THE EFFECT OF SEVERE MALNUTRITION, AND SUBSEQUENT RE-FEEDING ON DIGESTIVE FUNCTION IN HUMAN SUBJECTS, WITH SPECIAL REFERENCE TO GASTRIC ACID AND PANCREATIC ENZYME SECRETION, AND PROTEIN SYNTHESIS

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For my Family

"We ought, in every instance, to submit our reasoning to the test of Experiment, and never to search for truth, but by the natural road of Experiment and Observation."

Lavoisier

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DECLARATION

I, Trevor Anthony Winter, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part thereof has been, is being, or is to be submitted for another degree to this, or any other University.

Signed:

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1. Winter TA, Marks T, Callanan M, O'Keefe SJD, Bridger SA. Impaired pancreatic secretion in severely malnourished patients is a consequence of primary pancreatic dysfunction. Nutrition 2001;17:230-235.

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1. Winter TA, De Coito P, O'Keefe SJD, Bridger S, Marks T. Entero-pancreatic function in patients with severe malnutrition due to anorexia. Gastroenterology 1999;116:G2567

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AMA	Arm muscle area
AMC	Arm muscle circumference
ATP	Adenosine triphosphate
BAO	Basal acid output
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
ECL	Enterochomaffin-like
FFA	Free fatty acids
IPSID	Immunoproliferative small intestinal disease
KIC	Ketoisocaproic acid
MAC	Mid arm circumference
MAO	Maximum acid output
MPFS	Mucosal protein fractional synthesis
REE	Resting energy expenditure
RQ	Respiratory quotient
SA	Specific activity
SA Max	Maximum specific activity
TFA	Total fatty acids
TST	Triceps skinfold thickness
WBP	Whole-body protein

ABSTRACT

THE EFFECT OF SEVERE MALNUTRITION, AND SUBSEQUENT RE-FEEDING ON DIGESTIVE FUNCTION IN HUMAN SUBJECTS, WITH SPECIAL REFERENCE TO GASTRIC ACID AND PANCREATIC ENZYME SECRETION, AND PROTEIN SYNTHESIS

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Severe malnutrition may adversely affect digestive function, and result in the difficulties frequently encountered when attempting to re-feed such patients. To investigate the effects of undernutrition, and subsequent re-feeding, on digestive function in human patients, severely malnourished patients were studied before, and after a period of intensive nutritional support. The contribution of co-existent disease to the digestive dysfunction was investigated by stratifying the patients into those with associated overt disease (*disease patients*; n = 18), and those in whom the undernutrition was due to anorexia nervosa (*anorexia patients*; n = 8). Results were evaluated in comparison to a control group of 17 normal healthy volunteers.

Gut absorption was assessed by means of D-xylose and fat absorption studies, and by determination of stool frequency, stool weight and faecal fat excretion whilst on a standard diet. Duodenal mucosal morphology was studied, and fractional synthesis rates determined by means of L-[1-^{14}C]leucine incorporation into the tissue protein. Respiratory quotient (RQ), and resting energy expenditure (REE) was measured, and L-[1-^{14}C]leucine whole-body protein (WBP) kinetic studies performed. Both enteral meal- and intravenous hormone (pentagastric and cholecystokinin-8)-stimulation of gastric acid and pancreatic enzyme secretion was investigated, and pancreatic enzyme (amylase and trypsin) synthesis and zymogen stores studied by means of L-[1-^{14}C]leucine incorporation into enzyme protein.

The mean body mass index (BMI) of the malnourished patients was 13.39 kg/m^2. The group demonstrated significant digestive dysfunction with impaired D-xylose and fat absorption, and increased stool frequency, stool weight and faecal fat excretion. Villous atrophy was evident in 36% of malnourished patients, however, gastric and duodenal mucosal protein fractional synthesis rates were normal. Despite having similar impairment of gut absorption, as assessed by D-xylose

absorption, the *anorexia patient* group did not demonstrate clinical evidence of digestive dysfunction. The malnourished patients, in particular the *disease patient* group, tended to have a lower RQ compared to controls. REE was significantly lower in the malnourished patients when expressed in absolute terms, however, when expressed per kg/body weight, levels were significantly higher than the control value. WBP flux and synthesis was also significantly lower in the malnourished patients when expressed in absolute terms. Expressed per kg body weight, the *anorexia patient* group had similar WBP flux and synthesis to controls, whereas the *disease patient* group had significantly reduced levels. Gastric acid and pancreatic enzyme secretion was significantly impaired in the malnourished patients, with maximal acid output reduced to 26% of the normal value, and pancreatic enzyme (amylase, lipase and trypsin) secretion reduced to 36% of normal. Comparison of the enteral meal- and hormone (pentagastrin and cholecystokinin)- stimulated studies indicated that the impairment in secretion was principally consequent to primary gastric pariental and pancreatic acinar cell dysfunction. Comparison of the *disease patient* group with the *anorexia patient* group showed similar reduction in the secretion of amylase and trypsin. However, lipase secretion, although significantly impaired in the *disease patient* group, was relatively preserved in the *anorexia patient* group. Lipase output correlated inversely with both faecal fat excretion and stool frequency indicating that pancreatic enzyme insufficiency contributed significantly to the digestive impairment in malnourished patients, and possibly explaining the lack of clinical expression of digestive dysfunction in the *anorexia patient* group. Pancreatic enzyme synthesis studies indicated impaired synthesis of amylase and trypsin, with zymogen stores reduced to 32% and 41% of normal. The reduction in enzyme synthesis was particularly evident in the *disease patient* group.

Following the period of intensive nutritional support, mean BMI of the malnourished patients increased to 15.93 kg/m^2. The patients demonstrated a significant improvement of D-xylose and fat absorption, and reduction in stool frequency. There was a significant increase in RQ, and REE, expressed per kg body weight, was now similar to controls. The *disease patient* group showed a significant improvement in WBP flux and synthesis. Although basal acid output was unchanged following feeding, there was a significant improvement in pentagastrin-, but not enteral meal-stimulated acid output, although values remained significantly lower than controls. Secretion of pancreatic enzymes was also significantly improved, with amylase and lipase, but not trypsin output returning to normal values. Pancreatic enzyme synthesis studies indicated normalisation of amylase synthesis and zymogen stores. There remained evidence of persistent impairment of trypsin synthesis, which was principally evident in the *disease patient* group.

The studies demonstrate significant impairment in digestive function in severely malnourished patients. Clinical expression of this dysfunction, however, does appear to relate to the presence of co-existent disease. Nutritional support results in marked improvement of the digestive processes.

CHAPTER 1

INTRODUCTION

1.1 The Ravages of Famine

> *"I am mourning on my high throne for the vast misfortune, because the Nile flood in my time has not come for seven years! Light is the grain; there is a lack of crops and of all kinds of food. Each man has become a thief to his neighbours. They desire to hasten and cannot walk. The child cries, the youth creeps along as does the old man; their souls are bowed down, their legs are bent together and drag along the ground and their hands rest in their bosoms. The council of the great ones in the court is but emptiness. Torn open are the chests of provisions, but instead of contents there is air. Everything is exhausted."*

(Inscription in an ancient Egyptian tomb on the island of Sahal, 3rd dynasty, c2686-2613 BC)

Since antiquity, mankind has been plagued by famine, war, pestilence and disease. The transition from hunting and gathering, to agriculture and animal husbandry began approximately 12 000 years ago in the Near East and Mediterranean Region. This resulted in the development of large communities from the previous small nomadic groups, and thus began the competition between population growth and demand, and available food supply.[1,2] Such communities were particularly vulnerable to the unpredictability of climate and crop failures, and depredations from neighbouring tribes.[3] The ecological changes associated with this transition have been related to higher levels of stress from undernutrition and infectious disease.[4] Diets barely adequate in average years, often lead to starvation in adverse (usually drought) years. Paleopathological study of the archaeological remains of pre-historic man has revealed the immortalised evidence of adverse nutritional states (paleonutrition). Abnormalities, including dental enamel hypoplasia, Harrison's lines in long bones (growth arrest), Wilsons bands in teeth (enamel micro-defects), as well as porotic hyperostosis, have been described.[5-9] Porotic hyperostosis, (after Angel 1966,[10]) was first recognised over one hundred and fifty years ago, and is a condition of bony changes causing porosity of the cranium, usually occurring along the frontal, parietal and occipital bones, as well as the

orbital roof (*cribra orbitalia*). It is considered a "nutritional stress indicator", consequent to chronic anaemia in childhood, coupled with protein deficiency, and pathogen load,[9,11] and may result from the bone marrow becoming hyperplastic to compensate for the diminished red cells. A similar, but not identical picture can be caused by thalassaemia. There appears to be a strong correlation between the occurrence of porotic hyperostosis and the introduction of cereal grains in the Neolithic period, and subsequent subsistence on cereal grains, particularly maize.[12] Dependency on maize has been identified as a primary underlying cause of iron-deficiency anaemia in these prehistoric communities, particularly in the post-weaning period of childhood. Although maize is a source of iron, the phytate content in corn cultivated at the time inhibits intestinal absorption, leading to anaemia.[13] Malnourished pregnant women give birth to low birth-weight infants with limited haemoglobin stores. This results in an increased susceptibility to infections/infestations, and iron deficiency, particularly in the post-weaning period.[9,11] Evidence of porotic hyperostosis has been reported in 25% of the skulls of mummified Peruvian children, dating back more than 8000 years.[13]

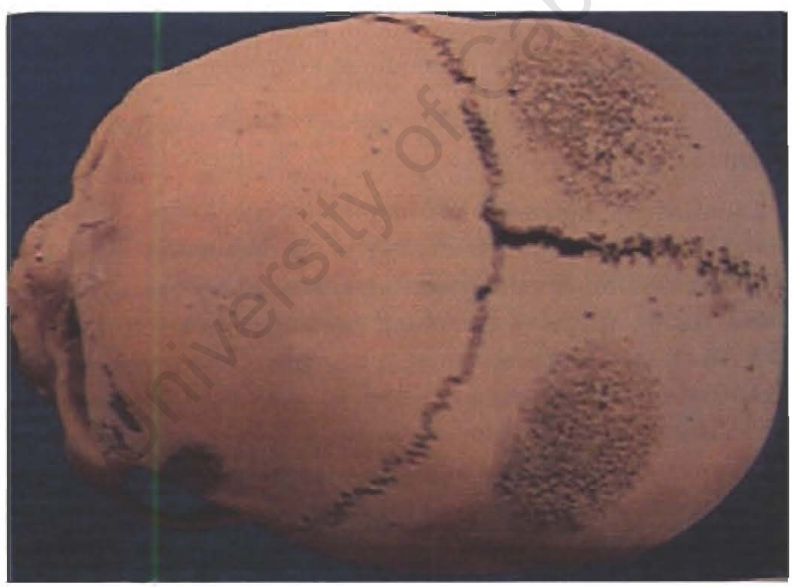


Figure 1.1: Bitemporal porotic hyperostosis.

Throughout recorded history there are vivid accounts of the consequences of malnutrition. Probably the earliest recorded famine was the Stele famine in Egypt, more than 5000 years ago.[14] The writer Ipuwer reported:

> "*Towns are destroyed and Upper Egypt has become an empty waste...He that layeth his brother to the ground is everywhere to be seen.*"

Figure 1.2: Wall carving of a famine scene: 5th Dynasty; pyramid complex of King Unas, Saqqâra

Famine victims, with their wasted bodies and protuberant ribs are graphically depicted on the causeway of the pyramid of Unas at Saqqâra (5th Dynasty, c2375-2345 BC), and foreshadowed Egypt's entry into the First Intermediate Period.[15] This Period was characterised by crop failures, civil disorder, political instability, and the fall of the Old Kingdom. As inscribed:

> "*The entire country had become like a starved locust.*"

An inscription on the tomb of Ankhtifi, c2000 BC,[14] further illustrates the drastic effects of starvation, resulting in the total disintegration of moral and social life.

> "*All of Upper Egypt was dying of hunger to such a degree that everyone had come to eating his children.*"

Biblical accounts also report starving people resorting to cannibalism. As recorded in 2 Kings 6;28, during the famine caused by the siege of Samaria by King Ben-Hadad of Aram:

> "*This woman said to me, 'Give up your son so we may eat him today, and tomorrow we'll eat my son.' So we cooked my son and ate him. The next day I said to her, 'Give up your son so that we may eat him,' but she had hidden him.*"

The devastation caused by the Kashmir famine in India in the years 917-18 is described dramatically in Kalhana's *Rajatarangini*:[16,17]

> "*One could scarcely see the water in the Vistasta [Jehlam], entirely covered as the river was with corpses soaked and swollen by the water in which they had long been lying. The land became densely covered with bones in all directions until it was like one great burial ground, causing terror to all beings.*"

China had 1829 famines recorded between 108 BC and 28 AD It is estimated that famines resulted in the deaths of 40 000 people in England in 310 AD, and a further 40 000 in London in 1235 and 1257[18] Two-thirds of the population of Italy perished in 1347, and one-third of the population of Paris died in 1438 Five hundred thousand people succumbed from famine in Russia in 1600, 168 000 in Bohemia in 1770 and 9 million in Northern China in 1877.[17] The 20th Century has been no exception, with 30 million deaths reportedly occurring in the Chinese famine of 1959-1962.[19] Hunger disease remains prevalent today, with World Health Organisation (WHO) figures indicating that 28% of all children under 5 years of age are underweight, 35% are stunted, and 8% are wasted.[18] The Nordic Conference on Environment and Development in 1987 estimated that 500 million people in the world are undernourished.[20] The problem is not restricted to the Developing World, with surveys suggesting that as many as 50% of hospitalised patients in the Developed World, are nutritionally depleted.[21-25]

1.2 The Development of Nutritional Physiology

Despite their graphic illustrations and descriptions of the consequences of malnutrition, the ancient Egyptians did not seem to have a formal theory of the relationship between nutrition and health.[3] As detailed by his first biographer, Soranus of Ephesus, A.D. 93-138, Hippocrates of Cos, the Asclepiad, was born on the island of Cos on the twenty-seventh day of the Dorian month of Agrianos (the eighth day of the local calendar), under the "monarchy" of Abriadas, in the first year of the Eighty-Fourth Olympiad (460 BC).[26] Hippocrates is considered the "Father of Medicine" and was the first to distinguish Medicine from Mysticism. He emphasised the importance of conclusions drawn from careful observation, and noted in his Aphorisms Section II, 35:[27]

"*In all maladies those who are fat about the belly do best; it is bad to be very thin and wasted there.*"

The works of his school were collected as a group of aphorisms and treatises in the third century BC, and formed part of the *Corpus Hippocraticum* for the library at Alexandria. Hippocrates observed in his treatis *Of the Epidemics*, that, in most diseases, there was a natural tendency to cure:[28]

"*Nature is the Doctor.*"

If the patient's constitution were supported by simple means, recovery would generally follow. Great attention was paid to the role of diet in health and illness. Two Hippocratic treatises, *On Diet*, and *On Sound Diet*, contain considerable good advise on the maintenance of good health by sensible eating, and in his On *Regimen in Acute Diseases* he advocated the use of a diet of ptisan (barley gruel), and hydromel (honey and water) for ill patients.[29] In *On the Physician* Hippocrates also made specific reference to the importance of nutritional well-being not only for patients, but also for the care-givers:[28]

"*For the physician it is undoubtedly an important recommendation to be of good appearance and well fed, since people take the view that those who do not know how to look after their own bodies are in no position to look after those of others.*"

Hippocrates in his *On Airs, Waters and Places* also advised physicians:[29]

"*To observe how men live, what they like, what they eat and what they drink [...] All this a doctor must know in order to understand local complaints and be in a position where he can prescribe suitable treatments for them.*"

The weakness of Hippocratic teaching lay, however, in its lack of anatomy and physiology, which are the foundations of modern medicine. Greek respect for the dead precluded human dissection, and knowledge of anatomy was derived from animal experimentation. As introduced by Empedocles (504-432 BC), the Greeks believed that there were four elements, fire, air, earth and water, and four elemental properties, hot, cold, moist and dry.

Hot and dry made fire
Hot and wet made air
Cold and dry made earth
Cold and wet made water

Likewise there were considered to be four humours, also related to the four elemental properties:

Hot and dry made yellow bile
Hot and wet made blood
Cold and dry made black bile
Cold and wet made phlegm

The Hippocratic School related health and disease to the balance of the four humours in the body. Nutrition was considered important for the maintenance of this balance. A fever developed when the body had a surplus of ingested food. This belief resulted in the practice of bleeding, purging and starving patients with fever, a policy that persisted well into the 19th Century.

The Hippocratic teaching was promoted, and further developed, by the powerful figure of Claudius Galen in the second century AD, who also considered the relationship between nutrition and the "humours":

> "*A clear proof of this may be found in the different humours produced by each article of food; some produce a melancholic blood, others a phlegmatic blood, or blood containing a fair amount of pale so-called yellow bile: some again produce pure blood.*"

Galen advocated the concept of the therapy of opposites, *"contraria contraries"*, applying heat if the disease resulted from cold, and starvation and purgatives for conditions where the body was considered overburdened. This policy clearly emanated from the teachings of Hippocrates (Aphorisms Section II, 22):

> "*Diseases which arise from repletion are cured by depletion, and those that arise from depletion are cured by repletion; and in general, diseases are cured by their contraries.*"

Although it has been considered that Galen, in fact, added little to the ancient doctrines taught by the Greeks,[30] his dogmatic nature is clearly illustrated by his statement in 160 AD:

> "*I have done so much for medicine as Trajan did for the Roman Empire, as when he built bridges and roads through Italy. It is I, and I alone, who will reveal the true path of medicine. It must be admitted that Hippocrates already staked out this path, but he did not go as far as one would have wished; his writings have faults and lack essential distinctions, his knowledge of certain topics is insufficient, and he is often unclear as the old tend to be. In sum: he prepared the way, but I have made it passable.*"

The Hippocratic/Galenic ideas remained unchallenged for the next fifteen hundred years, and are echoed in the teachings of the Salerno Medical School, founded in Italy in the eleventh Century by Benedictine monks, and published in its *Regimen Sanitatis Salernitanium*:[31]

> "*Four humours reigne within our bodies wholly,*
> *And these compared to the four elements,*
> *The Sanguine, Choller, Flegme and Melancholy,*
> *The latter two are heavie, full of fence,*
> *The other two are more jovial, quick and jolly,*
> *And may be likened thus without offence,*
> *Like ayre both warm and moist is sanguine cleare,*
> *Like fire doth Choler hot and dry appeare,*
> *Like water cold and moist is Flegmatique,*
> *And Melancholy cold, dry earth is like.*"

> "*Against these several humours overflowing*
> *As several kinds of Physics may be good*
> *As diet, drink, hot baths whence sweat is growing*
> *With purging, vomiting and letting blood*
> *Which taken in due time, not overflowing*
> *Each malady's infection is withstood.*"

The appreciation of the role of nutrition in health is indicated by the verse:

> "*Good dyet is a perfect way of curing:*
> *And worthy much regard and health assuring:*
> *A King that cannot rule him by his dyet.*
> *Will hardly rule his Realme in peace and quiet.*"

The Medieval era was largely a time of stagnation in medical science, and domination by astronomy and superstition. In the words of Carl Voit (1831-1908):

> "*One usually regards this period of the world as intellectually barren, during which only a blind imitation of the old and senseless scholasticism prevailed.*"

Evidence of the persistence of the ancient Greek view of medicine is illustrated by portrayal of the Doctor of Physich (based on John Gaddesdon, Professor of Medicine at Oxford) by Chaucer (1340-1400):

> "*He knew the cause of every malady,*
> *Were it of cold or hot or moist or dry,*
> *And where it engendered and of what humour,*
> *He was a very perfect practisour.*"

The work of Renaissance figures such as Andreas Vesalius (1514-63) *De Humani Corporis Fabrica Libri Septum*, Gabriele Fallopio (1523-62) *Observationes Anatomicae* and *Opera Omnia*, Gerolamo Fabrizio d'Acquapendente (Fabricus, 1533-1619), and William Harvey (1578-1657) *De Motu Cordis et Sanguinis in Animalibus*, however, revolutionised anatomy, exposing the fundamental flaws of Galen's theories, particularly pertaining to the flow of blood in the body (Galen believed that blood ebbed and flowed in the body, with communication between the two ventricles of the heart through invisible pores). These findings laid the foundations for modern physiology, and formed the basis for the understanding of how food is absorbed, distributed and metabolised. Richard Lower (1631-91), who was a member of the Oxford Group, which included Thomas Willis (1621-75), Robert Boyle (1627-91), Robert Hooke (1635-1703), John Mayow (1643-79) and Sir Christopher Wren (1632-1723), was the first to successfully transfer blood from one animal to another. In his *Treatise on the Heart*, Lower commented on the physiology of food in the body:

> *"For when chyle (gut lymphatic fluid) is mixed with the blood mass, it does not lose its nature and character with such rapidity as to give up its whiteness at once [...] it circulates with the blood like milk [...] If venesection is performed a long while after a meal, there appears no appearance of milk and serum only will be seen floating on the surface of the blood clot [...] The watery saline portion of it is separated through the kidneys, and got rid of by insensible transpiration or sweating: the burnt up portion is deposited in the liver [...] The cause of our life consists of this alone, that the blood, in its continuous passages throughout the whole of the body carries around heat and nutriment to all the organs, and that ever fresh chyle passes into the blood in due measure and amount, restoring with equivalent supplies the daily loss of blood fluid and refreshing it with its continuous inflow."*

René Descartes (1596-1650) in his *Tractatus de Homine*, possibly the first work on physiology, envisaged the human body as a machine activated by the heat collected by the blood. The blood carried the "vital spirit", and enabled the brain to receive impressions (sensations) of external objects, and also those of the soul, which Descartes clearly distinguished from organic matter. Extensive work into respiration, and its similarities to combustion was conducted in the eighteenth century. Joseph Black (1728-99) discovered carbon dioxide in 1757, Henry Cavendish (1731-1810), hydrogen 1766, Daniel Rutherford (1749-1819), nitrogen in 1772, and Joseph Priestley (1733-1804) isolated oxygen (which he referred to as dephlogisticated air) in 1772. The prevailing theory at the time, as proposed by George Ernest Stahl (1660-1734), considered that substances gave off "phlogiston" when burned,[31] and that the function of respiration was to deprive the blood of phlogiston.[32] Cavendish believed that the "inflammable air" (hydrogen) he collected by placing zinc, iron or tin into sulphuric or hydrochloric acids was pure "phlogiston," and, in 1783, went on to show that ignition of one volume of "phlogiston" with two parts of Priestley's "dephlogisticated air" resulted in the production of water. It, however, took the great mind of Antoine-Laurent Lavoisier (1743-94), prior to his execution by guillotine in the French Revolution, to penetrate the mystery of Priestleys "dephlogisticated air". He extended the findings, and provided a key role in our modern understanding of metabolism, with his description of the uptake of the gas, which he now referred to as oxygéne, by the blood in the lungs, with subsequent consumption in the tissues, with the production of heat (*calorique*), and "fixed air" (carbon dioxide). He concluded "*La respiration est donc une combustion.*"

The conclusion of Lavoisier's last scientific communication to the Acadèmie des Sciences is as true today as it was then:[30]

> "*Up to the present time we have learned only to conjecture as to the cause of a great number of diseases and as to a means of their cure. Before hazarding a theory we propose to multiply our observations, to investigate the phenomena of digestion and to analyse the blood in both health and disease. We will draw upon medical records and the light and experience of learned physicians who are our contemporaries and it will be only when we are thus completely armed that we will dare to attack a revered and antique colossus of prejudice and error.*"

The nineteenth Century saw a rapid development of knowledge of the various foodstuffs. In 1827, William Prout (1785-1850), of muriatic acid fame (see Chapter 2.1), classified foods into the subgroups saccharinous (carbohydrates), oleaginous (fats) and albuminous (protein). Justus von Liebig (1803-73), who described the use of chloroform for anaesthesia in 1831, and his student Carl Voit (1831-1908), described protein metabolism with the production of urea, and defined the concept of nitrogen balance. Joseph Francois Magendie (1783-1855), who is regarded as the founder of modern physiology, demonstrated the effects of various drugs such as morphine and strychnine on animals, and gave a scientific basis for the use of these drugs. His student Claude Bernard (1813-78) described the glycaemic function of liver by observing that sugar was found in the blood coming from the liver of a dog, whether the dog was fed meat or sugar, and went on to discover liver glycogen. His work on the digestive process, using a pancreatic fistula model, described how digestion was initiated in the stomach, and completed in the intestine under the influence of pancreatic juice, with the breakdown of fats, starch and protein. His most important contribution, however, was his *Leçons sur les phenomenes de la vie* 1878, in which he stated his concept of the "*miliéu intérieur*" ("internal environment") in which metabolic processes were maintained in equilibrium to achieve homeostasis. This paved the way for the discovery of hormones, and is now accepted as one of the fundamental principles of physiology.

