi:10.1007/bf01068419

Schulzke, J.-D., Tröger, H., & Amasheh, M. (2009). Disorders of intestinal secretion and absorption. *Best Practice & Research Clinical Gastroenterology*, *23*, 395-406. doi:10.1016/j.bpg.2009.04.005

Seidner, D. L., Joly, F., & Youssef, N. N. (2015). Effect of Teduglutide, a Glucagon-like Peptide 2 Analog, on Citrulline Levels in Patients With Short Bowel Syndrome in Two Phase III Randomized Trials. *Clinical and Translational Gastroenterology*, *6*, e93. doi:10.1038/ctg.2015.15

Selamnia, M., Robert, V., Mayeur, C., Delpal, S., & Blachier, F. (1998). De novo synthesis of arginine and ornithine from citrulline in human colon carcinoma cells: Metabolic fate of L-ornithine. *Biochimica et Biophysica Acta*, *1425*(1), 93-102. doi:10.1016/S0304-4165(98)00056-7

Sellmann, C., Jin, C. J., Engstler, A. J., De Bandt, J. P., & Bergheim, I. (2017). Oral citrulline supplementation protects female mice from the development of non-alcoholic fatty liver disease (NAFLD). *European Journal of Nutrition*, *56*(8), 2519-2527. doi:10.1007/s00394-016-1287-9

Selvaggi, G., Weppler, D., & Tzakis, A. (2003). Liver and gastrointestinal transplantation at the University of Miami. *Clinical Transplants*, 255-266.

Senn, S. (2002). Cross-over trials in clinical research. 2nd edn. Wiley, Chichester

Sevinc, E., Akar, H. H., Sevinc, N., Arslan, D., Sezgin, G. C., & Kendirci, M. (2015). Amino acid levels in children with celiac disease. *Nutrición Hospitalaria*, *32*(1), 139-143. doi:10.3305/nh.2015.32.1.9066

Shanbhogue, L. K. R., & Molenaar, J. C. (1994). Short bowel syndrome: Metabolic and surgical management. *British Journal of Surgery*, *81*(4), 486-499. doi:10.1002/bjs.1800810404

Shanga, G. M. (2003) Analysis of crossover designs when treatments have unequal variances. Kansas State University, Manhattan.

Sheehe, P. R., & Bross, I. D. J. (1961). Latin squares to balance immediate residual, and other order, effects. *Biometrics*, *17*(3), 405-414. doi:10.2307/2527834

Shim, S., Jang, W. S., Lee, S. J., Jin, S., Kim, J., Lee, S. S., Bang, H. Y., Jeon, B. S., & Park, S. (2014). Development of a new minipig model to study radiation-induced

gastrointestinal syndrome and its application in clinical research. *Radiation Research*, *181*(4), 387-395. doi:10.1667/rr13207.1

Shin, S., Fung, S.-M., Mohan, S., & Fung, H.-L. (2011). Simultaneous bioanalysis of l-arginine, l-citrulline, and dimethylarginines by LC-MS/MS. *Journal of Chromatography B*, *879*(7-8), 467-474. doi:10.1016/j.jchromb.2011.01.006

Shuttleworth, C. W. R., Burns, A. J., Ward, S. M., O'Brien, W. E., & Sanders, K. M. (1995). Recycling of L-citrulline to sustain nitric oxide-dependent enteric neurotransmission. *Neuroscience*, *68*(4), 1295-1304. doi:10.1016/0306-4522(95)00193-M

Shuttleworth, C. W. R., & Sanders, K. M. (1996). Involvement of nitric oxide in neuromuscular transmission in canine proximal colon. *Proceedings of the Society for Experimental Biology and Medicine*, *211*(1), 16-23. doi:10.3181/00379727-211-43950C

Shuttleworth, C. W. R., Conlon, S. B., & Sanders, K. M. (1997). Regulation of citrulline recycling in nitric oxide-dependent neurotransmission in the murine proximal colon. *British Journal of Pharmacology*, *120*(4), 707-713. doi:10.1038/sj.bjp.0700949

Siqueira, L. T., Ferraz, A. A., Campos, J. M., De Lima Filho, J. L., Albuquerque, M. C., de Lima Aires, A., Ribeiro, M. H., Cavalcanti, M. T., De Lima, B. C. C., & Ferraz, E. M. (2010). Analysis of plasma citrulline and intestinal morphometry in mice with hepatosplenic schistosomiasis. *Surgical Infections*, *11*(5), 419-426. doi:10.1089/sur.2009.066

Soeters, P. B., Hulsewe, K. W., Deutz, N. E. P., Luiking, Y., & Dejong, C. H. C. (2003). Gastrointestinal disease. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 405-418). London: CRC Press. doi:10.1201/9780203010266.ch25

Squellerio, I., Tremoli, E., & Cavalca, V. (2011). Quantification of arginine and its metabolites in human erythrocytes using liquid chromatography-tandem mass spectrometry. *Analytical Biochemistry*, *412*(1), 108-110. doi:10.1016/j.ab.2011.01.018

Sterne, J. A., Sutton, A. J., Ioannidis, J. P., Terrin, N., Jones, D. R., Lau, J., Carpenter, J., Rucker, G., Harbord, R. M., Schmid, C. H., Tetzlaff, J., Deeks, J. J., Peters, J., Macaskill, P., Schwarzer, G., Duval, S., Altman, D. G., Moher, D., & Higgins, J. P. (2011). Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *British Medical Journal*, *343*, d4002. doi:10.1136/bmj.d4002

Stockwell, G. A. (1893). Citrullin Suppositories. *Medical Age*, *11*, 322.

Straathof, J. W., Adamse, M., Onkenhout, W., Lamers, C. B., & Masclee, A. A. (2000). Effect of L-arginine on lower oesophageal sphincter motility in man. *European Journal of Gastroenterology and Hepatology*, *12*(4), 419-424.

Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., Moher, D., Becker, B. J., Sipe, T. A., & Thacker, S. B. (2000). Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *Journal of the American Medical Association*, *283*(15), 2008-2012. doi:10.1001/jama.283.15.2008

Sureda, A., Cordova, A., Ferrer, M. D., Tauler, P., Perez, G., Tur, J. A., & Pons, A. (2009). Effects of L-citrulline oral supplementation on polymorphonuclear neutrophils oxidative burst and nitric oxide production after exercise. *Free Radical Research*, *43*(9), 828-835. doi:10.1080/10715760903071664

Suzuki, K., Kanamori, Y., Sugiyama, M., Komura, M., Terawaki, K., Kodaka, T., Takahashi, M., Fukami, E., & Iwanaka, T. (2012). Plasma citrulline may be a good marker of intestinal functions in intestinal dysfunction. *Pediatrics International*, *54*(6), 899-904. doi:10.1111/j.1442-200X.2012.03739.x

Tangphao, O., Grossmann, M., Chalon, S., Hoffman, B. B., & Blaschke, T. F. (1999). Pharmacokinetics of intravenous and oral L-arginine in normal volunteers. *British*

Journal of Clinical Pharmacology, *47*(3), 261-266. doi:10.1046/j.1365-2125.1999.00883.x

Thibault, R., Avallone, S., Orsonneau, J., Wyart, V., Darmaun, D., & Letessier, E. (2008). P351 Nutritional status, aminoacids and micronutrient plasma concentrations in the mid-term follow-up of roux-en-y gastric bypass for morbidly obese patients. *Clinical Nutrition Supplements*, *3*(Supplement 1), 178. doi:10.1016/S1744-1161(08)70413-7

Thibault, R., Avallone, S., Orsonneau, J., Le Cam, G., Wyart, V., Krempf, M., Darmaun, D., & Letessier, E. (2009). Assessment of Plasma Citrulline, Iron, Vitamine D and Nutritional Status in the Short-Term Follow-Up of Roux-En-Y Gastric Bypass for Morbidly Obese Patients. *Obesity Surgery*, *19*(8), 957-958. doi:10.1007/s11695-009-9904-9

Thibault, R., Flet, L., Vavasseur, F., Lemerle, M., Ferchaud-Roucher, V., Picot, D., & Darmaun, D. (2011). Oral citrulline does not affect whole body protein metabolism in healthy human volunteers: results of a prospective, randomized, double-blind, cross-over study. *Clinical Nutrition*, *30*(6), 807-811. doi:10.1016/j.clnu.2011.06.005