A "fortuitous accident", exploited by the American doctor, William Beaumont (1785-1853), involved a young fur trapper, Alexis Saint Martin, who was accidentally shot in the stomach with a musket in 1822. The patient survived, but was left with a permanent gastro-cutaneous fistula. Access to the gastric lumen afforded Beaumont the unique opportunity to observe the digestive process directly, and over the course of the period 1822 to 1833 he studied the

many aspects of gastric physiology.[33] Although without formal academic training, his research was impeccably methodical, and he provided accurate information, leading to theories, which are still valid. Beaumont observed the contractions of the stomach, investigated the manifestations of hunger and thirst, described the appearance and properties of gastric juice and confirmed the presence of acid. He noted the valve-like function of the pylorus, and that the gastric peristalsis had two functions: to mix the food with gastric secretions, and to enable the stomach to empty the products of digestion. His work with Alexis St Martin was documented in his classic monograph, *Experiments and Observations on the Gastric Juice, and the Physiology of Digestion*, published in 1833.[33]

In 1825, Francois Leuret and Jean Lassaigne of Paris, although still of the opinion that gastric acid was lactic, demonstrated that acid introduced into the duodenum of dogs, stimulated pancreatic secretion. Seventy years later, in 1895, Ivan Leukich Dolinsky, a student of Pavlov, rediscovered the effect of gastric juice in stimulating pancreatic secretion. William Bayliss, and his brother-in-law, Ernest Starling, extended Dolinsky's thesis on January 16, 1901, and as recorded by Charles Martin:[34]

> "*In an anaesthetized dog, a loop of jejunum was tied at both ends and the nerve supplying it dissected out and divided so that it was connected with the rest of the body only by its blood vessels. On introduction of some weakened HCl into the duodenum, secretion from the pancreas occurred and continued for some minutes. After this had subsided a few cubic centimetres of acid were introduced into the denervated loop of jejunum. To our surprise a similarly marked secretion was produced. I remember Starling saying: 'Then it must be a chemical reflex'. Rapidly cutting off a further piece of jejunum he rubbed its mucous membrane with sand in weak HCl, and injected it into the jugular vein of the animal. After a few moments the pancreas responded by a much greater secretion than had occurred before. It was a great afternoon!*"

Bayliss and Starling concluded that acid liberates a chemical agent from the gut mucosa, and that this messenger is conveyed to the pancreas via the bloodstream where it stimulates secretion. The term "hormone"(Greek, "hormàein" - I arouse to activity), first suggested by William Hardy, was adopted by Starling, and formed the basis of the science of endocrinology. Bayliss and Starling called this first hormone "secretin".

In 1906, perhaps stimulated by the work of Bayliss and Starling, John S. Edkins (1863-1910) described a "gastric secretin" derived from the gastric antral mucosa.[35] Simon Komarov (1892-1964), whilst working with Babkin, eventually isolated the hormone in 1938, which he termed "*gastrin*":

> "*In all cases, without exception, the pyloric preparation, injected in quantities equal to 5 gm of mucosa, elicited a copious secretion of gastric juice, which was characterised by high acidity and low peptic power and which was not affected by atropine even in large doses.*"

In 1928, Ivy and Oldberg discovered cholecystokinin/pancreozymin.[36] Secretin was shown to be responsible for the secretion of a bicarbonate-rich pancreatic fluid,[37] and cholecystokinin/pancreozymin, an enzyme rich fluid,[38] as well as contraction of the gall bladder. The "biochemical era" began in 1964 with the determination of the amino acid sequence of gastrin by Gregory et al,[39] secretin in 1970,[40] and cholecystokinin/pancreozymin in 1971.[41]

Studies into the neural control of the stomach began nearly two thousand years ago when Marinus in the first century AD studied the anatomy of the vagus nerve. Galen expanded the work of Marinus by commenting on the possible function of the nerves to the stomach:

> "*The stomach must have very accurate perception of the need for food and drink. Hence, most of these nerves seem to be distributed to its so-called [cardiac] orifice and afterwards to all the other portions of it as far as its lower end.*"

The term *vagus* or "wandering part" was applied to the nerve, although in the 19th Century the possibly more appropriate term, *pneumogastric,* appeared in the French and Italian literature, and subsequently in the English and American. The German literature, however, retained the term *vagus*, and with the prominence of German scientific research, by the end of the 19th Century the term *pneumogastric* had fallen from common usage.

Following experimentation in dogs, B. C. Brodie in 1814 noted:

> "*...that the suppression of secretion in all of them was to be attributed solely to the division of the nerves: and all of the facts which have been stated sufficiently demonstrate, that the secretions of the stomach are very much under the control of the nervous system.*"

The study of the effects of the vagus nerve was, however, dominated by Ivan Pavlov (1849-1936). His theory of "*nervism*" described:

> "*A physiological theory, which tries to prove that the nervous system controls the greatest possible number of bodily activities.*"

Using his ingenious models of the gastric fistula, the "*Pavlov pouch*", as well as a pancreatic fistula, Pavlov extensively investigated the cephalic phase of both gastric and pancreatic secretion, and also described the "*conditioned reflex*". He concluded:

> "*It is obvious that the effect of feeding was transmitted by nervous channels to the gastric glands.*"

Pavlov's work was published in his book *The Work of the Digestive Glands*, translated into English in 1902.[42]

The concept of a hormonally mediated regulatory system, as promoted by the observations of Bayliss and Starling, as well as Edkins, was, therefore, a considerable issue for Pavlov and the supporters of the "*nervism*" doctrine. This was particularly in view of the statement by Bayless and Starling in 1902, following their inability to repeat Pavlov's observations:

> "*The nervous process [of pancreatic secretion] is superfluous and therefore improbable.*"[43]

Boris Babkin, a member of the Pavlov school later commented:

> "*I think that it was in the fall of 1902 that Pavlov asked V.V. Savich to repeat the secretin experiments of Bayliss and Starling. The effect was self-evident. Then, without a word, Pavlov disappeared into his study. He returned half an hour later and said: 'Of course, they are right. It is clear that we did not take out an exclusive patent for the discovery of truth.* "'[44]

Pavlov in his Nobel Lecture, December 12, 1904,[45] further acknowledged the dual regulatory processes, stating:

> "*The system of the organism, of its countless parts, is united into a single entity in two ways: by means of a specific tissue which exists solely for the purpose of maintaining interrelations, that is, the nervous tissue, and by means of body fluids bathing all body elements.*"

CHAPTER 2

LITERATURE REVIEW

2.1 THE PHYSIOLOGY OF GASTRIC SECRETION

2.1.1 Historical Perspective

The ancient Greeks considered digestion to be a process of concoction, or cooking, by which food was converted to chyle, and then to the four humours (blood, phlegm, yellow, and black bile). Hippocrates referred to this process as *pepsis*, believing that it was accomplished through the action of the heat of the stomach. Galen extended this theory, and proposed that the process of concoction occurred sequentially in the stomach, intestine and liver, until the food was ultimately converted into blood.

There was little further development in the understanding of the digestive process until the 16th Century. Paracelsus, (Philippus Aureolus Theophrastus Bombastus von Hohenheim; 1493-1541), proposed a break from Galaenic thinking, publically burning the books of Galen and the Arab 7th Century physician Avicenna. He rejected the mysticism of humours and health, and wrote in his *Opus Chyrurgicum*, 1565:

> "*Very few surgeons have exact knowledge of diseases and their causes; but my books are not written like those of other physicians, copying Hippocrates and Galen; I have composed them on the basis of experience.*"

Paracelsus believed there to be acid in the stomach, which was necessary for the digestive process. He referred to the acid as "*acetosum ensurium*" (hungry acid), and that its action was to prevent the formation of precipitations and concretions in the body.

Jean Baptiste van Helmont (1577-1644) also believed that digestion commenced in the stomach under the influence of acid (gastric ferment), and thereafter, a number of other

fermentation processes, including that of bile in the duodenum, occurred. He also commented on the neutralising effect of the alkaline gall in the duodenum.

At the end of the 17th Century, there remained two main schools of thought regarding the digestive process. One school maintained that digestion was a process of trituration (grinding), and the other perceived it to be a process of chemical action, induced by substances secreted by the stomach. In 1752, Rene de Rèaumur (1683-1757) demonstrated that food, placed within small hollow metal tubes swallowed by his pet buzzard, showed evidence of having dissolved without putrefaction, when recovered from the bird. By enclosing sponges in his metal canisters, Rèaumur also demonstrated that the gastric secretion was acidic. Lazzaro Spallanzani (1729-1799) repeated the studies of Rèaumur on a number of different animals, including himself. Although he initially asserted that gastric juice was neutral, he proposed his "theory of chemical solution", in which he considered the chymification of food by the stomach to be a chemical process. He also noted the antiseptic properties of gastric juice preventing fermentation or putrefaction of food. Subsequent work performed by Spallanzani revealed the presence of "marine acid" in the gastric juice. However, despite these findings, confusion remained regarding the nature of gastric secretions. Young, in 1803, following experiments on the bullfrog, confirmed the presence of acid in the stomach, but concluded that it was phosphoric acid.[46] The controversy was finally resolved in 1823 by William Prout (1785-1850). Although acknowledging that gastric secretions were acidic, Prout initially thought that it was phosphoric acid. However, in his landmark paper, *On the Nature of Acid and Saline Matters Usually Existing in the Stomach of Animals*, he specifically identified "muriatic acid" (hydrochloric acid) in the gastric juice of several species, and was able to identify free and total acid. Prout also proposed that chloride may be secreted from the blood to the gastric lumen by electric means, and that secretion of acid by the stomach would be accompanied by the blood becoming alkaline. It would be more than one hundred years before the existence of the postprandial alkaline tide was confirmed.

William Beaumont (1785-1853) initiated formal physiological study of gastric function in humans with his classical experiments with Alexis St Martin, who had a permanent, post traumatic, gastro-cutaneous fistula. Over the course of twelve years, Beaumont studied gastric physiology extensively confirming the presence of gastric acid, noting that meat was digested more easily than vegetable matter, and studying the motility of the stomach.

Despite the extensive work done by Beaumont, the source of the gastric acid remained unclear. Resolution of this question required detailed study of the morphology, and fine structure of the stomach. Johannes Purkinje, in 1837, described the light microscopic features of the stomach. He was able to identify the gastric glands, and described the "parietal cells", so called because of their parietal position in the walls of the glands. Camillo Golgi extended the observations of parietal cell structure. In 1893 he demonstrated that stimulation of digestive activity was accompanied by a marked change in the morphological appearance of the gastric glands. In particular he noted the changes in the minute tubules (secretory canaliculi) of the parietal cells, leading him to postulate that the parietal cells were the source of gastric acid.

In 1836, Theodor Ambrose Schwann (1810-1882) described a water-soluble substance in gastric juice, which digested egg white. He called the factor "pepsin".[47] Wasman isolated pepsin in 1839, and in 1854 Epstein and Grutzner postulated the existence of pepsinogen. In 1867 Rudolf Peter Heinrich Heidenhain (1834-1897) began a systematic study of the digestive glands.[48] He noted that there were two kinds of cells in gastric glands: one secreting acid, and the other, pepsin. He called these cells *Belegzellen* and *Hauptzellen* respectively. Several years later John Newport Langley (1852-1925) translated this terminology into English as "border" and "chief" cells, and also used the word "oxyntic" to identify the role of acid secreting cells.[47] Between 1879 and 1882 Langley established the basic morphological and secretory characteristics of the pepsin secreting glands. Wilhelm Kuhn (1837-1900) theorised that since the stomach, itself, was not digested by pepsin, the substance must be produced in an inactive form (pepsinogen). He referred to these inactive forms of enzymes as "zymogens". Kuhn also identified trypsin in 1868, and is attributed with the first use of the term "enzyme".

2.1.2 Gastric Anatomy

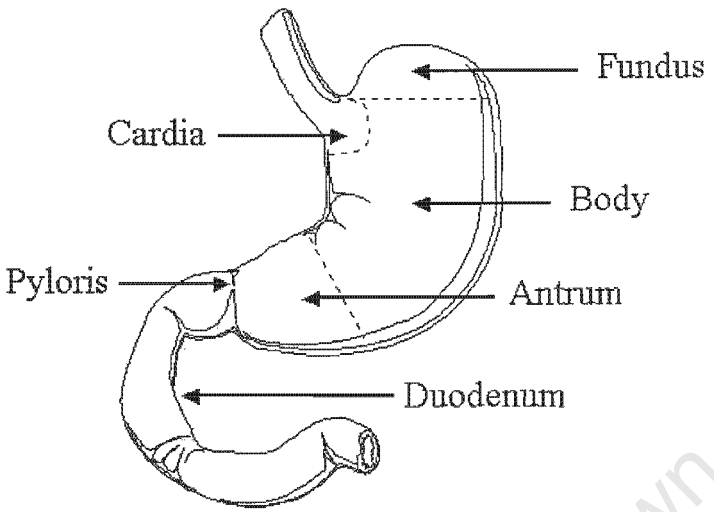


Figure 2.1.1: Gastric anatomy

The stomach is the most dilated part of the digestive tract, and serves as a temporary reservoir for ingested food, allowing for the initiation of digestion, and conversion of the food into a form suitable for entry into the small intestine, where the bulk of digestion and absorption takes place. Food enters the stomach through the oesophageal (or cardiac) orifice. The fundus is that part of the stomach lying above the cardiac orifice. The major part of the stomach comprises the body (or corpus), and an oblique line joining the most dependent part of the lesser curve, the incisura (or angularis), to the greater curve divides the body from the antrum. The pylorus forms the exit from the stomach, and entry into the duodenum.[49]

2.1.2.1 Gastric Structure

The wall of the stomach consists of the four usual layers: serous, muscular, submucous, and gastric mucosal membrane.

The Serosa (visceral peritoneum), covers the entire external surface of the organ, except for the lines of attachment of the greater and lesser omenta along the lesser and greater curvatures, and a small area on the posterior-inferior surface of the stomach, close to the

cardiac orifice, where the stomach is in direct contact with the under surface of the diaphragm.

The Muscular Layer lies below the serosa, and consists of three layers of visceral muscular fibres: longitudinal, circular and oblique. The longitudinal fibres are the most superficial, and radiate from the cardiac orifice. The circular muscle fibres form a uniform layer over the whole of the stomach, internal to the longitudinal fibres, and aggregate into a ring at the pylorus, where they form the pyloric sphincter. The oblique fibres are internal to the circular layer, and are limited chiefly to the body of the stomach. Contraction of the muscular layers provides peristaltic activity within the stomach, thoroughly mixing, and grinding the food, returning some to the body of the stomach for further processing, and propelling some into the duodenum. The pyloric sphincter undergoes intermittent contraction, and relaxation, allowing entry of the gastric content into the duodenum in a highly controlled fashion.

The Submucosa consists of loose areolar tissue, connecting the muscular layer to the mucosa.

The Gastric Mucous Membrane is thick, smooth and velvety in appearance. When examined under a lens, the luminal surface of the mucous membrane has a honeycomb appearance with numerous small depressions, each about 0.2 mm in diameter. These constitute the gastric pits, the bottom of which, receive the orifices of the gastric glands. The surface of the mucous membrane and the gastric pits is covered with a single layer of columnar secretory epithelial cells, (surface mucus cells), which secrete mucus onto the surface of the stomach. The gastric mucus provides the function of lubrication, as well as protection against secreted acid and enzymes. The gastric glands consist of cardiac glands, main glands of body and fundus, and the pyloric glands.

The *cardiac glands* are few in number, and are confined to a small area adjacent to the cardiac orifice. Mucus secreting cells predominate, with few acid secreting (oxynic) and enzyme secreting (zymogen) cells.

The *main gastric glands* are are found in the body and fundus of the stomach. Three to seven glands open into each gastric pit. Each gastric gland consists of an isthmus, a neck and a base. The isthmus and neck of the gland is comprised predominantly of mucus secreting

cells. The acid secreting (oxyntic) parietal cells are distributed throughout the neck of the gland. They are large (20μm) conical cells containing abundant mitochondria, and a well developed cytoplasmic membrane system. The endocrine, enterochromaffin-like (ECL) cells are approximately 10μm in size, and are distributed throughout the distal third of the gland. The ECL cells contain numerous vacuoles, and are the main source of mucosal histamine, stored in granules dispersed throughout the cytoplasm. The chief (peptic or zymogenic) cells are present in the basal parts of the glands. These are cuboidal, protein synthesising cells, containing prominent secretory bodies, much rough endoplasmic reticulum, and a prominent Golgi complex. These cells are the source of the digestive enzymes (pepsin, and gastric lipase) in the stomach.

Two or three short convoluted *pyloric glands* open into each gastric pit in the antral zone of the stomach. Within each gland, the epithelial cells are predominantly mucous in type. Parietal (oxyntic) cells are sparse, however, G (gastrin secreting), and D (somatostatin secreting) cells are present.

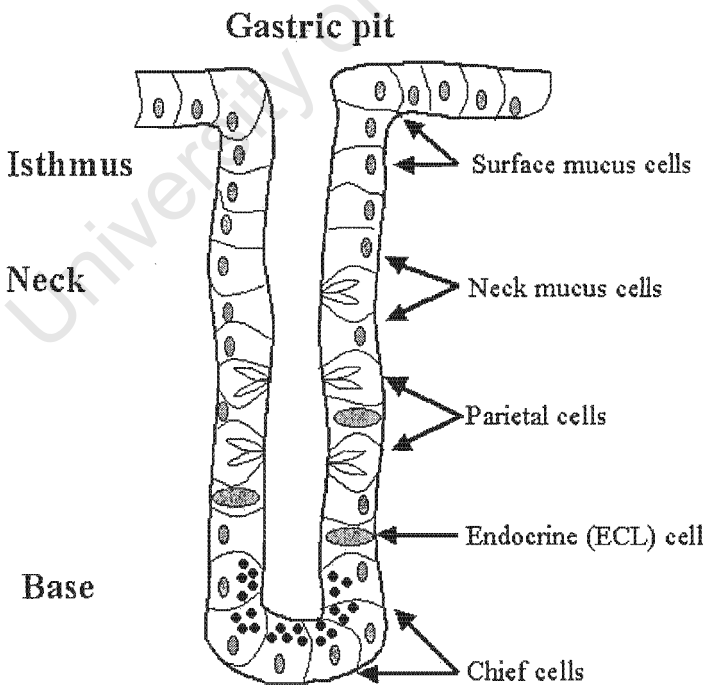


Figure 2.1.2: Schematic of a gastric gland

2.1.3 Physiology of Gastric Secretion

2.1.3.1 Acid Secretion

Stimulation of the gastric parietal cell to produce acid is mediated both centrally by vagal parasympathetic fibres producing actylcholine, and peripherally by the release of histamine from the mucosal enterochromaffin-like (ECL) cells (Figure 2.1.3).[47] The ECL cells represent between 0.5% and 1% of the cells of the stomach, and are distributed in the distal third of the gastric glands. Histamine is stored in acidic granules, dispersed throughout the cytoplasm of the ECL cell, and is released by exocytosis on stimulation of the cell by acetylcholine or gastrin. Secretion of histamine into the paracellular spaces stimulates the parietal cell to secrete acid. Gastrin is released from the antral G cells in response to aromatic amino acids, as well as in response to gastrin releasing peptide (GRP) released by the vagus. Decreased luminal pH in the stomach stimulates the D cells of the antral mucosa to secrete somatostatin, which inhibits the release of gastrin from the G cells, providing feed back control of acid secretion.

The normal adult human stomach contains approximately 1 000 million parietal cells. The parietal cell is a large (20μm), conical cell containing numerous mitochondria, a relatively sparse Golgi apparatus, and an extensive cytoplasmic membrane system. The cell contains receptors for histamine (H_2 receptor), acetylcholine (M_3 receptor) and gastrin (G receptor) on its surface. Binding of histamine to the H_2 receptor results in elevation of intracellular cAMP via a Gs protein. cAMP is the major second messenger involved in acid secretion. Stimulation of the M_3 receptor by acetylcholine is associated with a transient elevation of intracellular calcium. Although gastrin receptors have been identified on parietal cells, stimulation of which also results in release of intracellular calcium, the precise role of gastrin in directly stimulating the parietal cell, however, remains unclear. Blockage of the histamine receptor by an H_2 receptor antagonist effectively inhibits pentagastrin (an analogue of gastrin) stimulated acid secretion indicating that histamine is the chief mediator of acid secretion.

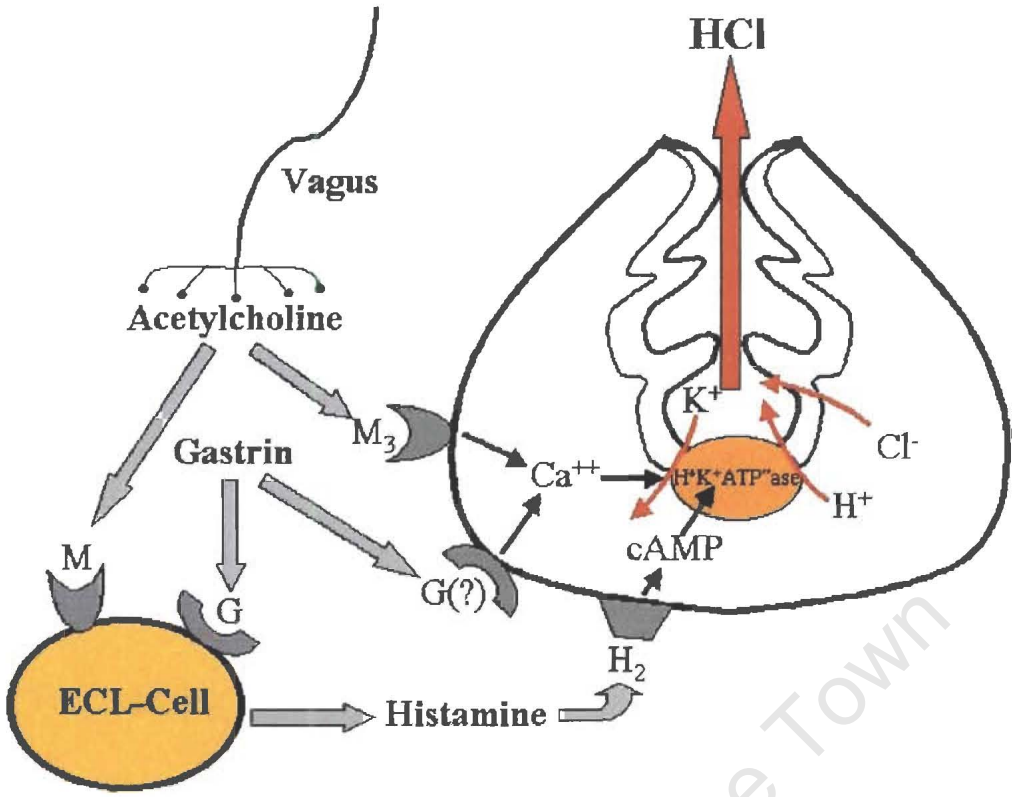


Figure 2.1.3: Stimulation of gastric acid secretion

Understanding of the precise mechanism by which the parietal cell produces acid was initiated by the studies of Ganser and Forte in 1973.[50] Following studies on the oxyntic cells of the bullfrog, they noted the presence of a unique protein in the parietal cells. The protein was a potassium-stimulated adenosine triphosphatase (K^+-ATP'ase), and was subsequently shown to be present in the parietal cells of other animals, including man. The protein uses energy derived from the splitting of ATP (supplied by the mitochondria in the parietal cell) to pump cytosolic hydrogen ions (H^+) into the secretory canaliculi, in exchange for potassium ions (K^+). This acid pump is therefore an H^+,K^+-ATP'ase, often referred to as the proton pump. On stimulation of the parietal cell, the apical membrane becomes permeable to K^+ ions, enabling these ions to diffuse down the concentration gradient from the cytosol into the secretory canaliculi. Activation of the proton pump results in the pumping of H^+ ions from the cytosol to the secretory canaliculi in exchange for the K^+ ions. Stimulation of the parietal cell also results in an increase of the apical membrane to Cl^- which, like the K^+ ions diffuse out of the cell into the secretory canaliculi, where they combine with the H^+ ions to form hydrochloric acid (HCl), which then passes into the lumen of the stomach. This action results

in the formation of a significant pH gradient, from 7.3 in the cell to 1 in the extracellular secretory canaliculi.

2.1.3.2 Pepsinogen Secretion

Pepsinogen consists of a group of aspartic proteinases, with a molecular weight of approximately 42 000.[51] It is secreted into the gastric juice by four different cells: the mucous neck cells and the chief cells in the fundus and body of the stomach, the pyloric gland cells in the gastric antrum, and the Brunners gland cells in the duodenum. The gastric chief cells secrete most pepsinogen. Seven isoenzymes of pepsinogen have been identified, numbered 1 to 7. Pepsinogen I (PGA) comprises isoenzymes 1 to 5, and pepsinogen II (PGC), isoenzymes 6 and 7. Pepsinogen I is found only in the gastric body, whereas pepsinogen II is found also in the antrum and duodenum. The pepsinogens are synthesised as a pre-pepsinogen, containing a 15-amino acid NH_2 – terminal sequence that is cleaved to produce pepsinogen.[52] The genes for human pepsinogen have been characterized,[53] and that for pepsinogen I located to chromosome II.[54] Pepsinogen is stored in zymogen granules within the cell. When the chief cell is stimulated, the granules fuse with the apical membrane, and the contents extruded by exocytosis.

Regulation of pepsinogen secretion is mediated by both intracellular cyclic adenosine monophosphate (cAMP) and calcium. Stimulation of the chief cell by both CCK and muscarinic agonists increase pepsinogen secretion by mobilisation of intra cellular calcium, whereas secretin, histamine and vasoactive inhibitory peptide (VIP) increase secretion by increasing intracellular cAMP. Prostaglandins and somatostatin decrease intracellular cAMP, thus inhibiting pepsinogen secretion (Figure 2.1.4).[55]

Studies in man have indicated that vagal stimulation is responsible for the basal secretion of pepsinogen. Histamine directly stimulates pepsinogen secretion via H_2-receptors, and gastrin probably acts indirectly through its stimulation of histamine secretion by the gastric ECL cells. The secretion of pepsinogen is inhibited by prostaglandin E_2 (PGE_2) and somatostatin.

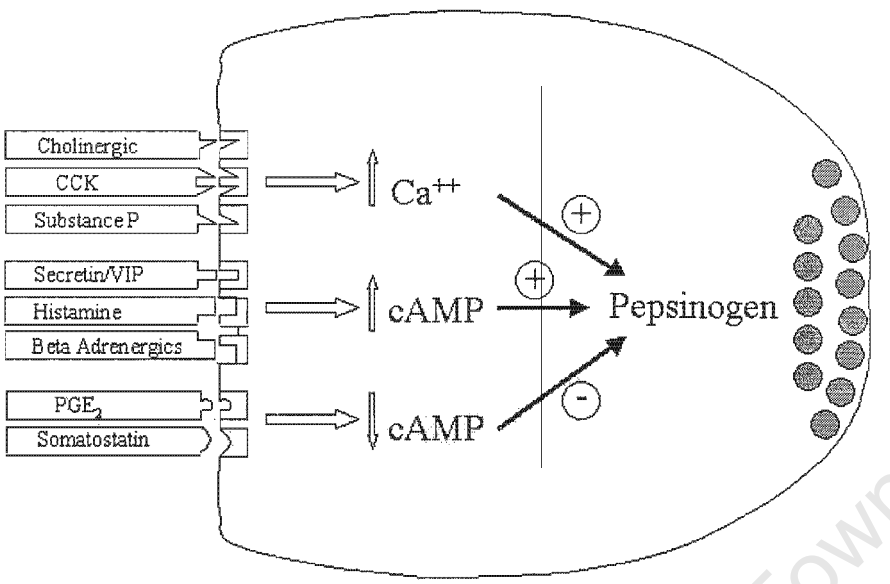


Figure 2.1.4: Control of pepsinogen secretion in mammalian chief cells (adapted from Muller 1990[55])

2.1.4 Function of Gastric Acid

The normal human stomach, under basal conditions secretes 1.5-2 mEq acid per hour, and approximately 25 mEq acid per hour under conditions of maximal stimulation.

The chief functions of gastric acid may be summarised as follows:

1. Provision of an antiseptic environment
2. Activation of pepsinogen
3. Activation of gastric lipase
4. Initiatiation of the digestive process
5. Facilitation of the absorption of iron
6. Facilitation of the absorption of vitamin B12

Gastric acid provides an antiseptic environment (the acid trap), preventing bacterial contamination of the upper gastro-intestinal tract. Recent studies have also indicated that dietary nitrate, and the development of salivary nitrite, results in effective antimicrobial

action in the acid environment of the stomach.[56,57] The acid milieu is a prerequisite for the action of the gastric protease enzyme pepsin in the initiation of protein digestion. The conversion of pepsinogen to pepsin occurs slowly at pH 5 to 6, but is almost instantaneous at pH 2. Pepsin is produced from pepsinogen by an auto-catalytic reaction. Pepsin initiates protein digestion, and is able to catalyse the hydrolysis of a wide range of peptide bonds. Optimal pH for peptic activity is 1.0 to 3.0. The gastric epithelial cells are protected from the action of pepsin by the adherent mucus layer, as well as the near-neutral pH at the mucosal surface consequent to the mucosal secretion of bicarbonate, which inhibits pepsin activity. Pepsin has a contributory role in the overall regulation of the digestive process, by producing the peptides, and amino acids that subsequently act as stimuli for the release of hormones such as gastrin and cholecystokinin (CCK).

Chief cells located in the fundic mucosa also secrete gastric lipase.[58] Secretion is stimulated by cholecystokinin, and gastric lipase activity is higher against short-and medium-chain triglycerides than against long-chain triglycerides. The activity of gastric lipase is dependent on the presence of acid, with an optimal pH range between 4.5 and 5.5, and activity rapidly declining at pH > 6.5. Like pepsin, gastric lipase is likely to play a contributory role in the overall regulation of digestion, by producing fatty acids, which are important stimulants for the secretion of CCK, which in turn stimulates pancreatic exocrine secretion. By maintaining an acid environment, which is a prerequisite for the function of both pepsin, and gastric lipase, hydrochloric acid secretion by the parietal cells plays a contributory role in the initiation of the digestive process (Figure 2.1.5).

The acidic environment facilitates the conversion of ferric iron to ferrous iron, under the influence of ascorbic acid secreted by the gastric mucosa, improving the intestinal absorption of iron. Gastric parietal cells also secrete intrinsic factor, which plays a vital role in the transport and absorption of cobalamin (vitamin B12). Release of cobalamin from dietary protein is dependent on the presence of acid in the stomach. The released cobalamin is initially bound to salivary R proteins, but in the proximal duodenum pancreatic proteases release the cobalamin, which is then bound to intrinsic factor. The cobalamin-intrinsic factor complex is resistant to further protease activity, allowing the cobalamin to be delivered to the distal small bowel where absorption occurs.

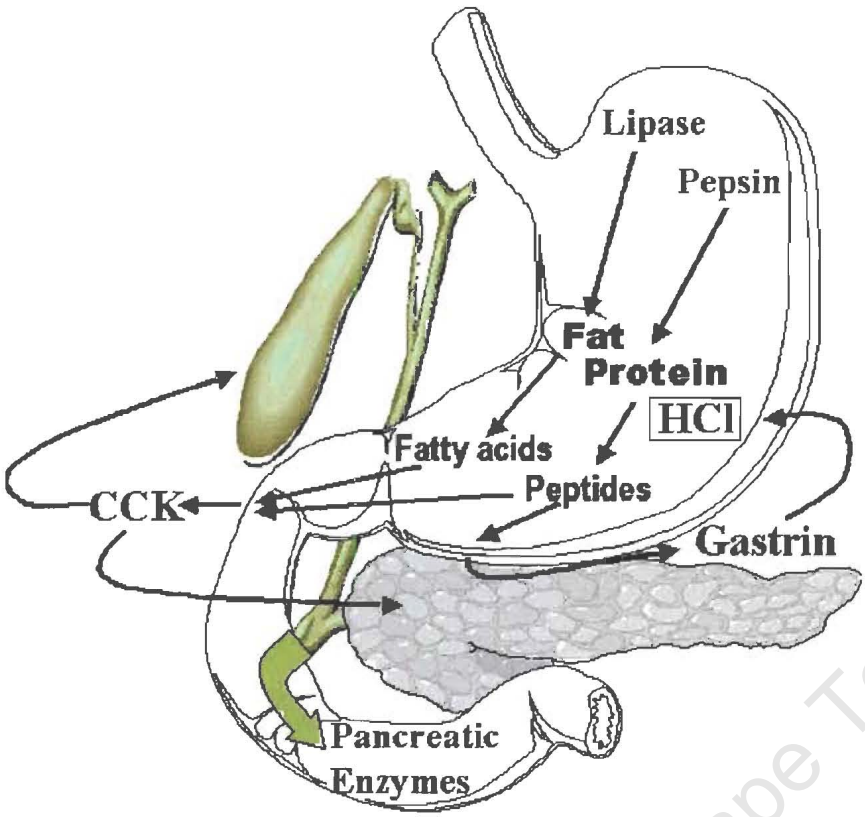


Figure 2.1.5: The interaction between gastric secretions and pancreatic function. Gastric lipase and pepsin, in the acidic environment of the stomach, catalyse the digestion of fats to fatty acids, and proteins to peptides respectively. Peptides subsequently stimulate the G cells of the gastric antrum to secrete gastrin, which stimulates the parietal cells to produce acid. Both fatty acids, and amino acids liberated from the peptides, stimulate the secretion of CCK from duodenal mucosal cells, which stimulates gall bladder contraction, and pancreatic enzyme secretion.

2.2 THE PHYSIOLOGY OF PANCREATIC SECRETION

2.2.1 Historical Perspective

The pancreas was first described by Herophilus of Chaikidon in about 300 BC, and Erasistratos (310-250 BC).[59] Herophilus also coined the term "duodenum", meaning twelve digits long. Rufus of Ephesus, approximately 400 years later, formally named the organ with the word "pancreas" derived from the Greek pan (all), and kreas (flesh). Although recognised as a distinct organ, its formal structure and function remained unknown until the 17th Century. Johann George Wirsung described the main pancreatic duct in 1642, and Abraham Vater the duodenal papilla in 1720. Santorini identified the accessory duct in 1724.

Reignier De Graaf (1641-73) initiated formal physiological study of the organ, and collected and studied pancreatic juice using a duck's quill inserted into the pancreatic duct of a dog. His findings were recorded in *De Succo Pancreatico*, 1664. On tasting the juice secreted by the pancreas, he, however, mistakenly declared it acidic. Despite J.C. Brunner declaring the pancreas a non-vital organ in 1688, Claude Bernard (1813-78), using a pancreatic fistula model, discovered that pancreatic juice was important for digestion (previously thought to only occur in the stomach), by demonstrating its effect in breaking down fats, starch and protein in the duodenum. Dolinsky, in Pavlov's laboratory in 1895, demonstrated that infusion of hydrochloric acid into the duodenum strongly stimulated the secretion of pancreatic juice. In line with his theory of "nervism', Pavlov interpreted this response as being mediated by a local nerve reflex. Bayliss and Starling in 1901, however, demonstrated that the response also occurred in denervated bowel, and was duplicated by the intravenous injection of an extract taken from the gut mucosa. This resulted in the identification of the hormone secretin. Ivy and Oldberg demonstrated that intestinal extracts stimulated gall bladder contraction in 1928.[36] The substance fulfilled the criteria for a hormone, and was termed cholecystokinin (CCK). In 1943, Harper and Raper demonstrated stimulation of pancreatic secretion by a similar intestinal extract, and proposed the name pancreozymin.[38] Mutt and Jorpes subsequently isolated a 33 amino acid polypeptide that has the properties of both CCK and pancreozymin, revealing that the two substances were, in fact, one hormone.[60,61] Isolation and chemical characterization of secretin was achieved by Mutt,

Jorpes, and Magnusson in 1970,[40] and that of CCK by Mutt and Jorpes in 1971.[41] These hormones are found in the upper small intestine, with the greatest concentration found in the duodenum. Secretin was shown to be responsible for the secretion of a bicarbonate-rich pancreatic fluid,[37] and CCK, an enzyme rich fluid,[38] as well as contraction of the gallbladder.

2.2.2 Structure of The Pancreas

The pancreas is a lobular structure, measuring 12-15 cm, weighing between 70 and 150 g and consisting of a head, uncinate process, neck, body and tail.[49,62] The main pancreatic duct (duct of Wirsung) traverses the pancreas from left to right, and receives the ducts of the various lobules, joining at almost right angles resulting in a "herring-bone pattern". At the neck of the pancreas, the main duct turns downwards, backwards and to the right, comes into relation with the common bile duct, and together pass obliquely into the wall of the descending part of the duodenum, where they unite to form the hepatopancreatic papilla. The accessory pancreatic duct (duct of Santorini) runs upward in front of the main pancreatic duct, to which it is connected by a communicating duct, and opens into the duodenum above the major duodenal papilla to form the minor duodenal papilla.

The pancreas consists of two distinct types of glandular tissue, and is both an exocrine and endocrine organ. The main mass of the pancreas, (90%), is exocrine tissue, in which there are embedded clusters of endocrine cells (the islets of Langerhans). The exocrine cells are primarily involved in the production of digestive enzymes, whereas the endocrine cells secrete the hormones insulin (B cells), glucagon (A cells), somatostatin (D cells), pancreatic polypeptide (PP) and vasoactive intestinal peptide (VIP), which have both local (within the pancreas) as well as distant effects.[63]

2.2.2.1 The Exocrine Pancreas

2.2.2.1.1 Basic Structure

The exocrine pancreas consists of lobular units, separated by loose connective tissue. The secretory (zymogenic) cells are pyramidal in shape, and are arranged in flask shaped

structures, each with a central narrow intercalated (intralobular) duct, lined by cuboidal (centroacinar) cells, to form the pancreatic acinus.[64] The intralobular ducts, drain into larger interlobular ducts lined with columnar epithelium, which course in the connective tissue septa, and ultimately empty into the main pancreatic duct.

Vascular supply of the pancreas is derived from the splenic and pancreatoduodenal arteries, and venous drainage is into the portal, splenic and superior mesenteric vessels. Innervation is from the parasympathetic system, the sympathetic system, and from local nerve plexuses in the small intestinal wall. The parasympathetic supply reaches the pancreas via the vagus, and also through the celiac ganglion, the splanchnic nerves and the mural plexuses of the duodenum.

2.2.2.1.2 Secretory Cells

The secretory cells constituting the pancreatic acinus are typical zymogen cells. These cells contain a basally situated nucleus, surrounded with basophilic cytoplasm. The ultra-structure consists of regular arrays of granular endoplasmic reticulum, interspersed with mitochondria and dense secretory granules. A prominent Golgi complex, surrounded by numerous larger secretory granules, is present in the apical supranuclear compartment of the cell. The large secretory granules contain the enzymic constituents of pancreatic secretion. These enzymes are in an inactive form whilst in the granules, and only become activated after release from the intralobular ducts.