Tooley, K. L., Howarth, G. S., & Butler, R. N. (2009). Mucositis and non-invasive markers of small intestinal function. *Cancer Biology & Therapy*, *8*(9), 753-758. doi:10.4161/cbt.8.9.8232

Través, C., García-Arumí, E., López-Hellín, J., Baena-Fustegueras, J. A., & López-Tejero, M. D. (2010). Concomitant apolipoprotein A-IV and citrulline plasma changes during short-term parenteral nutrition in surgical patients. *e-SPEN*, *5*(5), e219-e224. doi:10.1016/j.eclnm.2010.07.004

Tsagris, M., Frangos, C. C., & Frangos, C. C. (2013). Confidence intervals for Cronbach's reliability coefficient. Paper presented at the Recent Techniques in Educational Science, Athens, Greece. May 14-16, 2013

Tsuchioka, T., Fujiwara, T., & Sunagawa, M. (2006). Effects of glutamic acid and taurine on total parenteral nutrition. *Journal of Pediatric Surgery*, *41*(9), 1566-1572. doi:10.1016/j.jpedsurg.2006.05.021

Tuchman, M. (1989). Persistant acitrullinemia after liver transplantation for carbamylphosphate synthetase deficiency. *New England Journal of Medicine*, *320*(22), 1498-1499. doi:10.1056/NEJM198906013202219

Uchiyama, C., Mori, M., & Tatibana, M. (1981). Subcellular localization and properties of N-acetylglutamate synthase in rat small intestinal mucosa. *Journal of Biochemistry*, *89*(6), 1777-1786.

Urschel, K. L., Shoveller, A. K., Pencharz, P. B., & Ball, R. O. (2005). Arginine synthesis does not occur during first-pass hepatic metabolism in the neonatal piglet. *American Journal of Physiology - Endocrinology and Metabolism*, *288*(6), E1244-1251. doi:10.1152/ajpendo.00530.2004

Urschel, K. L., Shoveller, A. K., Uwiera, R. R., Pencharz, P. B., & Ball, R. O. (2006). Citrulline is an effective arginine precursor in enterally fed neonatal piglets. *Journal of Nutrition*, *136*(7), 1806-1813. doi:10.1093/jn/136.7.1806

Vadgama, J. V., & Evered, D. F. (1992). Characteristics of L-citrulline transport across rat small intestine in vitro. *Pediatric Research*, *32*(4), 472-478. doi:10.1203/00006450-199210000-00019

Vaira, D. (2014). Diagnosing small bowel malabsorption. *Internal and Emergency Medicine*, *9*(1), 1-2. doi:10.1007/s11739-013-1021-z

Van De Poll, M. C. G., Ligthart-Melis, G. C., Boelens, P. G., Deutz, N. E. P., Van Leeuwen, P. A. M., & Dejong, C. H. C. (2007a). Intestinal and hepatic metabolism of glutamine and citrulline in humans. *Journal of Physiology*, *581*(2), 819-827. doi:10.1113/jphysiol.2006.126029

Van De Poll, M. C. G., Siroen, M. P. C., Van Leeuwen, P. A. M., Soeters, P. B., Melis, G. C., Boelens, P. G., Deutz, N. E. P., & Dejong, C. H. C. (2007b). Interorgan amino acid exchange in humans: Consequences for arginine and citrulline metabolism. *American Journal of Clinical Nutrition*, *85*(1), 167-172. doi:10.1093/ajcn/85.1.167

van der Velden, W. J., Herbers, A. H., Feuth, T., Schaap, N. P., Donnelly, J. P., & Blijlevens, N. M. (2010). Intestinal damage determines the inflammatory response and early complications in patients receiving conditioning for a stem cell transplantation. *PLoS One*, *5*(12), e15156. doi:10.1371/journal.pone.0015156

van der Velden, W. J., Herbers, A. H., Bruggemann, R. J., Feuth, T., Peter Donnelly, J., & Blijlevens, N. M. (2013). Citrulline and albumin as biomarkers for gastrointestinal mucositis in recipients of hematopoietic SCT. *Bone Marrow Transplantation*, *48*(7), 977-981. doi:10.1038/bmt.2012.278

van Noord, D., Mensink, P. B., de Knegt, R. J., Ouwendijk, M., Francke, J., van Vuuren, A. J., Hansen, B. E., & Kuipers, E. J. (2011). Serum markers and intestinal mucosal injury in chronic gastrointestinal ischemia. *Digestive Diseases and Sciences*, *56*(2), 506-512. doi:10.1007/s10620-010-1303-5

van Vliet, M. J., Tissing, W. J., Rings, E. H., Koetse, H. A., Stellaard, F., Kamps, W. A., & de Bont, E. S. (2009). Citrulline as a marker for chemotherapy induced mucosal barrier injury in pediatric patients. *Pediatric Blood & Cancer*, *53*(7), 1188-1194. doi:10.1002/pbc.22210

van Wijck, K., Wijnands, K. A., Meesters, D. M., Boonen, B., van Loon, L. J., Buurman, W. A., Dejong, C. H., Lenaerts, K., & Poeze, M. (2014). L-citrulline improves splanchnic perfusion and reduces gut injury during exercise. *Medicine and Science in Sports and Exercise*, *46*(11), 2039-2046. doi:10.1249/mss.0000000000000332

Vanclée, A., Lutgens, L. C. H. W., Oving, E. B. H., Deutz, N. E. P., Gijbels, M. J. J., Schouten, H. C., & Bos, G. M. J. (2005). Keratinocyte growth factor ameliorates acute graft-versus-host disease in a novel nonmyeloablative haploidentical transplantation model. *Bone Marrow Transplantation*, *36*(10), 907-915. doi:10.1038/sj.bmt.1705157

Vecino Lopez, R., Andres Moreno, A. M., Ramos Boluda, E., Martinez-Ojinaga Nodal, E., Hernanz Macias, A., Prieto Bozano, G., Lopez Santamaria, M., & Tovar Larrucea, J. A. (2013). [Plasma citrulline concentration as a biomarker of intestinal function in short bowel syndrome and in intestinal transplant]. *Anales de Pediatría*, *79*(4), 218-223. doi:10.1016/j.anpedi.2013.02.007

Verdam, F. J., Greve, J. W., Roosta, S., van Eijk, H., Bouvy, N., Buurman, W. A., & Rensen, S. S. (2011). Small intestinal alterations in severely obese hyperglycemic subjects. *Journal of Clinical Endocrinology and Metabolism*, *96*(2), E379-383. doi:10.1210/jc.2010-1333

Vickery, H. B. (1941). Evidence from Organic Chemistry Regarding the Composition of Protein Molecules. *Annals of the New York Academy of Sciences*, *41*, 87-120. doi:10.1111/j.1749-6632.1941.tb35232.x

Vickery, H. B. (1972). The History of the Discovery of the Amino Acids II. A Review of Amino Acids Described since 1931 as Components of Native Proteins. *Advances in Protein Chemistry*, *26*, 81-171. doi:10.1016/S0065-3233(08)60140-0

Vidal-Casariego, A., Calleja-Fernandez, A., de Urbina-Gonzalez, J. J. O., Cano-Rodriguez, I., Cordido, F., & Ballesteros-Pomar, M. D. (2014). Efficacy of glutamine in the prevention of acute radiation enteritis: a randomized controlled trial. *Journal of Parenteral and Enteral Nutrition*, *38*(2), 205-213. doi:10.1177/0148607113478191

Vissers, Y. L. J., von Meyenfeldt, M. F., Luiking, Y. C., Dejong, C. H. C., & Deutz, N. E. P. (2008). Interorgan synthesis of arginine is down-regulated in tumor-bearing mice undergoing surgical trauma. *Metabolism*, *57*(7), 896-902. doi:10.1016/j.metabol.2008.02.003

Viswanathan, M., & Berkman, N. D. (2012). Development of the RTI item bank on risk of bias and precision of observational studies. *Journal of Clinical Epidemiology*, *65*(2), 163-178. doi:10.1016/j.jclinepi.2011.05.008

Viswanathan, M., Berkman, N. D., Dryden, D. M., & Hartling, L. (2013). Assessing Risk of Bias and Confounding in Observational Studies of Interventions or Exposures: Further Development of the RTI Item Bank. Rockville MD

Vokurka, S., Svoboda, T., Rajdl, D., Sedlackova, T., Racek, J., Koza, V., & Trefil, L. (2013). Serum citrulline levels as a marker of enterocyte function in patients after allogeneic hematopoietic stem cells transplantation - a pilot study. *Medical Science Monitor*, *19*, 81-85. doi:10.12659/MSM.883755

vom Dahl, S., & Häussinger, D. (2003). The role of amino acids in the control of proteolysis. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 275-290). London: CRC Press. doi:10.1201/9780203010266.ch17

Wada, M. (1930a). Über Citrullin, eine neue Aminosäure im Preßsaft der Wassermelone, Citrullus vulgaris schrad. *Biochemische Zeitschrift*, *224*, 420-429.