In comparison to the secretory acinar cells, the centroacinar cells lining the intralobular ducts have a comparatively empty cytoplasm. The ductal cells are primarily responsible for the production, and secretion of a bicarbonate-rich fluid, which on reaching the small intestine, neutralises gastric acid and promotes the activity of the pancreatic enzymes. The human sodium-bicarbonate co-transporter has recently been identified and cloned.[65] This protein is a 1079-amino acid protein, and like other transporters, has 12 transmembrane domains. Northern blot analysis has demonstrated expression of the sodium-bicarbonate co-transporter in the pancreatic acini and ductal cells, and to a lesser extent, in the kidney, brain, liver and stomach.[65]

2.2.3 Synthesis of Pancreatic Enzymes

Precursor amino acids are taken up into the acinar cell from the extracellular fluid by active transport, forming part of the intracellular amino acid pool. From this pool, the amino acids pass directly to the ribosomes of the rough endoplasmic reticulum (RER), on which the enzyme protein is formed. The classical electron microscopic autoradiographic studies of Jameson and Pallade determined the route and time course of the intracellular transport of pancreatic exocrine protein, and demonstrated the transport from one intercisternal compartment to another (Figure 2.2.1).[66]

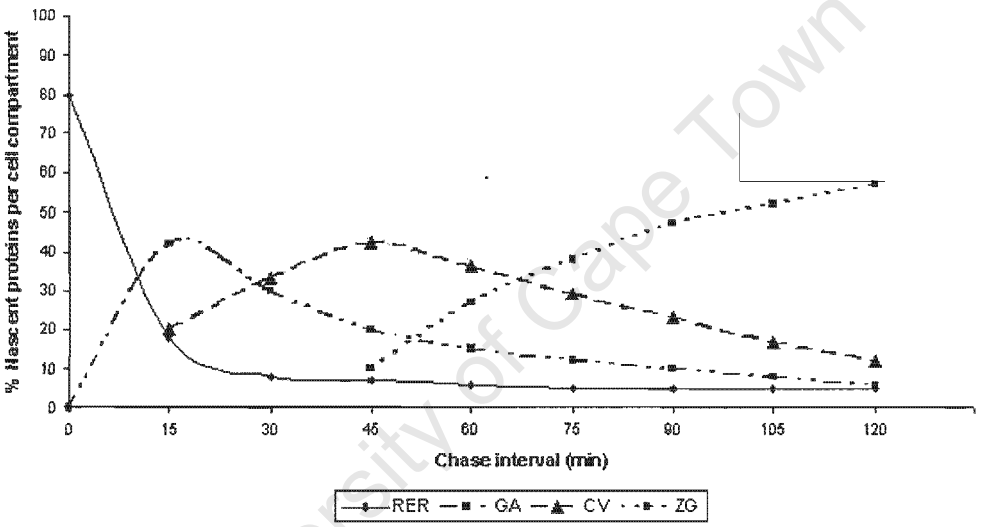


Figure 2.2.1: Kinetics of intracisternal transport of labelled exocrine proteins in the guinea pig pancreas. RER = Rough endoplasmic reticulum; GA = Golgi apparatus; CV = Condensing vacuole; ZG = Zymogen granules. Adapted from Jamieson and Palade[66]

The intracellular traffic of the exocrine proteins, as demonstrated by these autoradiographic studies, may be summarised as:

RER ⟶ Golgi ⟶ Condensing vacuole ⟶ Zymogen granules

Pancreatic proteins destined for secretion are synthesised as larger polypeptides (pre-secretory proteins) containing an additional peptide extension of 15-20 amino acid residues.[67]

The newly synthesised pancreatic enzymes are then transported through the outer membrane, and segregated in the cisternae of the RER. Intracisternal segregation of nascent secretory proteins is irreversible, and proteolytic removal of signal sequences results in conformationally stable protein.[68] The cisternal RER space is interconnected to other cisternal compartments of the cell (Golgi, secretory granules, lysosomes). The proteins then pass to the Golgi complex on the apical compartment of the cell, where further processing take place, including the addition of sugar molecules and divalent ions such as calcium to form glycoproteins such as amylase. Molecular sorting takes place in the Golgi complex, and results in the separation of acid hydrolases into lysosomal structures (primary lysosomes), and neutral or slightly alkaline hydrolases (exocrine enzymes) into condensing vacuoles and zymogen granules.[67] In the condensing vacuole, polypeptides are condensed into a prozymogen granule, which is ultimately covered by its own limiting membrane. Once the prozymogen granule obtains a limiting membrane, it is termed a zymogen granule, which contains all the various pancreatic enzymes. The limiting membrane is similar to the plasma membrane of the cell, and it protects the cytoplasm from the zymogen enzymes until they are discharged into the acinar lumen by exocytosis. Recent study has indicated that exocytosis of the enzymes involves transport of the zymogen granules to the apical secretory area of the cell along microtubules, under the influence of the mechanochemical enzyme, kinesin.[69,70] Fusion of the zymogen membrane with the apical portion of the plasma membrane then occurs, resulting in exposure and release of the zymogen enzymes to the intralobular ducts. Enzymes then pass to the interlobular ducts, the pancreatic duct, and then finally into the duodenal lumen.

2.2.4 Regulation of Pancreatic Enzyme Synthesis and Secretion

The pancreas secretes fluid, electrolytes, and enzymes into the proximal small bowel, where they are important for the digestion of fat, protein and carbohydrate. The pancreatic response following ingestion of a meal is regulated by cephalic, gastric, and intestinal stimuli.

The cephalic phase is initiated by behavioural cues, relating to the sight, and smell of food, and is mediated by the central nervous system. Sham feeding has been shown to result in a highly reproducible enzyme response, which amounts to approximately 50% of the maximal response.[71] It is generally assumed that vagal efferent fibres mediate the effects of cephalic

stimulation, however atropine, in small doses, has been found to have no effect on sham feeding induced pancreatic enzyme secretion in humans.[71] It is possible that there are vagal peptidergic efferent fibres, which might mediate pancreatic secretion.[72]

The gastric phase regulates pancreatic secretion in two ways. There is a direct neural pathway sensitive to gastric distension, and gastric emptying controls the entry of nutrients into the small intestine. Physical distension of the gastric antrum, following ingestion of food, elicits pancreatic secretion through a neural pathway involving the central nervous system.[73,74] The magnitude of stimulation is, however, relatively small compared to the maximal response of which the pancreas is capable.[73] The pancreatic response to gastric distension is blocked by atropine and vagotomy,[75] indicating that the gastric phase is mediated by vagal efferents.

The intestinal phase involves entry of nutrients into the proximal small bowel, inducing the most potent stimulation of pancreatic exocrine secretion. The products of digestion of fats (free fatty acids), and proteins (peptides and amino acids), rather than intact fats and proteins, appear to be primarily responsible for stimulation of pancreatic secretion.[76,77] Of the amino acids, the essential amino acids, phenylalanine, valine and methionine, appear to be the most potent stimulants.[76,77]

The primary stimulant of pancreatic exocrine secretion is the hormone cholecystokinin (CCK). CCK is produced in specialized gut endocrine cells (I cells) that line the upper small intestinal wall, and is secreted in response to food. As with most mucosal endocrine cells, CCK cells are flask shaped, with their apical surfaces open to the lumen of the gut.[78,79] The apical surface consists of microvilli, which sample the contents of the lumen of the gut. Concentrated on the basolateral pole of the cell are hormone–containing secretory granules, which on stimulation of the cell, discharge their contents into the paracellular, space from where the CCK can reach the bloodstream. CCK is also synthesised in neurones of the brain and gut. In the central nervous system, CCK may mediate the effects of satiety on the ventromedial hypothalamus.[80] In the peripheral nervous system, CCK-containing neurones are found in the myenteric, submucus and celiac plexi, in the circular muscle layers of both the small intestine and colon, in the vagi, and within nerves supplying the pancreas.[81] CCK stimulates postganglionic neurones in the gut to secrete acetylcholine, resulting in CCK's effects on gut motility. CCK has also been shown to delay gastric emptying. There is

evidence that, in both the exocrine and endocrine pancreas, CCK exerts its action via its effects on the nerves supplying the acinar and endocrine portions of the gland.[82] CCK, through its actions of induction of satiety, and stimulation of pancreatic exocrine synthesis and secretion, gallbladder contraction, and gut motility, coordinates the ingestion, digestion, and disposal of nutrients in a highly regulated fashion.[83]

The hormone was originally purified as a 33-amino acid peptide,[61] however it was subsequently recognised that various molecular forms of CCK exist in intestine, brain, other neural tissue, and blood, ranging from 4 to 83 amino acids in size. The molecular forms arise from a single CCK gene, and are the result of differences in post-translational processing.[84,85] Forms as small as CCK-8 have equivalent biological activity of larger forms, and recent studies have indicated that CCK-33, CCK-8, and perhaps an intermediate form are the predominant types found in human tissue.[86] CCK shares some homology with gastrin. This sequence similarity confers some gastrin-like biological activity to CCK, and weak CCK-like activity to gastrin.[87]

2.2.4.1 Dietary and Neural Regulation of CCK Secretion

The precise mechanism by which food stimulates pancreatic exocrine secretion via CCK remains enigmatic, and incompletely understood.[88] The structure, and localisation of the CCK secreting cells in the duodenum and upper jejunum indicates that they are capable of responding to food and digestive products present in the bowel lumen. Like other gastro-intestinal hormones, CCK is secreted into the bloodstream following ingestion of a meal.

Studies in humans indicate that the products of digestion, intraduodenal free fatty acids[89,90] and amino acids,[91] rather than the intact triglycerides or proteins are the most potent stimulants responsible for CCK release. Of the amino acids, phenylalanine appears to be the most potent for stimulating CCK secretion.[92-94] Carbohydrates (starch) do not appear to significantly influence the secretion of CCK.[95] Stimulation of the secretion of CCK is therefore dependent on the intraduodenal presence of the products of digestion of fat and protein, initiated by gastric lipase and protease, and by basal pancreatic secretion. Failure of CCK release after a meal has been demonstrated in patients with exocrine pancreatic insufficiency, which can be restored by the administration of pancreatic extracts.[96,97] The

intraduodenal presence of the products of fat digestion appears to be of particular importance. Inhibition of intestinal lipase by tetrahydrolipostatin (orlistat) prevents triglyceride hydrolysis, and results in an insignificant increase in CCK stimulation in response to a test meal.[90,98] In human, fatty acids with a chain of 12 carbon atoms or longer stimulate CCK release, whereas those with a chain length less than 11 carbon atoms do not.[99] Long-chain triglycerides are therefore potent stimulants of pancreatic secretion, whereas medium-chain triglycerides are not.

Intraduodenal instillation of 0.1mol/l HCl has been shown to result in a significant increase in CCK release.[100] However, a more physiological buffered solution of weaker acidity (pH 2.7) only induces a small increase in plasma CCK in humans.[101] This indicates that acid only plays a minor role in the stimulation of CCK release under normal physiological conditions.

2.2.4.2 Mechanism of CCK Release

Studies have indicated that the release of CCK, stimulated by amino acids or fatty acids, is dose-dependently reduced by an intraduodenal infusion of trypsin,[92,102,103] and is augmented by ingestion of protease inhibitors.[104,105] Infusion of the other pancreatic enzymes, lipase and amylase, has no effect on amino acid stimulated CCK secretion.[92] Diversion of proteolytic activity from the proximal small bowel by bile-pancreatic juice diversion has also been shown to augment CCK release and pancreatic exocrine secretion.[63,106] These findings indicate that the release of CCK from the upper small bowel is mediated via trypsin-sensitive CCK-releasing factors (CCK-RF), and that protease inhibition of its secretion represents feedback control.

Lu et al,[107] and Miyasaka et al,[108] demonstrated the existence of a factor in intestinal washes, which actively stimulated CCK release and pancreatic enzyme secretion in rats when infused into the duodenum. The factor has recently been purified and chemically characterized,[109,110] and has been termed luminal CCK-releasing factor (LCRF), to distinguish it from other CCK-releasing factors.

Herzig et al, extracted another CCK-RF from porcine intestinal mucosa, which has been shown to have a sequence identical to porcine diazepam-binding inhibitor (DBI), but

dissimilar to LCRF,[111] and has been shown to be stimulated by the presence of nutrients (5% peptone meal) in the proximal small bowel.[112] It is therefore apparent that there is more than one CCK-RF in the upper intestine. The relative contributions of the various CC-RF's to regulation of CCK release following ingestion of a meal, however, remain to be determined.[93] These peptides stimulate CCK-releasing cells to secrete CCK, unless free trypsin is present, in which case they are degraded prior to stimulation of CCK release.

In 1986, Iwai et al identified a 61 amino acid protein in rat pancreatic juice, which stimulated CCK release when introduced into the intestine.[113-115] In view of its ability to "monitor the intraduodenal environment for protein digestion", the protein was named "monitor peptide". Monitor peptide differs from the other CCK-RF's (LCRF and DBI) in that it is produced by the pancreatic acinar cells, and is secreted into the pancreatic juice, whereas LCRF and DBI are of intestinal origin. Monitor peptide also requires some level of pancreatic secretion for stimulating CCK release.[93]

Negative feedback control of CCK production by the proximal small intestine is provided by the proteolytic action of pancreatic enzymes (trypsin) on the various CCK-RF's. Intact protein in meals may therefore indirectly stimulate pancreatic secretion by competing with the CCK-RF's for pancreatic proteases. Bile salts have also been shown to be important modulators of CCK secretion. Studies have indicated that diversion of bile away from the duodenum,[116,117] or oral administration of the bile salt binding resin cholestyramine,[116] result in an increase in basal CCK levels. In view of the inhibitory effects of both bile and trypsin on enteral CCK release, the pancreatic response to intraluminal stimulants is likely to be submaximal.[93]

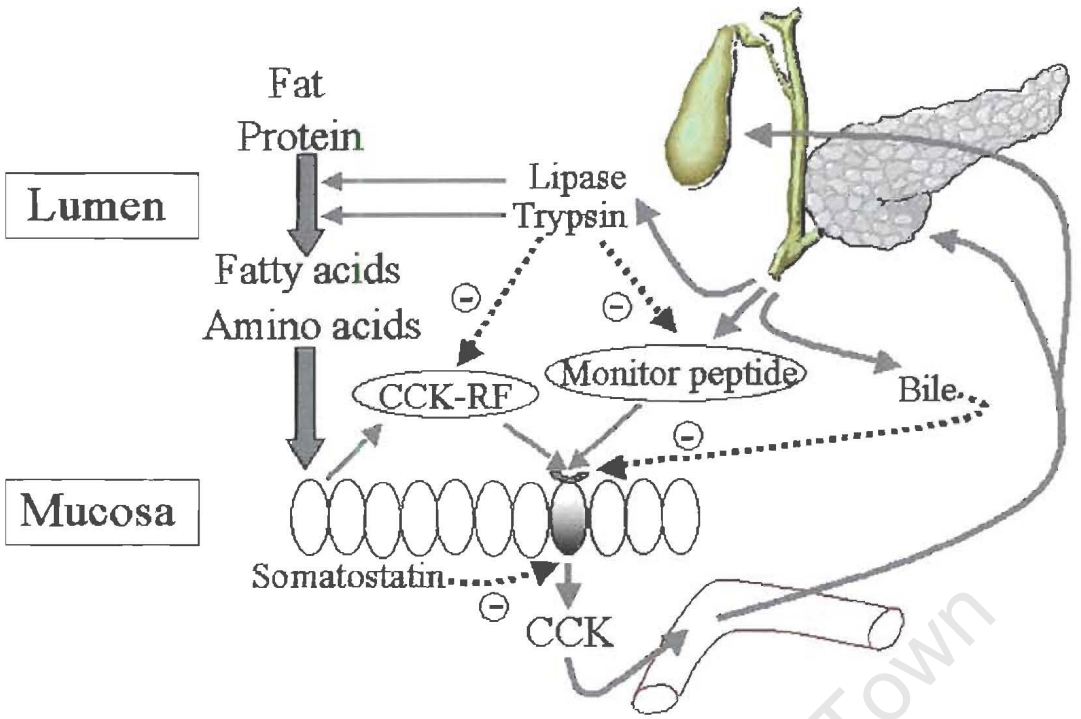


Figure 2.2.2: Intraluminal fatty acids and amino acids stimulate intestinal mucosal cells to secrete CCK-RF, which, in turn, stimulate the I-cells of the mucosa to secrete CCK. Under the influence of CCK, the pancreas secretes exocrine enzymes and monitor peptide, and the ball bladder discharges bile into the gut lumen. Monitor peptide further stimulates the I-cells to secrete CCK, whereas trypsin inactivates both CCK-RF and monitor peptide, and bile salts suppress the CCK secreting cell, providing negative feed back control. Somatostatin inhibits CCK secretion by the I-cell.

2.2.4.3 Effect of CCK on the Exocrine Pancreas

CCK appears to be the primary stimulant of pancreatic enzyme secretion, and exerts its action by binding to specific G-protein-coupled receptors (GPCR's). The CCK receptors are seven-transmembrane-spanning proteins coupled to heterotrimeric G-proteins. The 3 subunits of the G-protein are responsible for the intracellular actions, and ultimately the target tissue response. Most of the work on the function of CCK in the exocrine pancreas has been performed in rodents, which primarily express CCK-A receptors. The precise role and function of CCK-B/gastrin receptors, the predominant CCK-receptors in the human pancreas, is less clear. Saillan-Barreau et al,[118] however, demonstrated that CCK-B/gastrin receptors, introduced into the acinar cells of transgenic mice, functioned similarly to CCK-A receptors.

Stimulation of CCK receptors results in an increase in cytosolic Ca^{2+} in the apical pole of the cell, consequent to release from intracellular stores, as well as from plasma membrane pumps.[119] The increase in cytosolic Ca^{2+} spreads to the basal pole, where fusion of zymogen granules with exocytosis of enzyme in induced. The precise mechanisms by which cytosolic Ca^{2+} induces zymogen granule fusion and exocytosis remains unclear.[119]

In addition to a direct effect on the pancreatic acinar cell, CCK has been shown to have an indirect action, via stimulation of the cholinergic neural pathway in the pancreas.[82]

2.2.4.4 Other Factors Controlling Pancreatic Exocrine Secretion

Although CCK appears to have a primary role in the stimulation of pancreatic exocrine secretion, as illustrated in Table 2.2.1, a number of other factors have been shown to modulate secretion.

Table 2.2.1:
Regulation of Pancreatic Exocrine Secretion

Stimulation	Inhibition
Cholecystokinin	Glucagon
Secretin	Somatostatin
Gastrin	Pancreatic polypeptide
Neurotensin	Peptide YY
Insulin	Neuropeptide Y
Vasoactive intestinal polypeptide	Encephalin
Gastrin-releasing peptide	Calcitonin gene-related peptide
Bombesin	Pancreastatin
Adenosine 5-triphosphate	Thyrotropin-releasing hormone
Uridine triphosphate	Glucagon-like peptide-1
Histamine (H1 receptor)	Dopamine

2.2.4.4.1 Secretin

Secretin, the first hormone identified, is a primary regulator of pancreatic fluid and electrolyte secretion. Secretin receptors have been identified on pancreatic acinar and duct cells, but not on pancreatic islets or vascular structures.[120] Secretion of secretin by the mucosal cells of the proximal small bowel appears to be primarily controlled by gastric acid entering the duodenum, and results in the secretion of pancreatic fluid rich in bicarbonate.[121,122] Although the precise mechanism of action of secretin remains to be elucidated, the human pancreatic sodium bicarbonate cotransporter has recently been identified in pancreatic acinar and ductal cells.[65]

2.2.4.4.2 Glucagon and Glucagon-like peptide-1

Both glucagon and glucagon-like peptide –1 (GLP-1) have been shown to inhibit pancreatic exocrine secretion. The mechanism by which this occurs is presently unknown, but inhibition by GLP-1 may involve inhibition of parasympathetic stimulation from the central nervous system.[123]

2.2.4.4.3 The Role of Neurotransmitters

Many physiological processes use peptides as neurotransmitters (peptidergic neurotransmission), and many of the peptides found in the gastro-intestinal tract have been localised to the central nervous system. This makes complete separation of hormonal control and nervous control difficult, and often impractical. Gastrin-releasing peptide (GRP), and its analogue bombesin, is a neurotransmitter, hormone (paracrine), and growth factor, and has been shown to stimulate pancreatic secretion.[124,125] Calcitonin gene-related peptide (CGRP) has been found in both the central and peripheral nervous systems, and has been shown to inhibit bicarbonate and enzyme secretion by the pancreas.[126] Schmid et al recently identified M1 and M2 muscarinic receptors on isolated rat pancreatic acini.[127] Studies have indicated that cholinergic innervation and secretin may have synergistic effects on pancreatic secretion, which is inhibited by somatostatin.[128] Centrally administered dopamine has been shown to

inhibit basal pancreatic secretion,[129] and histamine[130] and triphosphate nucleotides[131] have stimulatory effects on pancreatic duct epithelial cells.

Physiological regulation of pancreatic exocrine secretion remains incompletely understood. The advent of molecular tools has increased our understanding of the processes involved, and is likely to lead to new, clinically significant, findings of the biochemical basis of this regulation.

2.2.5 Pancreatic Enzymes

The enzymes synthesized by the pancreatic acinar cells play an important role in the digestion of nutrients in the gut, converting the food particles into a form in which they can be absorbed. The pancreatic enzymes are hydrolases, lacking coenzymes, which can be conveniently classified according to the molecules they can hydrolyse. α-Amylase hydrolyses starch, proteolytic enzymes (trypsin, chymotrypsin, elastase, carboxpeptidase) hydrolyse proteins, lipolytic enzymes (lipase/co-lipase, carboxyl ester hydrolase, and phospholipase A2) hydrolyse fats, and nucleolytic enzymes (ribonuclease and deoxyribonuclease) hydrolyse nucleic acids.

2.2.5.1 Amylase

Human α-amylase has been isolated and purified.[132] It is a single polypeptide chain, with a molecular weight of 53 000 ± 1000. The enzyme is a glycoprotein, with a relatively small sugar (glucose) content. Human α-amylase is very similar to parotid amylase, with an identical amino acid sequence. Human amylase forms one of the main constituents of exocrine pancreatic secretion, comprising 10% of the total proteins in pancreatic secretion. Amylase hydrolyses the internal α-1,4 glucosidic linkages of polymers of glucose, producing branched dextrans.

2.2.5.2 Proteolytic Enzymes

Although most unicellular organisms can synthesize all the necessary amino acids from simple substances, higher animals have to rely on their diet to provide certain amino acids. Man requires approximately 1 g per day of each of eight amino acids, derived from the hydrolysis of protein in the gut.[133] These "essential" amino acids comprise phenylalanine, methionine, leucine, isoleucine, lysine, valine, threonine and tryptophan. Proteolysis is provided by proteases produced in the stomach (pepsin), the pancreas and the small bowel. In order to protect the acinar cell from the proteolytic effect of the enzymes, the proteolytic enzymes are produced in an inactive form (a zymogen), which require activation within the gut by a limited proteolysis. The activation of the proteases is a cascade phenomenon, the first step of which is the activation of trypsinogen to trypsin by an enzyme, enteropeptidase, produced by the enteric mucosa. Trypsin then completes the activation of trypsinogen, as well as the activation of chymotrypsinogen to chymotrypsin, proelastase to elastase, and procarboxypeptidase A and B to carboxypetidase A and B. Human pancreatic juice contains 2 trypsinogens, which comprise approximately 19% of the protein in the secretion. Trypsinogen 1 has a molecular weight of 23000, and trypsinogen 2 has a molecular weight of 25000. Under the influence of the proteases, both dietary, and luminal secreted proteins and peptides undergo limited, or complete proteolysis, resulting in free amino acids (40%), or oligopeptides.[134] The brush border peptidases of the gut continue to degrade oligopeptides to di-tripeptides and free amino acids, which are subsequently absorbed by the mucosa, and pass into the portal vein. Some peptides are taken up directly by the enterocytes, and the hydrolysis completed by cytosolic peptidases.

2.2.5.3 Pancreatic Trypsin Inhibitor

The trypsin inhibitors are a class of proteins found in pancreatic secretions, which are closely related to the enzymes. These proteins have the effect of stabilizing the pancreatic proteases during their intracellular transport within the acinar cell, by inhibiting any free trypsin liberated from the zymogen. Kunitz described the first in ox pancreatic secretions in 1936, and it has been shown to inhibit, not only trypsin, but also other endopeptidases such as chymotrypsin, thrombin, and kallikrein. This protein, however, appears to be unique to the ox.

Kazal et al in 1948 discovered the second pancreatic inhibitor, also in the ox.[135,136] The protein has a molecular mass of 60 000, contains 52 to 56 amino acids, and is now generally referred to as the "pancreatic secretory trypsin inhibitor". Pancreatic secretory trypsin inhibitor (PSTI) has been purified from the pancreatic secretions in man[137] and constitutes 0.3 –0.6% of the total protein in the juice. It has also been demonstrated in the mucus-producing cells of the gastro-intestinal tract, at the edge of benign gastric ulcers, in the lung and in the breast[138,139] In addition to his actions in preventing the premature activation of trypsin, PSTI protects mucosal mucus from degradation, and stimulates epithelial proliferation and healing in response to injury, possibly acting via the EGF-receptor.[139]

2.2.5.4 Lipase

Claude Bernard was the first to document that the contents of the lacteals of an animal, which had consumed a fatty meal, became milky below the point at which the pancreatic duct entered the duodenum. This indicated that the pancreatic juice contained a factor, which acted of the fats in the lumen of the small bowel, and allowed them to be absorbed. The digestion of fat is initiated by gastric lipase, but completed by pancreatic lipase and intestinal lipase. Pancreatic juice contains three lipolytic enzymes, lipase/colipase, phospholipase, and carboxyl ester hydolase. Lipase digests triglycerides, phospholipase, phospholipids, and carboxyl ester hydrolase, the esters of short chain fatty acids. Human pancreatic lipase has a molecular weight of between 46 000 and 48 000, and binds at the lipid-water interface of the insoluble dietary fats. The presence of amphipathic substances in the duodenum (bile salts, soaps, etc) interfere with this binding, but the pancreas also produces a small protein, "co-lipase", which allows lipase to be absorbed onto the lipid molecule in the presence of bile salts. Co-lipase binds first to the interfaces covered with bile salts, and then anchors lipase, which would otherwise be incapable of binding.

Lipase hydrolyses the two external chains of triglycerides first, producing from each triglyceride molecule, two free fatty acids, and one monoglyceride (Figure 2.2.3). Further hydrolysis involves the migration of the internal fatty acid chain to an external position, and therefore complete hydolysis of the triglyceride is slow, and the production of glycerol, under normal circumstances, is limited. The free fatty acids and monoglycerides are absorbed by

the gut mucosa, triglycerides are then reconstituted, and delivered to the circulation in the form of chylomicrons.

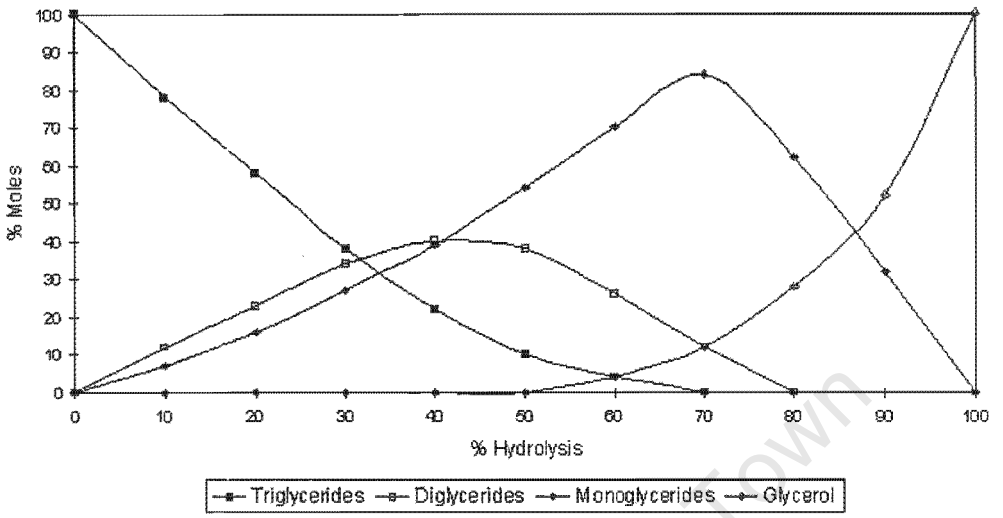


Figure 2.2.3: Relative proportions of triglycerides, diglycerides, monoglycerides and glycerol during lipolysis. Adapted from Constantin, Pasero and Desnuelle.[140]

2.2.5.5 Nucleolytic Enzymes

The nucleolytic enzymes are a group of phosphodiesterases, which hydrolyse phosphodiester bonds uniting the mononucleotides in nucleic acids. Ribonucleases constitute less than 0.1 % of the total protein in pancreatic juice, and are specific for the phosphodiester bond at the 3' position of pyrimidine nucleotides. Deoxyribonucleases act on deoxyribonucleic acid (DNA), liberating 5-monoesters, and act preferentially on the double helix of DNA. Human pancreatic deoxyribonuclease has been purified from pancreatic secretions, has a molecular weight of 30 000, and has been shown to have endonuclease activity on double- as well as single-strand DNA molecules.[141]

2.3 THE PHYSIOLOGY OF UNDERNUTRITION

> "*Wherefore, I say, that such constitutions as suffer quickly and strongly from errors in diet, are weaker than others that do not; and that a weak person is in a state very nearly approaching to one in disease; but a person in disease is the weaker, and it is, therefore, more likely that he should suffer if he encounters anything that is unseasonable.*"

Hippocrates: *On Ancient Medicine*

That deficiencies in diet, and nutritional status might adversely affect health was well appreciated by the ancient Greeks, and a variety of physiological derangements have subsequently been associated with the malnourished state.

In 1842, during the typhus epidemic, Robert Graves considered that the high mortality from the disease might, in fact, be related to the practice of bleeding, purging and starving patients with fever, a dogma, which had persisted since the days of Galen.[3] He therefore introduced a policy of actively feeding his patients, and the subsequent fall in mortality was dramatic. When questioned regarding the reasons for his success, he stated:

> "*You are not to permit your patient to encounter the terrible consequences of starvation because he does not ask for nutriment. Gentlemen, these results are due to good feeding [...] When I am gone, you may be at a loss for an epitaph for me. I give it to you in these words: 'He fed fevers'*"

Florence Nightingale also observed the consequences of undernutrition on the wounded in the Crimean war, and the positive results of ensuring adequate feeding:

> "*Every careful observer of the sick will agree in this, that thousands of patients are annually starved in the midst of plenty, from want of attention to the ways which alone make it possible for them to take food [...] I would say to the nurse, have a rule of thought about your patients diet; consider, remember how much he has had, and how much he ought to have today.*"

Much of our knowledge and understanding of the consequences of severe malnutrition on human physiology are based on careful observations during periods of starvation. A classic example is the studies of starvation by the twenty-eight Jewish Physicians in the Warsaw

ghetto, from February to July 1942. Despite knowing that their own days were numbered, their observations were carefully documented, and the manuscripts subsequently smuggled out of Poland. The manuscripts were discovered in a Warsaw library after the Second World War, and subsequently published under the title *Hunger Disease*.[142] These studies, although hampered by the rather rudimentary facilities available at the time, described meticulously a variety of physiological abnormalities in subjects who had lost up to 35% of their body weight due to starvation. Documented findings included cachexia, hypothermia, muscle weakness, diminished cardiac output, bradycardia, hypotension, oedema, an increased risk of venous thrombosis, anergy, achlorhydria and diarrhoea, as well as the psychological abnormalities of depression, apathy and immobility.[142] Incarceration in the Warsaw ghetto resulted in 43 000 deaths. Subsequent deportation resulted in a further 250 000 deaths.

An earlier study of the effects of undernutrition on human subjects was initiated by Benedict et al, and published in 1919, under the title *Human Vitality and Efficiency under Prolonged Restricted Diet*.[143] The Carnegie Nutrition Laboratory Experiment investigated the effect of a restricted diet, resulting in a loss of body weight of 10%, on basal metabolic rate, nitrogen and carbon balance, and work efficiency in 24 healthy young men. The weight loss was achieved by reducing intake to between 50% and 70% of estimated food requirements, and once the weight loss had been achieved, the basal ration was then supplemented to maintain caloric and nitrogen equilibrium. The study, therefore, was designed to investigate the human adaptive process to moderate undernutrition. Results of the study indicated a reduction in basal metabolism, with an 11.5% decrease in heat production. Nitrogen loss from the body paralleled weight loss, and there was a non-significant reduction in respiratory quotient from 0.8 to 0.78. The authors reported a decrease in blood pressure of approximately 20 mm Hg, and a reduction in pulse rate, however the semi-starvation did not affect the ability of the cardiovascular system to respond to exercise. Electrocardiographs (ECG's) were normal (apart from sinus-bradycardia). During the period of semi-starvation, muscle strength (hand dynamometry) was reduced 8-9%, and there was a marked reduction in work endurance.

In 1950, Ancel Keys, in the Minnesota Experiment, extended the findings of the Carnegie Experiment, and reported on a group of 32 healthy young men who underwent a period of semi-starvation, consuming a diet consisting of between 20% and 60% of their estimated requirements for a period of 24 weeks, followed by a period of nutritional restitution (Figure

2.3.1).[17] The period of semi-starvation resulted in an average weight loss of 23% (65% loss of body fat and 17% loss of fat free mass). It took 16 weeks of re-feeding for the body weight to return to normal, with an excess of fat (over baseline values) noted at 20 weeks. Figure 2.3.2 illustrates the effect of the semi-starvation, and subsequent refeeding on fitness (as assessed by the Harvard fitness test), and strength (hand dynamometry). Over the 24-week period of starvation, hand strength declined to 72% of baseline values, and fitness to 28%. Despite 20 weeks of nutritional rehabilitation, with return of the body weight to baseline values, strength and fitness remained impaired.

A variety of psychological assessments were conducted on the subjects, and as illustrated in Figure 2.3.2 subjects became increasingly depressed (as assessed by means of the Minnesota Multiphasic Personality Inventory) during the starvation period. Although there was improvement during the re-feeding period, the depression scores had not yet returned to baseline (control) levels after 20 weeks.

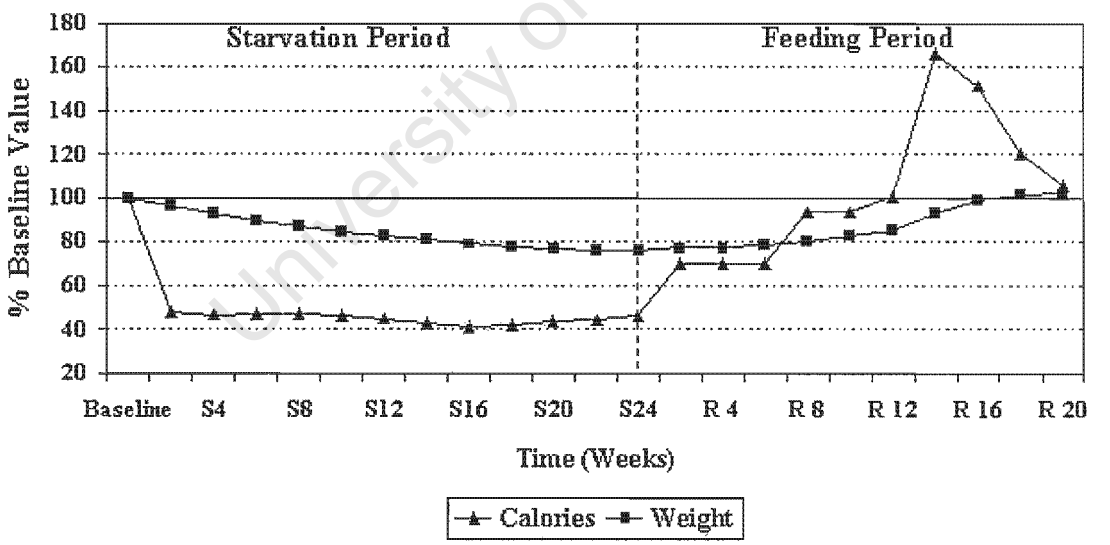


Figure 2.3.1: The Minnesota Experiment. Plot of caloric intake, and body weight (as a percentage of baseline levels) during the 24 weeks of semi-starvation (S), and 20 weeks of nutritional rehabilitation (R).

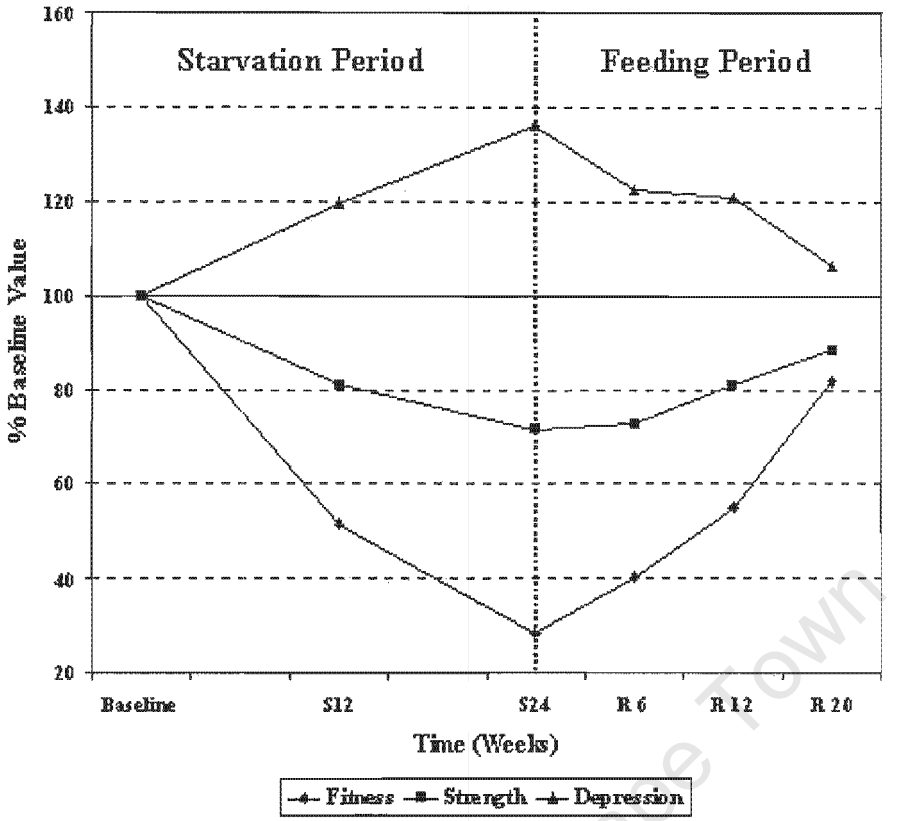


Figure 2.3.2: The Minnesota Experiment. Effect of semi-starvation, and nutritional rehabilitation, on muscle strength (hand dynamometry), fitness (Harvard Fitness Test), and psychological depression (Minnesota Multiphasic Personality Inventory), expressed as a percentage of baseline values.

2.3.1 Metabolic Consequences of Starvation

The normal resting energy requirement for an adult is approximately 25-30 kcal/kg/day in men and 20 to 25 kcal/kg/day in women.[144] The central nervous system consumes 25-33% of resting metabolic energy in adults, and up to 50% in children.[145] Tissues such as heart, kidney and muscle use free fatty acids and ketone bodies as fuel, whereas the brain is primarily dependent on glucose.[146]

Energy stores in the body are in the form of glycogen in the liver and skeletal muscle, tissue fat, and protein, predominately in skeletal muscle.[147] Glycogen stores are limited, providing less than a day's supply of energy. The most important endogenous fuel supply is tissue fat,

which the average normal human adult has approximately 15 kg,[147] which constitutes about 120 000 kcal, sufficient for about 60 days supply. Tissue protein may provide 24 000 kcal, enough for approximately 12 days supply. It should also be noted that fat is virtually anhydrous, and therefore an efficient energy source, with each kilogram of body fat providing approximately 8 000 kcal. Tissue protein, on the other hand, is associated with a high water content (75% of muscle is water), and each kilogram of muscle, consisting of 250 g protein, provides only 1 000 kcal. Muscle protein is therefore an inefficient source of energy, and is particularly vulnerable to the effects of starvation.

Experimental study of both short-, and long-term fasting has contributed considerably to our understanding of normal metabolic processes, and on the way that the body can adapt to food deprivation. Like other animals, humans are unable to significantly convert fatty acids to glucose, and therefore the primary sources of substrates for gluconeogenesis, principally to supply the brain with glucose, are muscle protein-derived amino acids, and glycerol from adipose tissue triglycerides.[145] During short-term fasting, over a 24 hour period, the tissues of a normal person will utilise approximately 1800 kcal derived from about 75 g of protein (primarily from muscle) and 160 g of triglyceride from adipose tissue.[147] Of the 180 g of glucose released from the liver (gluconeogenesis), 80% (144 g) will be completely oxidized by nervous tissue, mainly brain. The remaining 36 g are converted by glycolytic tissue (erythrocytes, leukocytes, bone marrow, renal medulla, peripheral nerves, and to a lesser degree, skeletal muscle) to lactate and pyruvate, which are carried to the liver and kidney to be re-synthesised into glucose (the Cori Cycle). The Cori Cycle spares gluconeogenesis from protein by limiting the complete oxidation of glucose to carbon dioxide.

If these pathways described during brief fasting persisted during prolonged food deprivation, there would be a rapid, excessive loss of protein to provide glucose. Fortunately, the brain can substitute a fat derived product, namely ketone bodies, for glucose, and with prolonged starvation, this progressively occurs.[147] The decreased requirement for glucose results in decreased hepatic gluconeogenisis, and an increase in the utilization of fat stores for energy (Figure 2.3.3).

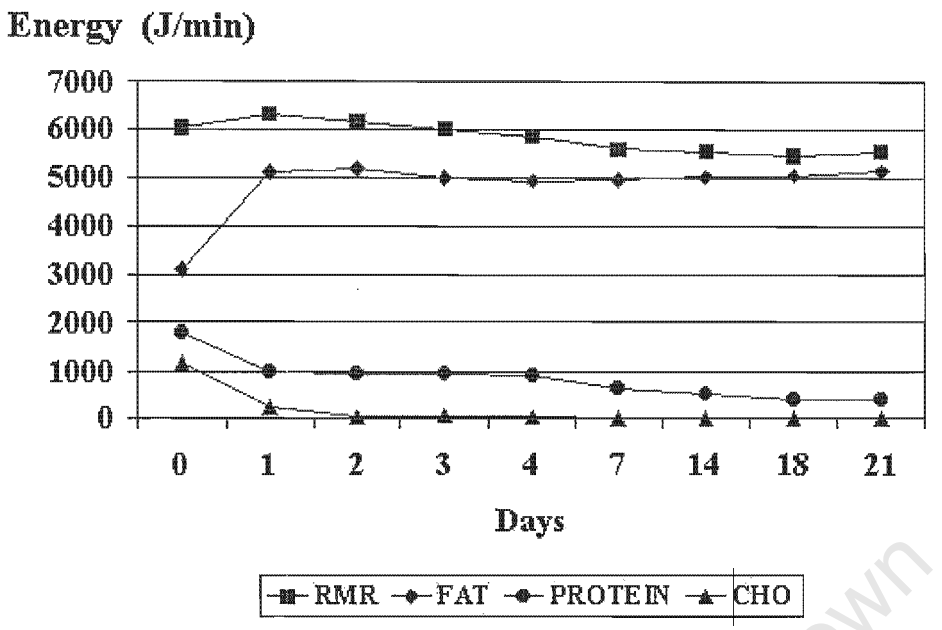


Figure 2.3.3: Energy requirements (resting metabolic rate; RMR), and the utilisation of carbohydrates (CHO), fat and aminogenic (protein) compounds during starvation. Adapted from Owen et al.[148]

The tricarboxylic (Krebs) cycle is, however, dependent on the availability of oxaloacetate and other intermediates in order for it to function. There is, therefore, an obligatory requirement for amino acids or glucose, derived from proteolysis, to accompany fatty acid oxidation to supply these intermediates and maintain functional activity of the cycle.[145,148]

Studies have indicated that late in starvation, metabolism of fat provides approximately 93% of the energy requirements, with the remainder derived from the oxidation of aminogenic compounds.[148] As the brain increasingly uses ketone bodies, and the liver reduces gluconeogenisis, the kidney produces increased amounts of glucose, primarily from the amino acid glutamine. This last alteration appears to make the kidney the chief gluconeogenic tissue in prolonged starvation.[147]

The metabolic adaptation to starvation is dramatically illustrated by the tragic consequences of the ten Irish Republican Army hunger strikers who died following a period of absolute food deprivation. One individual, who had sustained a gunshot injury, died after 45 days, and the other nine, after a mean of 61.6 days (range 57 to 73 days). At the time of death, it was

calculated that the individuals had lost about 40% of their body weight, comprising between 70% and 94% of their body fat stores, and approximately 19% of body protein, illustrating the protein sparing nature of the metabolic adaptation.[149] Unfortunately, once the fat stores are depleted, without additional nutrient intake, the human body may be unable to generate sufficient energy from the remaining protein stores to sustain life.[150] Excessive proteolysis can also cause death from gross fragmentation of myofibrils[151] and depletion of other lean body mass organs. A recent study of anorexic patients near death (mean body mass index 9.77 kg/m^2, fat-free mass ≈ 100% body weight) demonstrated that the patients generated only 410 kcal (or 34% of their resting energy requirement) from the metabolism of fat free muscle protein.[152] Interestingly, the study demonstrated an increase in resting energy expenditure (REE) in this pre-terminal state, possibly reflecting the metabolic consequences of resumption of gluconeogenesis (from muscle protein stores) by the liver (the "king penguin syndrome"), which may hasten their final demise. This study did, however, illustrate that despite such appalling nutritional deficiency, and despite previous reports indicating that a body mass index less than 13 in males, and 11 in females was usually fatal,[153] survival is possible if appropriate nutritional support is instituted without further delay.