Wada, M. (1930b). On the Occurrence of a New Amino Acid in Watermelon. *Proceedings of the Imperial Academy*, *6*(1), 15-17. doi:10.2183/pjab1912.6.15

Wada, M. (1933). Isolierung des Citrullins (δ-Carbamido-ornithin) aus tryptischen Verdauungsprodukten des Caseins. *Biochemische Zeitschrift*, *257*, 1-7.

Wagenmakers, A. J. M. (2003). Metabolism of branched-chain amino acids in man. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 123-134). London: CRC Press. doi:10.1201/9780203010266.ch8

Wakabayashi, Y., Yamada, E., Yoshida, T., & Takahashi, H. (1994). Arginine becomes an essential amino acid after massive resection of rat small intestine. *Journal of Biological Chemistry*, *269*(51), 32667-32671.

Wakabayashi, Y., Yamada, E., Yoshida, T., & Takahashi, N. (1995). Effect of intestinal resection and arginine-free diet on rat physiology. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *269*(2), G313-G318. doi:10.1152/ajpgi.1995.269.2.G313

Wakabayashi, Y. (2003). The glutamate crossway. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 135-152). London: CRC Press. doi:doi:10.1201/9780203010266.ch9

Walker, V. (2014). Ammonia metabolism and hyperammonemic disorders. *Advances in Clinical Chemistry*, *67*, 73-150. doi:10.1016/bs.acc.2014.09.002

Wallis, K., Walters, J. R. F., & Gabe, S. (2009). Short bowel syndrome: the role of GLP-2 on improving outcome. *Current Opinion in Clinical Nutrition and Metabolic Care*, *12*, 526-532. doi:10.1097/MCO.0b013e32832d23cd

Walrand, S., & Boirie, Y. (2003). Muscle protein and amino acid metabolism with respect to age-related sarcopenia. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 389-404). London: CRC Press. doi:10.1201/9780203010266.ch24

Wang, A., Ling, Z., Yang, Z., Kiela, P. R., Wang, T., Wang, C., Cao, L., Geng, F., Shen, M., Ran, X., Su, Y., Cheng, T., & Wang, J. (2015). Gut microbial dysbiosis may predict diarrhea and fatigue in patients undergoing pelvic cancer radiotherapy: a pilot study. *PLoS One*, *10*(5), e0126312. doi:10.1371/journal.pone.0126312

Wang, B.-S., Wang, X.-J., & Gong, L.-K. (2009). The Construction of a Williams Design and Randomization in Cross-Over Clinical Trials Using SAS. *Journal of Statistical Software*, *29*(1), 1-10.

Ware, L. B., Magarik, J. A., Wickersham, N., Cunningham, G., Rice, T. W., & Christman, B. W. (2013). Low plasma citrulline levels are associated with acute respiratory distress syndrome in patients with severe sepsis. *Critical Care*, *17*(1), R10. doi:10.1186/cc11934

Wedlake, L., McGough, C., Hackett, C., Thomas, K., Blake, P., Harrington, K., Tait, D., Khoo, V., Dearnaley, D., & Andreyev, H. J. N. (2008). Can biological markers act as non-invasive, sensitive indicators of radiation-induced effects in the gastrointestinal mucosa? *Alimentary Pharmacology & Therapeutics*, *27*(10), 980-987. doi:10.1111/j.1365-2036.2008.03663.x

Wernerman, J. (2003). Control of amino acid metabolism by counterregulatory hormones. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in*

Clinical Nutrition (2nd edn, pp. 201-210). London: CRC Press. doi:10.1201/9780203010266.ch13

Wessells, K. R., Hess, S. Y., Rouamba, N., Ouedraogo, Z. P., Kellogg, M., Goto, R., Duggan, C., Ouedraogo, J. B., & Brown, K. H. (2013). Associations between intestinal mucosal function and changes in plasma zinc concentration following zinc supplementation. *Journal of Pediatric Gastroenterology and Nutrition*, *57*(3), 348-355. doi:10.1097/MPG.0b013e31829b4e9e

Wiese, D., Lashner, B., & Seidner, D. (2008). Measurement of nutrition status in Crohn's disease patients receiving infliximab therapy. *Nutrition in Clinical Practice*, *23*(5), 551-556. doi:10.1177/0884533608323421

Wijnands, K., Castermans, T., Hommen, M., Meesters, D., & Poeze, M. (2015). Arginine and Citrulline and the Immune Response in Sepsis. *Nutrients*, *7*(3), 1426-1463. doi:10.3390/nu7031426

Wilburn, J., McKenna, S. P., Heaney, A., Rouse, M., Taylor, M., Culkin, A., Gabe, S., Burden, S., & Lal, S. (2017). Development and validation of the Parenteral Nutrition Impact Questionnaire (PNIQ), a patient-centric outcome measure for Home Parenteral Nutrition. *Clinical Nutrition*. doi:10.1016/j.clnu.2017.04.004

Williams, E. J. (1949). Experimental Designs Balanced for the Estimation of Residual Effects of Treatments. *Australian Journal of Scientific Research, Series A: Physical Sciences*, *2*(2), 149-168. doi:10.1071/CH9490149

Windmueller, H. G., & Spaeth, A. E. (1981). Source and fate of circulating citrulline. *American Journal of Physiology - Endocrinology and Metabolism*, *241*(6), E473-E480. doi:10.1152/ajpendo.1981.241.6.E473

Winkler, M. F. (2005). Quality of life in adult home parenteral nutrition patients. *Journal of Parenteral and Enteral Nutrition*, *29*(3), 162-170. doi:10.1177/0148607105029003162

Winkler, M. F. (2010). 2009 Lenna Frances Cooper Memorial Lecture: living with enteral and parenteral nutrition: how food and eating contribute to quality of life. *Journal of The American Dietetic Association*, *110*(2), 169-177. doi:10.1016/j.jada.2009.12.002

Winkler, M. F., Hagan, E., Wetle, T., Smith, C., Maillet, J. O., & Touger-Decker, R. (2010). An exploration of quality of life and the experience of living with home parenteral nutrition. *Journal of Parenteral and Enteral Nutrition*, *34*(4), 395-407. doi:10.1177/0148607110362582

Winkler, M. F., & Smith, C. E. (2014). Clinical, social, and economic impacts of home parenteral nutrition dependence in short bowel syndrome. *Journal of Parenteral and Enteral Nutrition*, *38*(1 Suppl), 32S-37S. doi:10.1177/0148607113517717

World-Medical-Association. (2013). World medical association declaration of helsinki: Ethical principles for medical research involving human subjects. *Journal of the American Medical Association*, *310*(20), 2191-2194. doi:10.1001/jama.2013.281053

Wu, G., Borbolla, A. G., & Knabe, D. A. (1994a). The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. *Journal of Nutrition*, *124*(12), 2437-2444. doi:10.1093/jn/124.12.2437

Wu, G., Knabe, D. A., & Flynn, N. E. (1994b). Synthesis of citrulline from glutamine in pig enterocytes. *Biochemical Journal*, *299*(1), 115-121. doi:10.1042/bj2990115

Wu, G., Flynn, N. E., Yan, W., & Barstow, D. G., Jr. (1995a). Glutamine metabolism in chick enterocytes: absence of pyrroline-5-carboxylase synthase and citrulline synthesis. *Biochemical Journal*, *306*(3), 717-721. doi:10.1042/bj3060717

Wu, G., & Knabe, D. A. (1995). Arginine synthesis in enterocytes of neonatal pigs. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *269*(3), R621-R629. doi:10.1152/ajpregu.1995.269.3.R621

Wu, G., Knabe, D. A., Yan, W., & Flynn, N. E. (1995b). Glutamine and glucose metabolism in enterocytes of the neonatal pig. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *268*(2), R334-R342. doi:10.1152/ajpregu.1995.268.2.R334