2.3.2 Undernutrition and the Limits of Human Survival

The degree of weight loss that can be sustained before death become imminent (*eine bestimmte Grenze*) remains controversial, and assessments are generally based on assessments of the weights of victims of starvation. The concept of lethal weight loss appears to have first been proposed by Chossat in 1843, who noted death would ensure in a variety of animals (mammals and birds) once they had lost 40-50% of their body weight.[18,153,154] In the Madras famine of 1877, a group of 30 individuals studied had a mean body mass index of 13.5 kg/m^2 at time of death, having lost approximately 28.4% of their estimated normal weight.[17] James, in 1988, classified a BMI above 18.5 kg/m^2 as normal, and below 16.0 kg/m^2 as severe or grade III chronic energy deficiency (CED).[155] He proposed a figure of 12 kg/m^2 as the lower level of human survival. Henry,[153] in reviewing a number of studies[156-165] including data from the Dutch Famine in 1945,[160] concluded that a BMI of around 13 kg/m^2 in males, and around 11 kg/m^2 in females was usually fatal. The lethal level of weight loss in humans does, however, show substantial variability. Krieger suggested that the rate of loss of

weight might influence mortality, with a lethal level of weight loss of about 40%, during acute starvation, and 50% during semi-starvation.[166] Survival has been recorded with body mass indices (BMI) as low as 9.77 kg/m.[152] It is important to differentiate between the absolute minimum BMI compatible with life, and percent loss of weight. Obesity appears to confer some protection against starvation, and obese individuals have been reported to be able to lose as much as 65-80% of their weight during therapeutic fasting over a 7-24 month period.[167]

The precise cause of death in patients suffering from severe malnutrition is often not clear, and may remain a matter of conjecture. Death may be insidious, and as documented by Fliederbaum:[168]

> "*Active, busy, energetic people are changed into apathetic, sleepy beings, always in bed, hardly able to get up to eat or to go to the toilet. Passage from life to death is slow and gradual, like death from physiological old age. There is nothing violent, no dyspnoea, no pain, no obvious changes in breathing or circulation. Vital functions subside simultaneously. Pulse rate and respiratory rate get slower and it becomes more difficult to reach the patient's awareness, until life is gone.*"

On the other hand, Zimmer, Weill and Dubois described the "disease of malnutrition" in nine thousand inmates of the internment camps in France, 1941 - 1942, and noted:[169]

> "*In other cases the patients were attacked more rapidly, in full career, as it were, and collapsed while walking or passed away in their sleep.*"

The cause of death was generally ascribed to "essential malnutrition", however, the suddenness of demise occurring in some starved persons did suggest a cardiac origin. Malnutrition has been associated with electrolyte imbalances, including sodium, potassium, calcium, magnesium and phosphate, all of which have an arrhythmogenic potential. Malnutrition may also result in vitamin deficiencies, particularly thiamine (beriberi), which may affect cardiac status. Previous study has also demonstrated that starvation may result in protein degradation with gross fragmentation of cardiac myofibrils, resulting in cardiac failure and arrhythmias.[151]

Malnutrition has been associated with cardiac abnormalities. Ancel Keys in his studies of the effect of semi-starvation on 32 healthy male volunteers noted bradycardia, hypotension and a 45% reduction in cardiac output.[17] Abnormalities were noted in the ECG's of the majority of subjects studied, with the changes reaching their maximum by the 12th week of semi-starvation, and recovering slowly during nutritional rehabilitation. The ECG changes associated with undernutrition included bradycardia, a loss of the normal physiological variation in rate, and a generalised decrease in amplitude of all the deflections (P wave, QRS complex, and T wave). The slow heart rate, in most cases, was due to a sinus bradycardia, however, two of their subjects developed a nodal rhythm. Keys and colleagues also noted a marked right axis shift of the QRS axis, and also described prolongation of the QT interval. Recovery of most ECG abnormalities was only partial following 12 weeks of refeeding, but all had returned to normal by 20 weeks, with some functions (heart rate, QTS axis), actually overshooting the control values. The authors concluded that although there appeared little correlation between the ECG changes and the functional state of the heart, the occurrence of significant changes in the ECG during semi-starvation could not be ignored.

Cunha et al recently confirmed prolongation of the QT interval in patients with malnutrition, and commented on the potential for life threatening arrhythmias in these individuals.[170,171] Although a number of their subjects had evidence of electrolyte abnormalities, they were unable to demonstrate a direct correlation between electrolyte status and QT-interval. It therefore remains unclear whether the abnormalities noted on the ECG's of undernourished patients are primarily a result of the malnourished state, or whether they are secondary to electrolyte disturbances.

2.3.3 Effects of Undernutrition on Gastro-Intestinal Structure and Function

Gastro-intestinal disorders feature prominently in victims of starvation and malnutrition.[17] Diarrhoea was recorded during the Irish potato famine of 1847,[172] and was listed as a major cause of death in the Indian famine of 1877-78 and of 1897-99.[173] Diarrhoea was particularly prevalent in children during the Russian famine of 1898-99,[174] and was widespread in the famine areas of Russia in 1921-22.[175] The observations during the siege of Kut, during the First World War, are of particular importance, as the food intake and period of restriction were known.[176] For the period December 4, 1915, to April 29, 1916, the average intake for

the Indian troops at Kut was 1 110-1 550 kCal. Diarrhoea began in early March, and became progressively worse as the siege continued. Out of a total of 1 500 troops, diarrhoea was considered to be the major cause of death of 200 individuals.

The consequences of mass starvation were most evident in the concentration camps of the Second World War. In the Belsen camp 40 000 starved persons were crowded together under most unhygienic conditions. At the time of liberation, almost all inmates had diarrhoea, which, in the most severely emaciated, was reported refractory to treatment, and usually resulted in death.[17] During 1944, of the 771 repatriates from political concentration camps in Germany admitted to the Salpêtrière Hospital, Paris, all were malnourished, and 67 died. Twenty-nine of the deaths were directly attributed to "diarrhoea and cachexia"[177] Helweg-Larsen et al described three different types of diarrhoea associated with the severe malnutrition observed in the concentration camps.[178] A progressive, afebrile "hunger diarrhoea", an "infectious diarrhoea", and an "alimentary diarrhoea" induced by feeding.

Diarrhoea was particularly prevalent in the children of the Warsaw Ghetto, and often preceded the appearance of oedema.[179] The diarrhoea was frequently severe, with the stools often colitic (bloody) in nature. Unfortunately, no bacteriological studies were performed to determine whether the diarrhoea was infective in nature. Evidence of undigested food in the stool was noted, suggesting maldigestion. Lame and colleagues also commented on the high prevalence of diarrhoea in the concentration camps, noting that 20 to 40 stools a day were not uncommon.[180] Bacteriological search generally failed to identify any causative organisms, and sulphonamides and vitamins were of no benefit. Ingestion of large amounts of food often intensified the diarrhoea, often with fatal results. The authors postulated that the diarrhoea was a consequence of atrophy of the digestive glands, and a disturbance of water metabolism.

Although Keys and colleagues, in their study of 32 healthy individuals, did not document any significant gastro-intestinal disorders during the period of semi-starvation, a number of their subjects did experience "gastric distress" during the rehabilitation period.[17]

2.3.3.1 Effects of Undernutrition on Gastric Function

The physicians of the Warsaw ghetto observed almost total achlorhydria in malnourished subjects, both in the fasting state, as well as after a gastric stimulus (alcohol and caffeine).[168] They also commented on the rarity of gastritis and peptic ulcers in their patients, possibly as a consequence of the reduced gastric acid. Fliederbaum noted:

> "*It is interesting that the vagus nerve is so highly active in the circulatory system* (subjects were noted to be hypotensive and bradycardic) *and has such low activity as to be almost paralysed in the functions of the stomach.*"

Keys studied basal and histamine (0.1 mg/kg) stimulated gastric acid secretion in 10 of his subjects at the end of the period of semi-starvation.[17] Five of the subjects had no free acid in the fasting state, whereas 5 had normal acidity. All the histamine-stimulated tests were reported to be within normal limits. Gracey and colleagues reported reduced basal acid output in 9 of 14 malnourished infants and children. All their patients demonstrated impaired response to pentagastrin stimulation.[181] The authors commented that reduced gastric acid probably contributes towards bacterial overgrowth and diarrhoeal diseases in malnourished children. Likewise, Gilman et al reported significantly reduced basal and stimulated (betazole) acid secretion in 35 severely malnourished children, compared to a group of 20 better-nourished children.[182] Following a three week period of nutritional rehabilitation, during which their weight-for-height improved from 61% (±9.0%) to 81% (±11%), although gastric secretion volumes improved, both basal and stimulated acid output remained impaired. The authors documented colonisation of the gastric juice by gram-negative organisms in 26 of the 32 (81%) malnourished children, but in none of the better-nourished group. Only in those children, in whom gastric acid output improved in response to refeeding, did the incidence of bacterial colonisation decrease.

2.3.3.2 Effects of Undernutrition on Pancreatic Function

Although the physicians in the Warsaw ghetto observed undigested food in the stools of severely malnourished subjects, they noted that diastase was present in the urine. This led them to assume that pancreatic function in "hunger disease" was not damaged.[168] The finding of undigested food in the stool, however, indicated significant maldigestion, implying

deficiency of the digestive (pancreatic) enzymes. As pointed out by Winick in his commentary of the work, this appears to be a case of investigators allowing the results of a crude biochemical test outweigh that of their clinical observation![142]

Subsequent work has indicated malnutrition is associated with pancreatic abnormalities. Davies, in a classical paper on kwashiorkor, reported pancreatic atrophy, particularly affecting the acinar cells.[183] Véghelyi et al noted fibrosis of the pancreas in children dying from kwashiorkor during the siege of Budapest, and commented that the first sign of dietary deficiency was a failure of the exocrine secretion of the pancreas.[184] More detailed pathological studies,[185-187] including electron microscopy,[188] have reported extreme atrophy of exocrine cells, disorganisation of the acinar structure, marked reduction of zymogen granules, vacuolation, cystic dilatation of the ducts, and an increase in fibrous tissue.

Studies of functional abnormalities have reported impaired pancreatic exocrine secretion in malnourished patients. Thompson et al, Barbezat et al, and Sauniere et al reported low levels of pancreatic enzymes (amylase, lipase and trypsin) in children with kwashiorkor,[185,189-191] and Gomez et al, Barbezat et al and Danus et al, likewise, demonstrated low levels in marasmic children.[189,192,193] Lipase and trypsin appeared to be most affected, whereas volume of secretion and bicarbonate were normal, suggesting abnormality of protein synthesis. Barbezat et al found a significant correlation between enzyme output and serum albumin concentration, with deficiency of enzyme production only evident when the serum albumin was below 30 g/l.[189] Nutritional replacement resulted in normalisation of pancreatic enzyme secretion.[185,189,193]

There have been relatively few studies of pancreatic function in malnourished adults. Jackson and Linder reported poor production of bicarbonate and enzymes in an individual, "Toni", with severe malnutrition following massive small bowel resection.[194] Tandon et al reported low bicarbonate, lipase and protease, but normal amylase secretion in adults with protein-energy malnutrition.[195,196] Dietary therapy resulted in almost complete recovery of bicarbonate and lipase and an improvement in protease secretion.[196,197] O'Keefe et al documented 48% reduction in trypsin secretion, 80% reduction in amylase secretion, and 86% reduction in lipase secretion, in response to secretin/pancrozymin stimulation, in a severely malnourished patient with intestinal lymphoma.[198] In their study, pancreatic enzyme secretion remained impaired despite a 6 week period of nutritional support, and an increase in

the patient's weight from 22 to 46 kg. One year later, at a weight of 60 kg, repeat pancreatic function tests revealed marked increase in pancreatic enzyme secretion, compared to previous measurements, with return to normal values.

Studies of the effects of malnutrition on pancreatic endocrine structures have produced conflicting results. Some investigators have reported no change, suggesting that the islets of Langerhans are less affected than the exocrine tissue.[184,199,200] Other studies in rats have found reduction in total islet cell mass, and size of the individual B-cells,[201,202] and reduced plasma insulin levels.[203] In humans, although malnutrition has been reported to accentuate the beta cell dysfunction of type 2 diabetes,[204] and may predispose an individual to diabetes,[205] this phenomenon appears to be relatively isolated to tropical areas where it has been referred to as J (Jamaica)-type, or malnutrition related diabetes mellitus (MRDM). Other studies of malnourished individuals have failed to confirm the presence of MRDM.[206-208] It would appear that the islet cells of the pancreas are less susceptible to the effects of malnutrition than are the acinar cells.

The pancreas is an extremely metabolically active organ, producing between 6 and 20g of enzymes per day.[209] It is therefore particularly vulnerable to disease states, and the effects of malnutrition may be profound with fibrosis, acinar atrophy and marked reduction in secretion.[189,195,210,211] The islet cells, however, do appear to be relatively preserved.

2.3.3.3 Effects of Malnutrition on Gut Mucosa

The gastro-intestinal tract has a complicated mucosal structure, specifically designed for optimal digestion and absorption of nutrients. The small intestinal mucosa is arranged into finger-like villi, the surface of which consists of enterocytes and scattered goblet cells. A variety of digestive enzymes, particularly disaccharidases are produced by the mucosa, and the large surface area provided by the villi and microvilli is essential for adequate absorption. The mucosal cells are formed from mitotically active undifferentiated cells in the crypts of Lieberkuhn, at the bases of the villi. They migrate up to the tips, where they are sloughed into the intestinal lumen in large numbers. The average life of mucosal cells is 3-5 days, and the secretion of protein into the lumen due to cell sloughing has been calculated to be as great as 30 g/day.

Malnutrition, by impairing protein synthesis, may cause a decrease in intestinal cell mass, villus size, crypt size and mitotic index resulting in mucosal atropy, and the compromising of absorptive ability.[212-214] Food also has a direct trophic effect on the gastro-intestinal mucosa, and the route of nutrient administration, and the availability of certain nutrients directly influence gut structure.[215]

Glutamine is the most abundant free amino acid in the body, constituting more than 60% of the free intracellular amino acid pool.[216] Most of this glutamine is synthesized and stored in skeletal muscle. Glutamine is an important energy source and precursor for purine and pyrimidine biosynthesis for cells having a high turnover rate. This includes cells such as enterocytes and those involved in the immune system. The importance of glutamine as an energy source for the gut has been illustrated by studies showing that, in the post-absorptive state, the small bowel extracts 20% to 30% of the circulating glutamine.[217] It is also an important source of glucose during periods of starvation. Glutamine has been considered a non-essential amino acid, as human tissues, particularly skeletal muscle and lung, are able to biosynthesise glutamine from glutamate. Although it is the most abundant amino acid, plasma levels rapidly decrease following injury. In metabolically stressed individuals the capacity for producing endogenous glutamine may be overwhelmed, resulting in deficiency.[216,217] This deficiency may subsequently result in derangements of intestinal structure and function, as well as compromising the immune system.[216-219] It has therefore been proposed that glutamine should be rather considered a partially, or conditionally, essential amino acid.[220]

Short chain fatty acids, together with hydrogen and carbon dioxide, are produced in the colon by anaerobic bacterial fermentation of unabsorbed carbohydrate and dietary fibre. The most important fatty acids produced are the 2, 3, and 4-carbon forms, acetate, propionate and butyrate, generated in roughly the ratio of 60%, 20% and 20%. Butyrate is the most important fuel for the colon, approximately half of the propionate produced, is used by the liver for gluconeogenisis, whilst most of the acetate is metabolised by muscle or converted to long-chain fatty acids and ketones in the liver.[221] Short chain fatty acids are readily absorbed by the colonic mucosa. The absorption of sodium and water is enhanced,[222,223] and they have been shown to be trophic to the intestinal mucosa.[224,225]

Studies in rats have indicated that malnutrition can result in up to 50% reduction in gut mucosal mass, predominantly within the proximal small intestine.[212,226] This atrophy occurs not only in starvation, but also with intravenous nutrition, when enteral nutrition is withheld.[227] Although it does occur in humans, it does appear to be somewhat less than in animal models, with reductions in mass of between 10% and 15% reported.[228] Histopathological studies in malnourished individuals have demonstrated changes in gut mucosal structure, ranging from near normal morphology to sub-total villous atrophy. Barbezat et al described varying degrees of mucosal atrophy in 17 malnourished children, with approximately 50% having evidence of lactase deficiency.[229] In a similar study of 24 children with protein energy malnutrition, Romer et al reported that all had grade I to II villous atrophy, the degree of which did not appear to directly correlate with the degree of malnutrition.[230] The authors also noted significant reduction in mucosal lactase activity, which did seem to relate to the severity of the malnutrition. In contrast, maltase and sucrase deficiencies were only evident in the more severely malnourished patients. A study by Nichols et al also reported malnourished infants to have an advanced degree of villous atrophy (grade 3), which, in their study, correlated with the degree of undernutrition.[231] Lactase messenger RNA was reduced to 32%, and sucrase to 65% of normal values, and lactase and sucrase enzyme activities were diminished. In view of the hypolactasia being associated with a much lower lactase than sucrase messenger RNA abundance, it was considered that the deficiencies were unlikely to be explained solely by a loss of enterocytes, as a consequence of the villous atrophy. The authors concluded that malnutrition appears to specifically suppress lactase gene transcription or messenger RNA stability in infants.

James investigated absorption of sugars from the intestine of malnourished children.[232] Ten children were studied, all of whom were shown to have impaired glucose absorption, eight had reduced lactose absorption and six diminished sucrose hydrolysis and absorption. Jejunal biopsies revealed varying degrees of mucosal abnormality, and those children with the most severe mucosal damage had the lowest rate of sugar absorption. The malabsorption of disaccharide was related to the impairment of hydrolysis, and not to the malabsorption of the monosaccharide product. Presence of unabsorbed sugar in the bowel produced an increase in the net movement of water into the lumen, which may result in diarrhoea. The author also commented that secondary effects from the bacterial fermentation of unabsorbed carbohydrate, and inhibition of water absorption by the colon by bile salts washed into the

colon might also contribute to diarrhoea in malnourished children. Nutritional rehabilitation resulted in reversal of the abnormalities.

Sullivan et al performed jejunal biopsies on 40 malnourished children and also reported a spectrum of mucosal change, ranging from "normal" to "flat".[233] The morphologic changes, in this study, did not relate to clinical, biochemical, or anthropometric data. All biopsies, however, revealed evidence of inflammatory cell infiltration, suggesting an intestinal reaction to some environmental antigen (dietary, microbial, or both) of the cell-mediated type. Campos et al conducted histological, and ultrastructural study of the jejunal mucosa in 7 marasmic children.[213] Four patients had evidence of subtotal villous atrophy, and three were reported as "near normal". Electron microscopy revealed short or branched microvilli in 4 patients, and increased intraepithelial lysosomes in 2. The authors also reported an increase in theliolymphocytes, excessive epithelial cell extrusion and abnormalities in the appearances of the mucosal plasma cells suggesting possible local deficiency in immune function.

The integrity of the gastro-intestinal mucosa is a key element in the prevention of translocation of bacteria and toxins from the enteric lumen to the systemic circulation.[234] Furthermore, the gut is the body's largest immune organ, and nutrients have fundamental and regulatory influences on the immune response of the gastro-intestinal tract, and, therefore, on host defence.[235] Breakdown of the gut barrier and the mucosal immune system may result in systemic endotoxaemia. Endotoxaemia results in decreased glutamine uptake, consequent to diminished glutaminase within the intestine, and may result in organ dysfunction with further impairment of the barrier, the clotting system, and immune system.[236,237] Studies in animal models have demonstrated bacterial translocation occurring in malnutrition, haemorrhagic shock, thermal injury, endotoxaemia, trauma, and intestinal obstruction, however, the clinical relevance to humans remains unclear.[227]

Welsh et al demonstrated a significant increase in intestinal permeability, as measured by the lactose:manitol test, in malnourished patients, indicating that the intestinal barrier was significantly compromised.[238] Mucosal biopsies revealed morphologic, immuno-histochemical, and molecular evidence of activation of lamina propria mononuclear cells and enterocytes, and a heightened acute phase response. Malnutrition has been associated with increased systemic antibodies to food protein, indicating translocation of antigen across the gut barrier, and a defective mucosal immune system.[239] Reynolds et al studied 20

malnourished patients in comparison to 15 well-nourished patients.[240] Although, on biopsy, they were unable to demonstrate morphological abnormality of the distal duodenal mucosa, they noted systemic antibodies (IgG) to the enteric antigens gliadin and/or beta-lactoglobulin in 15 of the malnourished patients, but none of the well-nourished patients. The antibody-positive malnourished patients had significantly better nutritional status than antibody-negative patients, suggesting that malnutrition had an adverse effect on the immune response. Previous studies by Sullivan et al reported anergy to intradermal challenge with either purified protein derivative or candidin in 70% of 40 malnourished children.[233]

Malnutrition has been associated with bacterial contamination of the upper gastro-intestinal tract.[241] This may be consequent to achlorhydria,[242] as well impaired gut motility.[243] Bacterial overgrowth has been associated with fat malabsorption due to deconjugation of bile salts, impairment of disaccharidase activity with reduced carbohydrate absorption and subsequent increased osmotically active bacterial fermentation products, and vitamin B12 deficiency.[244,245] These factors may result in diarrhoea and further deterioration of the nutritional state. Defects of gut mucosal barrier function and immunity, induced by the malnourished state, may also increase the risk of translocation of both bacteria and toxins, with the potential for septic complications and endotoxaemia, although the clinical relevance of this in humans remains uncertain.[237]

2.3.4 Physiological Consequences of Weight Loss

Studies have indicated a close relationship between physiological function and lean body mass. Significant physiological impairment with increased postoperative complications and a prolonged hospital stay are associated with a 20% loss of body protein.[23,24,246] A 10% body weight loss relates to a loss of between 15.2 and 20.8% body protein, 15% weight loss to between 19.2 and 24.8 % protein loss, and 20% body weight loss to between 23.0 and 29.0% protein loss. Therefore, in previously healthy individuals, a weight loss of 15% would relate to a body protein loss of approximately 20%, and a significantly increased risk of postoperative complications.[247] However, John Kinney has previously reported that physiological derangements begin to appear at between 5 and 10% body weight loss, and in the presence of disease, these are likely to be clinically significant.[248]

2.3.5 Effects of Undernutrition on Body Composition

Undernutrition results in the loss of variable proportions of fat, tissue protein, and minerals, along with changes in the extra-cellular fluid compartment of the body. Oedema is frequently, although not universally, observed, and the studies by Keys[17] and Henschel[249] reported that the extra-cellular fluid compartment increased from approximately 24% in normal men, to 34% following semi-starvation. More recent work from Barac-Nieto has also reported a relative increase in the total body water of malnourished individuals, with a proportionately greater increase in extracellular fluid and a reduction in intracellular fluid,[250] even in the absence of oedema.[251] As previously discussed, fat stores are preferentially utilised during starvation, with the studies performed by Keys indicating that fat, expressed as a percentage of body weight, fell from 13.9% to 5.2% after 24 weeks of semi-starvation.[17] This represented loss of 6.9 kg of fat, or 71% reduction of initial body stores. On the other hand, "active" tissue mass, (body mass minus fat, water and bone), although reduced in absolute values (39.9 kg vs. 29.2 kg), remained proportionately similar to control values, when expressed as a percentage of body weight (57.5% vs. 55.5%). Barac-Nieto et al compared the contribution of muscle cell mass and non-muscle or visceral cell mass to the fat free mass, and demonstrated that in moderate malnutrition (mean body mass index 19.6 kg/m^2) the reduction in muscle cell mass was in proportion to the decrease in total body cell mass.[250] In more severely malnourished individuals (mean body mass index 17.5 kg/m^2), the reduction in muscle mass (41%) was proportionately greater than that of body cell mass, while visceral mass was only minimally affected (<1.5%).

2.3.6 Effects of Acute Undernutrition on Basal Metabolic Rate

Studies of experimental semi-starvation have demonstrated a reduction of basal metabolic rate associated with weight loss.[17,143,252] This decrease in basal metabolic rate may be explained on the basis of loss of active tissue mass, as well as a decrease in the activity of metabolic active tissue (increase in metabolic efficiency). Various investigators have considered the relative contributions of these two factors to the decreased basal metabolic rate in undernourished patients. Taylor and Keys noted that a decrease in metabolically active tissue accounted for 65% of the reduction in basal metabolic rate, and "metabolic adaptation" accounted for 35%.[253] Conversely, based on a second set of studies, Grande et al

found that a decrease in metabolic activity of the active tissue mass contributed 65%-73% to the reduction in basal metabolic rate.[252] These differences, however, may be explained by the differences in the duration of semi-starvation in the two studies. In the study reported by Taylor and Keys, the patients received an energy-restricted diet for 24 weeks, whereas the study by Grande et al did not exceed 3 weeks. Reassessment of the data by James and Shetty indicated that the fall in basal metabolic rate during the first two weeks of energy restriction was mainly a result of increased metabolic efficiency, which was then essentially unchanged for the remaining 22 weeks of semi-starvation.[254] With prolonged undernutrition, a greater contribution to the fall in basal metabolic rate was attributed to decrease in mass of active body tissue.

2.3.7 Mechanisms of Metabolic Adaptation in Acute Undernutrition

Reduction in basal metabolic rate following energy restriction therefore appears to occur in two phases. In the first phase, the decrease in metabolic rate is greater than can be attributed to loss of active tissue, and therefore represents an increase in metabolic efficiency of the active tissue. Following 2-3 weeks, the degree of this adaptation remains constant, and further decrease in basal metabolic rate is a consequence of loss of active tissue. The biochemical and physiological mechanisms accounting for the metabolic adaptation are not fully understood. Rolfe and Brown estimated that mitochondrial oxygen consumption contribute to approximately 90% of basal metabolic rate.[255,256] A futile cycle of pump and proton leak across the mitochondrial inner membrane accounts for 20%, and 80% of the oxygen consumption is coupled to ATP synthesis. Of the ATP synthesised, 25%-30% is used for protein synthesis, 19-28% by $Na^{+}K^{+}$ ATPase, 4-8% by Ca^{++} ATPase, 8% by actinomycin ATPase, 7-10% by gluconeogenises, and 3% by ureagenesis. How changes in these processes independently contribute to the metabolic adaptation to acute fasting is not known.[257]

Factors such as hormones, and substrate alterations may influence this metabolic adaption.[258] Several hormones have been shown to be sensitive to the levels of energy intake, and changes in sympathetic nervous system activity and catecholamines, thyroid hormone metabolism, insulin and glucagon play an important role in the metabolic response to energy restriction. These changes are directed not only at decreasing metabolic activity, but are also essential for

the mobilization of endogenous substrates and fuels during periods of restricted availability of exogenous energy sources. Sympathetic tone is decreased, as is insulin secretion, and changes in peripheral thyroid hormone metabolism occur, with reduced production of triiodothyronine (T3) from thyroxine (T4). Other hormones, such as glucagon, growth hormone and glucocorticosteroids also influence these changes, and together with reduced insulin secretion, promote endogenous substrate mobilization resulting in lipolysis and an increase in fatty acids and ketone bodies (Figure 2.3.4). These physiological changes are aimed at increasing "metabolic efficiency" and endogenous fuel supply at the time of energy deficiency.[257]

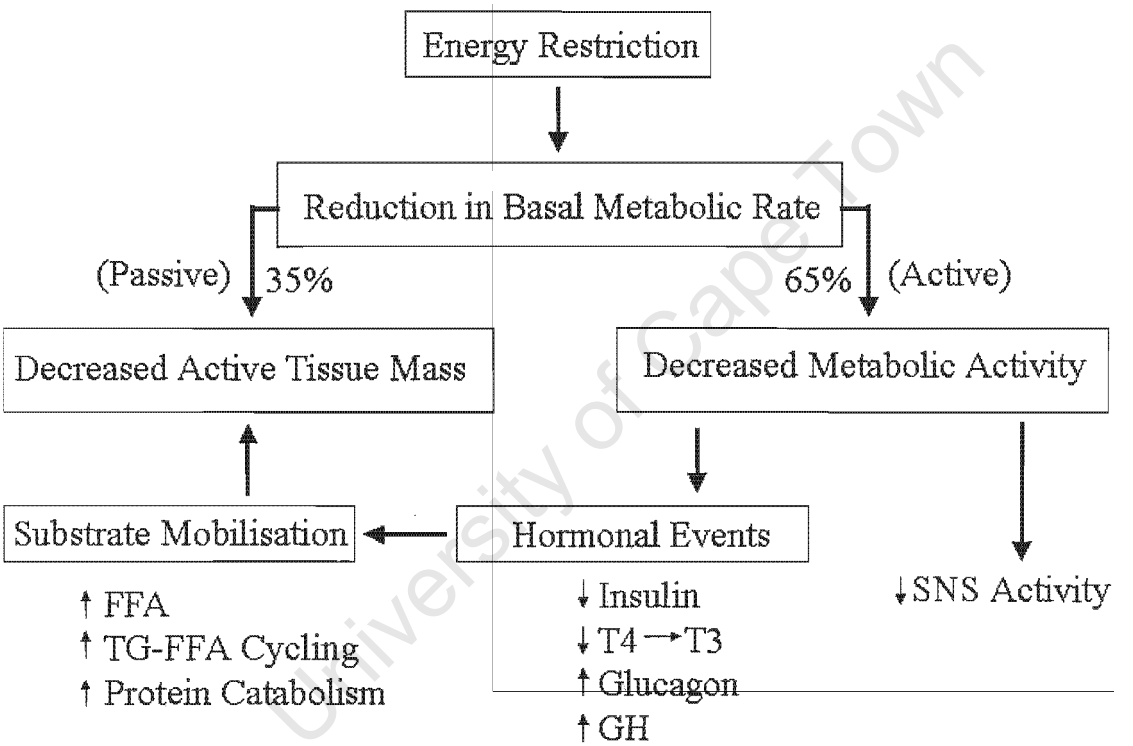


Figure 2.3.4: Possible mechanisms involved in the adaptive reduction of basal metabolic rate during short-term energy restriction. (FFA = Free Fatty Acids; TGA = Triacylglycerols; T4 = thyroxin; T3 = triiodothyronine; GH = growth hormone; SNS = sympathetic nervous system). Adapted from Shetty.[257]

2.3.8 Basal Metabolic Rate in Chronic Undernutrition

In contrast to the studies of the effects of acute semi-starvation in otherwise healthy subjects, several investigators have failed to demonstrate evidence of increased metabolic efficiency in chronically undernourished patients. Ashworth reported a 12% reduction in basal metabolic rate in chronically undernourished Jamaican subjects, but was unable to demonstrate enhanced metabolic efficiency.[259] Similarly, Soares and Shetty were unable to demonstrate metabolic adaptation in chronically undernourished Indian men, and, in fact, noted that when expressed per unit of active body mass, basal metabolic rates appeared higher than in well nourished individuals.[260,261] The authors also noted that, although in absolute terms, total body protein synthesis was lower than in well-nourished controls, when expressed per kg body mass there was no significant difference. Reports by McNeill et al, Carbonnel, and a recent study by Ferro-Luzzi et al also provided no evidence of metabolic adaptation in stable, undernourished individuals.[262-264] It has been noted, however, that differences in basal metabolic rate expressed per unit active tissue mass, or fat free mass, may not reflect differences in metabolic efficiency, since neither active tissue mass or fat free mass is a single uniform metabolic compartment.[257] Skeletal muscle, although comprising approximately 40-50% of the body weight, contributes only 18-22% to the basal metabolic rate.[265,266] The combined weight of the brain and liver comprise only 3-5% of the total body weight, yet utilise up to 40% of energy.[267] A disproportionate loss of the relatively less active muscle, with preservation of the metabolically active visceral tissue, may therefore result in an apparent increase in metabolic rate when expressed in relation to the total active tissue mass.[257] In patients with mild to moderate undernutrition, muscle mass is more likely to be reduced than visceral mass, resulting in an apparent increased basal metabolic rate expressed per kg fat free mass. With more severe forms of undernutrition, mobilisation of tissue from the visceral mass may result in a decreased basal metabolic rate per kg fat free mass (or active tissue mass).

2.3.9 Effects of Undernutrition on Whole-Body Protein Turnover and Synthesis

Studies of the effects of starvation and malnutrition on whole-body protein turnover have produced apparently conflicting results. Golden et al reported significantly lower whole-body protein turnover, synthesis and breakdown, assessed by ^{15}N-glycine administration, in 5

malnourished infants, compared to values after recovery.[268] Hoffer et al studied the effects of a very low calorie diet administered for three weeks in obese patients and noted a 20-40% reduction in whole-body protein turnover (as assessed by plasma leucine and alanine flux).[269] Hoffer et al also investigated the effects of consumption of a meal on protein dynamics, and reported a 35% increase in leucine flux, and a 77% increase in leucine oxidation compared to postabsorptive values.[270] A 23% reduction of total body protein synthesis, following a one-week period of fasting, was reported by Norton et al.[271] They, however, reported normal total body protein synthesis and turnover in three malnourished patients. Holt et al, likewise, reported similar whole-body protein synthesis rates in malnourished cystic fibrosis patients with stable pulmonary disease, compared to normal healthy children, whereas those patients with active infectious pulmonary disease had significantly lower synthesis rates.[272] The results indicated that the increased metabolic demands during acute disease, as well as possibly hypoxia, in the absence of sufficient energy resulted in markedly reduced protein synthesis. A more recent paper by Carbonnel et al studied a group of malnourished patients with non-neoplastic disease (mean body mass index 15.8 kg/m^2).[263] Although their results indicated reduced rates of whole-body protein turnover, when expressed in absolute terms (g/d), compared to healthy controls, when expressed as g/kg/d the rates were similar. Carbonnel et al also investigated the effects of refeeding (TPN infusion) on protein dynamics in malnourished patients, and noted that the rates of whole-body protein turnover and breakdown did not increase significantly when expressed per kg body weight, but increased slightly when expressed per kg fat free mass.[273] There was no change in synthesis or oxidation. Macallan et al reported that whole-body protein turnover rates, expressed per kg body weight, were greater in malnourished patients without tuberculosis, but not in patients with tuberculosis, compared to healthy controls.[274] Protein oxidation rates (in response to feeding) were greatest in the tuberculosis patients, resulting in a reduced protein balance. The authors comment that this indicated an "anabolic block" which may contribute to the wasting often evident in tuberculosis, and other chronic inflammatory states.

These studies indicate that there is a variable hypometabolic adaptive response, both in terms of resting energy expenditure and whole-body protein dynamics, dependant on the cause and severity of the malnutrition, and the presence of co-existent disease. Acute starvation of healthy individuals does appear to result in reduction in resting energy expenditure and protein synthesis, whereas hypometabolic adaptation may not be evident in chronically malnourished patients.[275]

2.4 MEASUREMENT OF PROTEIN SYNTHESIS

The word "protein" is derived from the Greek "proteios", which means "of the first rank", and was first coined by Jöns J Berzelius in 1838 to emphasize the importance of this class of molecules. All proteins, found in bacteria to man, are constructed from the same set of 20 L-isomer amino acids. This "alphabet" of amino acids is at least 2 billion years old.[276]

The body of a 70 kg man contains approximately 12 kg protein and 220 g free amino acids.[277] Skeletal muscle consists of about 7 kg protein and 130 g free amino acids. There is a continuous turnover (synthesis and breakdown) of protein, the "protein turnover cycle", which, in health, is in balance. When measured in the overnight fasted state, skeletal muscle contributes 27-29% to whole-body protein turnover,[278] rising to between 35% and 45% during ingestion of mixed meals and following strength training.[277,279] Regulation of synthesis and degradation of protein is crucial for the maintenance of cellular viability, regulation of growth and cellular protein mass, and the control of enzyme levels. Total body turnover of protein in a healthy adult is approximately 300g of protein per day and accounts for 20% of basal energy expenditure.[280] However, in conditions of starvation, trauma, sepsis and disease there may be a progressive loss of protein as a consequence of an inadequate intake of amino acids, and/or a catabolic wasting of muscle to provide fuel for the brain (gluconeogenesis) as well as to generate amino acid precursors for wound healing, tissue repair and synthesis of antibodies and acute phase reactants. In fact, patients with major trauma and sepsis may lose up to 50% of muscle protein over a two-week period. The importance of protein and amino acid metabolism in response to a variety of catabolic states mandates an understanding of the process.

Much of our knowledge of the regulation of protein metabolism at the whole-body as well at the level of organ and specific protein levels is based on the direct and indirect measurement of protein synthesis and breakdown. Methods of investigation of protein turnover have generally made use of assessment of the exchange rates of amino acids, reflected by their isotope labelled tracers, between various tissues.

The availability of radioactive isotopically labelled amino acids resulted in the first attempts to measure rates of whole-body protein turnover in humans. ^{3}H and ^{14}C emit β rays

(electrons), which cause flashes of light in a liquid scintillant. These can be detected with a photo multiplier tube as disintegrations per minute (dpm).

$$^{14}C \longrightarrow {}^{14}N + e^{-} \rightsquigarrow \text{Scintillation Fluid} \rightsquigarrow \text{Photo Multiplier Tube}$$

The energy spectra of ^{3}H and ^{14}C are different, with ^{14}C radiation having a much higher average energy. It is possible to gate the detector and therefore allow distinction between the two isotopes and thereby permit dual labelling techniques with ^{3}H and ^{14}C.

The use of radioactive isotopes have now been largely replaced by the use of stable isotopes which, as they are not a source of ionising radiation, are effectively safe and non-toxic making them suitable for repeat and long term studies in adults, as well as studies in children, and even in pregnant women.[281,282] Stable isotopes, and the tracer probes containing them, differ from the normal isotope only in their atomic or molecular mass. They can be separated on this basis in a mass spectrometer, either as native gases or as molecules, which are volatilised, and their isotope ratio gives a direct measure of labelling or enrichment.

Use of stable isotopes, however, does have some disadvantages. There is a relatively high natural background for most stable isotopes (1% for C and 0.3% for N) making detection of low levels of enrichment more difficult than for the radioisotopes. Methods for detection and quantification of the stable isotopes are also generally less sensitive than those for the radioisotopes. Also costs of production of molecules labelled with stable isotopes are high as is the cost of the instrumentation (mass spectroscopy) to measure them. The doses of the radioactive tracers used in the assessment of biological functions such as protein synthesis are so low that the safety aspects of them in mature, non-pregnant, adults are negligible, and their use therefore remains appropriate in many circumstances.

Measurement of protein synthesis is possible by assessment of the incorporation of a labelled amino acid into protein over time, in relation to the labelling of the precursor pool from which the amino acid is derived. The general model of whole-body protein metabolism is illustrated in Figure 2.4.1.

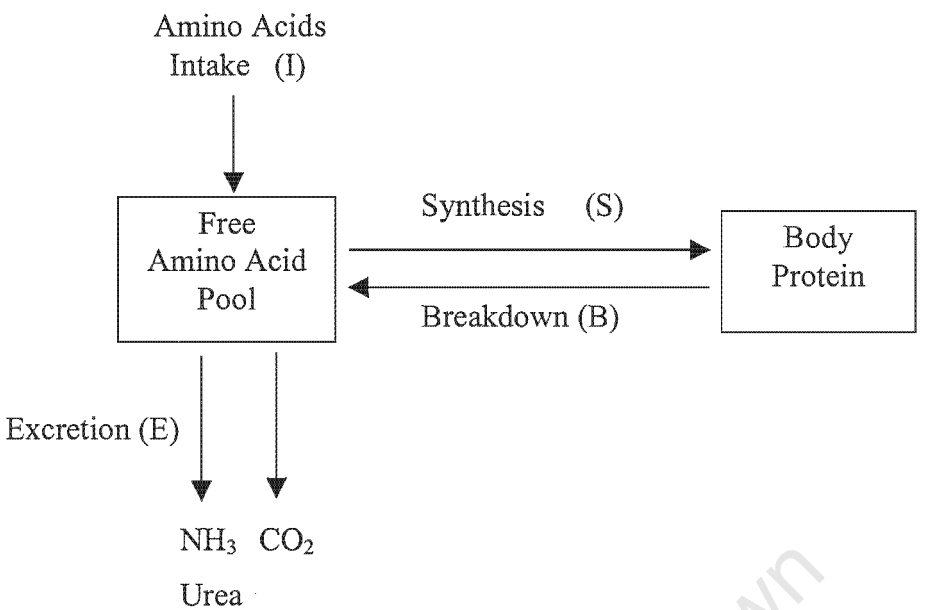


Figure 2.4.1: General model of whole-body protein metabolism

2.4.1 Measurement of Protein Synthesis by Continuous Infusion of Tracer Amino Acid

2.4.1.1 Whole-Body Protein Synthesis

Under steady-state conditions, constant infusions of tracer amounts of labelled amino acid causes the specific radioactivity (specific activity; SA) of the amino acid in the plasma to rise rapidly to a plateau at which time the rate of entry (RE) of the amino acid into the plasma is equal to the rate of loss (RL) from the plasma, and is termed flux (Q). Entry of amino acids into the plasma is consequent to dietary intake (I), and from the endogenous breakdown of tissue protein (B). Loss of amino acids from the plasma is a result of synthesis of new protein (S), as well as excretion of urea and ammonia, and from oxidation to CO^2 (E). Therefore, at steady state:

$$Q = RE = RL$$

$$RE = I + B$$

$$RL = S + E$$

$$Q = I + B = S + E$$

[^{15}N]glycine was the first stable isotope used for the quantification of whole-body protein turnover in vivo, and was initially given as a continuous intragastric infusion until a plateau had been reached in the labelling of urea in the urine.[283,284] This enrichment was assumed to represent the ^{15}N enrichment in a single nitrogen (N) metabolic pool from which exchange occurs with whole-body protein. Q can be calculated from the formula:

$$Q\ (\mu mol/h) = \frac{i}{E_{urea}}$$

where i is the infusion rate of the labelled isotope (dpm/h), and E_{urea} is the ^{15}N enrichment of the urinary urea (dpm/umol urea).

At steady state:

$$Q = S + E = B + I$$

As E (excretion) and I (intake) can be measured, S (synthesis) and B (breakdown) can be calculated. A critical assumption of this method is that all amino acids mix in one N compartment, and therefore end with the same enrichment. It has, however, been demonstrated that there are in fact several N compartments, leading to differences in the ^{15}N enrichment of urea and NH_3, as well as in the various amino acids.[285] These factors have resulted in inconsistencies in measured protein turnover rates using different [^{15}N]-labelled amino acids, and the [^{15}N]glycine method is currently not often used.

Leucine is an essential amino acid, that is, it is not produced in human tissues. Also, as opposed to the other essential amino acids that are metabolised primarily in the liver, leucine, together with the other branched chain amino acids valine and isoleucine, is catabolised primarily in muscle, the body's chief protein reservoir.[286-290] These features make leucine a good choice for the assessment of whole-body protein turnover, either as the radioisotope [^{14}C]leucine,[291,292] or the stable isotope [^{13}C]leucine.[286] The leucine tracer is given as a primed continuous infusion, which results in a steady state plateau enrichment of plasma leucine within 2 hours.

Using [^{14}C]leucine as the tracer, the flux (Q) can be calculated from the equation:

$$Q\ (\mu mol/h) = \frac{i}{Ep}$$

where i is the infusion rate of the isotope (dpm/h), and Ep is the plasma ^{14}C specific activity at isotopic plateau (dpm/μmol leucine) .

Using the stable isotope [^{13}C]leucine as the tracer, the flux (Q) can be calculated from the equation:

$$Q\ (\mu mol/h) = i\left[\frac{Ei}{Ep} - 1\right]$$

where i is the [1-^{13}C]leucine infusion rate (μmol/h), Ei is the enrichment of the [1-^{13}C] infused (atom % excess); and Ep the [1-^{13}C]leucine enrichment in plasma at isotopic plateau (atom % excess).

Oxidation, and excretion of leucine can be determined by the measurement of isotope enrichment of expired CO_2 at isotopic steady state. It is measured from the relationship between the rate of excretion of isotope in the breath carbon dioxide and the specific activity of $^{14(13)}$C leucine in the blood.

$$E\ (mmol/h) = \frac{\text{Excretion rate } ^{14(13)}CO_2 \text{ in breath (dpm/h)}}{0.8 \text{ . Plasma } [^{14(13)}\text{C}]\text{leucine specific activity (dpm/mmol)}}$$

assuming that 80% of metabolic CO_2 is excreted in the breath.[293,294]

The synthesis rate can then be calculated from the equation:

$$Q = S + E$$

In the post-absorptive, fasted state, intake (I) during the course of the study is zero, therefore, from the equation:

$$Q = B + I$$

flux (Q) is equivalent to the protein breakdown (B).