Wu, G. (1996). An important role for pentose cycle in the synthesis of citrulline and proline from glutamine in porcine enterocytes. *Archives of Biochemistry and Biophysics*, *336*(2), 224-230. doi:10.1006/abbi.1996.0552

Wu, G., Knabe, D. A., Flynn, N. E., Yan, W., & Flynn, S. P. (1996). Arginine degradation in developing porcine enterocytes. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *271*(5), G913-G919. doi:10.1152/ajpgi.1996.271.5.G913

Wu, G. (1997). Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *272*(6), G1382-G1390. doi:10.1152/ajpgi.1997.272.6.G1382

Wu, G., Davis, P. K., Flynn, N. E., Knabe, D. A., & Davidson, J. T. (1997). Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. *Journal of Nutrition*, *127*(12), 2342-2349. doi:10.1093/jn/127.12.2342

Wu, G. (1998). Intestinal mucosal amino acid catabolism. *Journal of Nutrition*, *128*(8), 1249-1252. doi:10.1093/jn/128.8.1249

Wu, G., Meininger, C. J., Kelly, K., Watford, M., & Morris, S. M., Jr. (2000). A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *Journal of Nutrition*, *130*(8), 1914-1919. doi:10.1093/jn/130.8.1914

Wu, G., & Morris, S. M., Jr. (2003). Arginine metabolism in mammals. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 153-168). London: CRC Press. doi:10.1201/9780203010266.ch10

Wu, G., Jaeger, L. A., Bazer, F. W., & Rhoads, J. M. (2004). Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *Journal of Nutritional Biochemistry*, *15*(8), 442-451. doi:10.1016/j.jnutbio.2003.11.010

Wu, G., Bazer, F. W., Davis, T. A., Kim, S. W., Li, P., Rhoads, J. M., Satterfield, M. C., Smith, S. B., Spencer, T. E., & Yin, Y. (2009). Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*, *37*, 153-168. doi:10.1007/s00726-008-0210-y

Yajima, Y., Hirasawa, T., & Saheki, T. (1982). Diurnal fluctuation of blood ammonia levels in adult-type citrullinemia. *Tohoku Journal of Experimental Medicine*, *137*(2), 213-220. doi:10.1620/tjem.137.213

Young, V., & Tharakan, J. (2003). Nutritional essentiality of amino acids and amino acid requirements in healthy adults. In: L. A. Cynober (Ed.), *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition* (2nd edn, pp. 439-470). London: CRC Press. doi:10.1201/9780203010266.pt4

Yu, H. C., Tuteja, S., Moon, J. I., Kleiner, G. I., Conanan, L., Gaynor, J. J., Kato, T., Levi, D. M., Nishida, S., Selvaggi, G., Gandia, C., Weppler, D., Esquenazi, V., Ruiz, P., Miller, J., & Tzakis, A. G. (2005). Utilization of dried blood spot citrulline level as a noninvasive method for monitoring graft function following intestinal transplantation. *Transplantation*, *80*(12), 1729-1733. doi:10.1097/01.tp.0000188168.45003.63

Yu, Y.-M., Ryan, C. M., Burke, J. F., Tompkins, R. G., & Young, V. R. (1995). Relations among arginine, citrulline, ornithine, and leucine kinetics in adult burn patients. *American Journal of Clinical Nutrition*, *62*(5), 960-968. doi:10.1093/ajcn/62.5.960

Zezulová, M., Bartoušková, M., Hlídková, E., Adam, T., Krčmová, L. K., Červinková, B., Solichová, D., Zlevorová, M., Cwiertka, K., Friedecký, D., Vrána, D., & Melichar, B. (2016). Citrulline as a biomarker of gastrointestinal toxicity in patients with rectal carcinoma treated with chemoradiation. *Clinical Chemistry and Laboratory Medicine*, *54*(2), 305-314. doi:10.1515/cclm-2015-0326

Ziegler, T. R., Benfell, K., Smith, R. J., Young, L. S., Brown, E., Ferrari-Baliviera, E., Lowe, D. K., & Wilmore, D. W. (1990). Safety and metabolic effects of L-glutamine administration in humans. *Journal of Parenteral and Enteral Nutrition*, *14*(4 Supplement), 137S-146S. doi:10.1177/0148607190014004201