Using isotope labelled leucine to assess protein turnover, the flux (Q), breakdown (B), synthesis (S) and excretion (E) are expressed as μmol leucine/day. This may be converted to g protein/day by assuming that leucine comprises 8% of human body protein.

2.4.1.2 The Amino Acid Precursor Pool

Central to the debate on the best model for assessment of protein turnover is the definition of the isotope enrichment of the pool from which amino acids are derived for protein synthesis (the "precursor pool"). It is generally accepted that intracellular aminoacyl-tRNA represents the most appropriate amino acid pool (Figure 2.4.2).

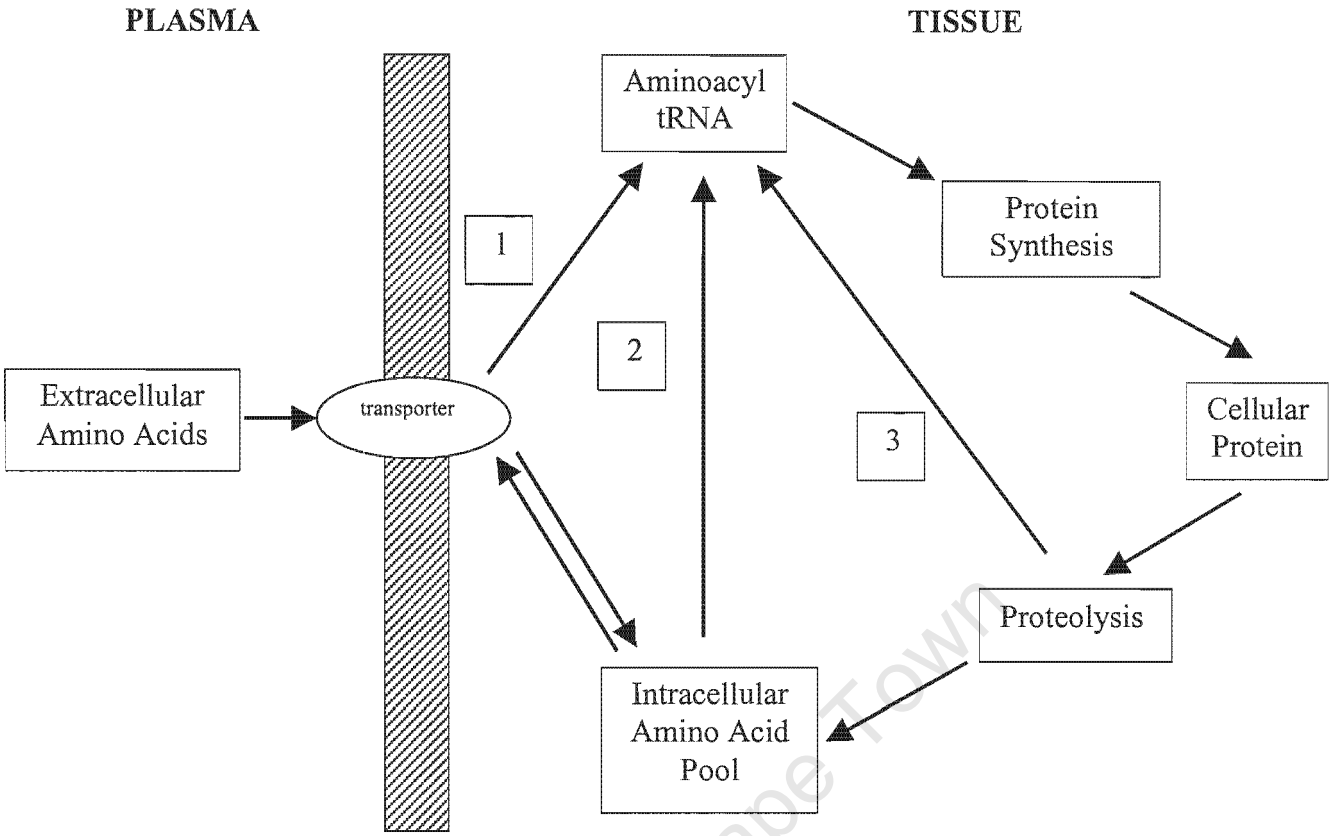


Figure 2.4.2: Source of amino acids for intracellular protein synthesis. Amino acids for the charging of tRNA for protein synthesis by ribosomes may be derived from 3 possible sources. 1) Direct from the plasma via membrane transporters, 2) from the intracellular amino acid pool and 3) directly from recycling from proteolysis of cellular protein. With tracer amino acid labelling of the extracellular (plasma) amino acid pool, should the source of amino acids for protein synthesis be predominately derived from the extracellular pool (source 1), then the enrichment of the aminoacyl-tRNA pool would be similar to that of the plasma pool. With the intracellular pool as the predominant source (source 2), then the labelling of the aminoacyl-tRNA would be lower than that of the plasma pool due to dilution by unlabelled amino acids derived from intracellular proteolysis. Should source 3 predominate, the labelling of the aminoacyl-tRNA would then be lower than the labelling of the intracellular amino acid pool. The relative proportions of these 3 pathways are likely to vary according to tissue types, and different metabolic and nutritional states.

The measurement of aminoacyl-tRNA is, however, technically difficult, due to its very short half-life, and small size of pool,[295] leading to the adoption of surrogates for this pool. Initial studies utilized the measurement of the specific activity of the amino acid in plasma.[286,291,292]

However, as illustrated in Figure 2.4.2, enrichment of the intracellular compartment is likely to be lower than the extracellular compartment due to the intracellular release of unlabelled amino acids due to protein breakdown. The ratio of enrichment between intracellular and extracellular compartments depends on the rate of tissue protein turnover, with rapidly turning over tissues having proportionately lower levels of intracellular enrichment. As the source of amino acids for protein synthesis is likely to be intracellular, enrichment of the intracellular pool would more closely approximate the precursor pool.[296]

Direct access to the intracellular pool may be difficult, or not possible, for a variety of tissues. However, intracellularly leucine undergoes rapid reversible transamination to its α-keto acid, α-ketoisocaproate (KIC), and subsequent irreversible decarboxylation Figure 2.4.3). In mammalian species, the primary regulatory site of leucine metabolism is the decarboxylation step.[297] The rapid, non-rate limiting transamination of intracellular leucine results in an intracellular leucine-KIC pool, which is in equilibrium. Both leucine and KIC cross the cell membrane into the extracellular (plasma) space, and the specific activity of the plasma KIC would therefore reflect the specific activity of the intracellular leucine. The common intracellular pool of leucine-KIC is demonstrated by studies showing that infusion of [^{3}H]leucine and [^{14}C]KIC results in the rapid appearance in the plasma of [^{3}H]KIC and [^{14}C]leucine.[298] Furthermore, within 10 minutes of stopping the simultaneous infusion of [^{3}H]leucine and [^{14}C]KIC in humans, the plasma specific activity (SA) of the reciprocal products, [^{14}C]leucine and [^{3}H]KIC were identical, and decayed together.[299]

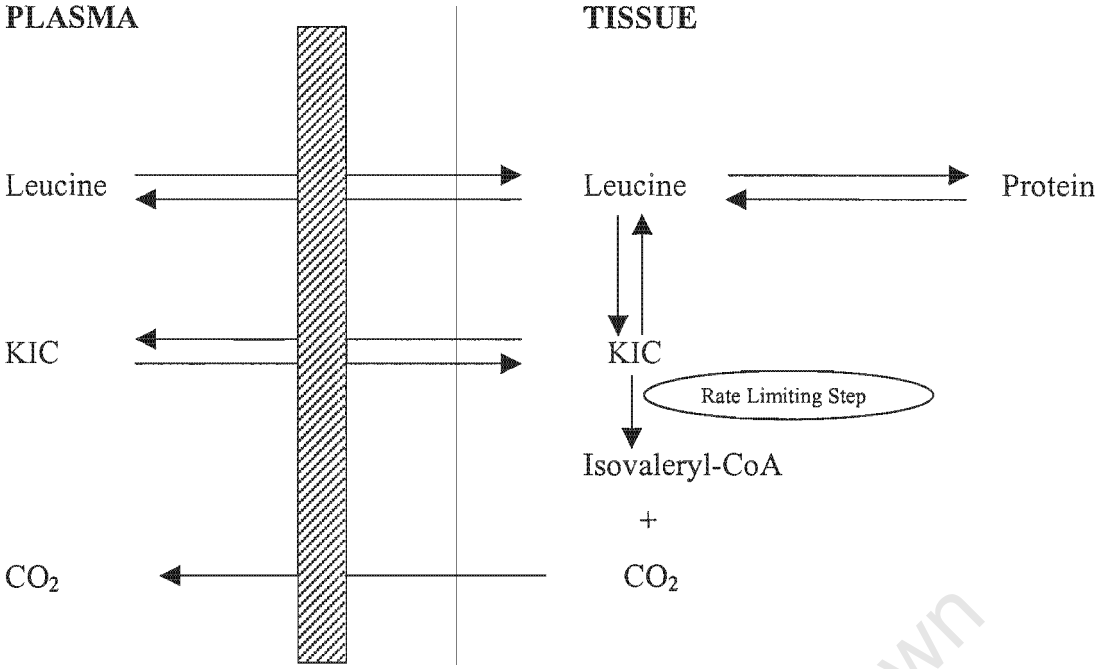


Figure 2.4.3: Plasma leucine is taken up by tissues and undergoes rapid reversible transamination to KIC to form a common pool. As intracellular leucine is the only source of KIC, at steady state during L-[1-^{13}C]leucine infusion, for those tissues, which release KIC into plasma, the enrichment of the released KIC will match the enrichment of intracellular leucine.

In view of the intimate relationship between intracellular leucine and its ketoacid, the use of reciprocal pool specific activities has been suggested to model leucine metabolism. In these models, the intracellular leucine pool is assessed by either the measurement of enrichment of the plasma KIC following a primed continuous infusion of labelled leucine, or conversely, the enrichment of plasma leucine following the infusion of labelled KIC (Figure 2.4.4).[299,300]

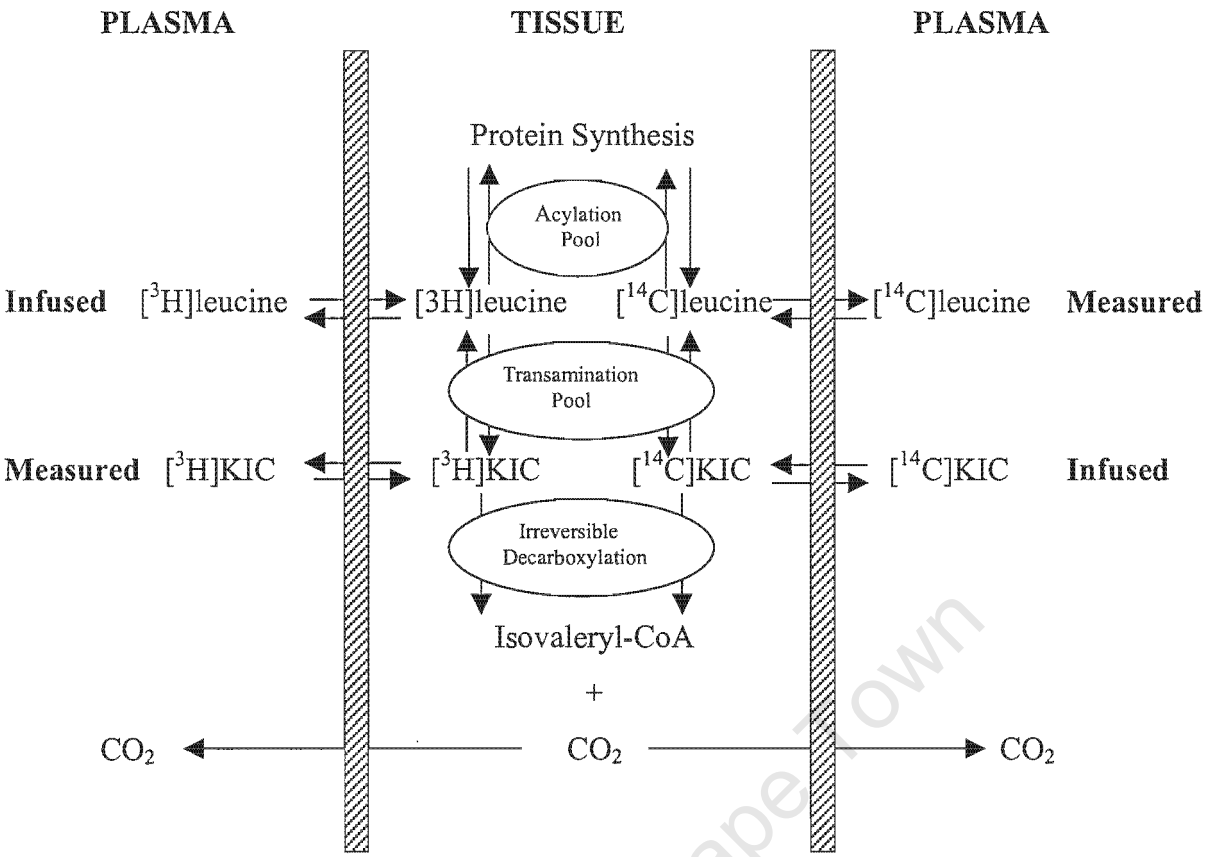


Figure 2.4.4: Infusion of either labelled leucine, or its ketoacid into the extracellular space allows passage of the labelled substrate into the intracellular pool where it enters the transamination pool. This results in the subsequent production of the reciprocal product (KIC or leucine), which may then cross the cell membrane to re-enter the extracellular (plasma) space. The rapidity and reversibility of the process allows equalization of the pools, and measurement of the reciprocal product in the plasma would be expected to give a good assessment of enrichment of the intracellular transamination pool.

There is, however, a remarkably constant relationship between the SA's of plasma leucine and that of the plasma KIC during labelled leucine infusion with a study by Matthews et al showing the enrichment of plasma KIC being approximately 77% (±1%) lower than that of the plasma leucine.[296] This indicates that entry of plasma leucine into cells accounts for approximately 77% of the intracellular leucine flux, with the remaining 23% derived from leucine release from intracellular protein breakdown. Use of plasma leucine SA as an index of the precursor pool would therefore result in an assessment of total body protein turnover 23% lower than if plasma KIC SA were used.

Although plasma KIC enrichment may reflect intracellular leucine enrichment, its use in the assessment of protein synthesis makes the assumption that both leucine transamination and acylation to aminoacyl-tRNA occur in the same intracellular pool. If this were indeed the case, then the enrichment of KIC would equal the enrichment of leucyl-tRNA. However, studies have indicated that there is more than one intracellular compartment, and it is possible that leucine transamination and acylation take place in different intracellular cytoplasmic pools.[301,302] Ljungqvist et al assessed enrichment of leucine and KIC in plasma and muscle tissue fluid during a continuous infusion of [^{13}C]leucine.[303] Their results indicated that although the ratio of enrichment of plasma leucine to plasma and tissue KIC remained constant, there was significantly higher enrichment of plasma leucine and plasma and tissue KIC compared to the enrichment of leucyl-tRNA. The enrichment of tissue leucine was similar to the leucyl-tRNA. The enrichment of KIC was 17% lower than that of the plasma leucine, but 43% higher than that of the leucyl-tRNA indicating that the transamination pool received more leucine from extracellular sources than the acylation pool. Therefore, use of KIC as a surrogate for the precursor pool would still underestimate muscle protein synthesis by approximately 43%. Furthermore, a mixed meal containing 14 mg protein/kg resulted in a decrease of the ratio of leucyl-tRNA to that of plasma leucine and KIC, whereas that of leucyl-tRNA to tissue fluid leucine remained constant. This indicated that after a mixed meal, leucine derived from extracellular sources was transaminated in preference to leucine derived from protein breakdown within muscle cells. These results indicate that in the non-fasted state, use of enrichment of plasma KIC (or plasma leucine) is unreliable in the assessment of protein synthesis, and that use of tissue fluid leucine enrichment (which is simpler to measure than leucyl-tRNA enrichment) is most appropriate as a surrogate of leucyl-tRNA enrichment. Most of the evidence currently available indicates that the aminoacyl-tRNA labelling is generally found, in vivo, to be intermediate between the intracellular and plasma free amino acid labelling (Table 2.4.1).[304]

Table 2.4.1:

Relationship Between the Labelling of the Various Amino Acid Pools in Muscle, Heart and Liver. Labelling of Aminoacyl-tRNA is taken as 100% (Adapted from Rennie MJ 1994[304])

Tissue	Plasma Amino Acid	Intracellular Amino Acid	Plasma KIC	Species	Tracer
Muscle	128	92	112	Man	Leu [295]
	130	93	104	Pig	Leu [295]
	124	79	109	Rat	Leu [295]
	129	115	-	Pig	Leu [305]
	167	73	-	Rat	Lys [306]
Heart	119	103	-	Pig	Leu [305]
	100	100	-	Rat	Leu [307]
	97	89	-	Rat	Phe [308]
Liver	121	62	-	Rat	Val [309]
	200	50	-	Rat	Leu [310]
	172	59	-	Rat	Leu [295]

Similarly, exercise appears to affect the metabolism of the various amino acid tracers. Using leucine as a tracer, protein oxidation was measured to be 2-to 3-fold higher during exercise of endurance-trained athletes ingesting carbohydrates than in the resting period, whereas protein breakdown was not affected.[311] However, using phenylalanine as tracer, protein oxidation, synthesis and breakdown were similar at rest, during exercise and during recovery. Urea production, measured by plasma tracer dilution, also did not increase during exercise. These discrepancies may be consequent to an increased uptake and oxidation of branched-chain amino acids (leucine, isoleucine and valine) in muscle during exercise,[312,313] As the size of the free amino acid pool may not be constant, this would invalidate the steady state assumption, and lead to an overestimation of the protein oxidation, and therefore an underestimation of protein synthesis.[277]

2.4.2 Measurement of Protein Synthesis by "Flooding Dose" Methodology

In order to overcome the "precursor pool" problem, the flooding dose method was developed. Studies indicated that increasing the extracellular concentration of amino acids increased intracellular concentrations, and reduced the difference between the specific activity of traced amino acid in the extracellular and intracellular pools.[314-316] The increased flux of labelled amino acid across the cell membrane reduced the dilution effect of amino acid released by intracellular protein degradation. The technique involves the bolus injection of large doses of a mixture of labelled and unlabelled amino acids.[317,318] Following the bolus infusion, the tracer enrichment decreases, but is nearly identical in the plasma and intracellular pools, and is assumed to be identical to all aminoacyl-tRNA pools in all tissues. The method has found widespread use for studies in vitro, both in cell culture,[319,320] and in infused tissues.[314,321] In vitro studies have demonstrated similar enrichment in the medium, intracellular amino acid and aminoacyl-tRNA for most,[315,320,322,323] but not all tissues.[324,325] The technique was subsequently adopted for studies in animals,[316,326-330] and with the use of stable isotopes, humans.[317,331] Advantages of the method include not only a better definition of the precursor pool specific activity, but also a much shorter period than that required with continuous infusion before measurements can be taken (1-2 h compared to 4-20 h in humans). This permits acute changes in tissue protein synthesis to be investigated. Disadvantages include the costs associated with the much larger doses of infused amino acid/tracer required, as well as concern regarding the discrepancy of results when compared with the continuous infusion method.

Several studies have indicated that the values of protein synthesis in various tissues obtained by the flooding dose method are usually higher, sometimes substantially so, than the value obtained by the continuous infusion method (Table 2.4.2), with a greater variability of result. The differences between the two methods may be a result of the definition of the precursor amino acid pool for protein synthesis. In the flooding dose protocol, it is assumed that aminoacyl-tRNA pool is labelled to the same extent as that measured in the primary pool, ie the plasma free amino acid pool. In the primed dose, constant infusion method, the precursor is usually assumed to be sufficiently close to the labelling of KIC (or free leucine) in the plasma, to allow them to be used as surrogates. Although there have been a number of studies comparing the enrichments of the various pools during constant infusion of labelled amino acid (Table 2.4.2), there is less information available regarding the relationship

between the labelling of human tissue amino acyl-tRNA, and the various free amino acids (both intra- and extra-cellular) during a flooding dose protocol.[304]

Table 2.4.2:

Comparison of protein fractional synthetic rates in various tissues of the rat assessed by either continuous infusion or flooding dose

Tissue	Constant Infusion (%/d)	Flooding Dose (%/d)
Muscle	4.7	6.9 [332]
Gut Mucosa	218	136 [333]
Liver	59	87 [333]

It is, however, unlikely that differences in the labelling of the various pools with the two methods could account for the 1.5- to 2-fold difference in the postabsorptive skeletal muscle protein synthesis reported (Table 2.4.2). There is evidence that the infusion of large doses of certain amino acids, as used in the flooding dose method, may directly influence protein synthesis. Using valine as the tracer, flooding with leucine has been shown to increase the fractional synthetic rate of mixed muscle protein from 1.0 to 1.6 %/d,[334] and that of albumin from 6.0 to 10.3%/d.[335] These responses are possibly mediated through the effects of amino acid infusion on the secretion of hormones such as insulin (leucine and phenylalanine are insulinotropic).[277] Leucine has also been reported to increase the stability of aminoacyl-tRNA,[336] and therefore may increase the incorporation of labelled amino acid into protein. On the other-hand, use of tracer quantities of amino acid, as in the constant infusion method, has been shown to have little, or no effect on the overall metabolism of the amino acid, or protein, or of metabolism in general.[298]

2.4.3 Measurement of Protein Synthesis by Arterio-Venous Balance Methods

The Fick Principal, using arterio-venous concentration differences, can be utilized to investigate metabolism of tissues and organs. The principal was first used to determine the cardiac output from the whole-body consumption of oxygen according to the calculation;

$$\text{blood flow} = \frac{\text{uptake}}{\text{arterial concentration - venous concentration}}$$

The uptake of a substance into the tissues from the blood can therefore be calculated from the equation:

$$\text{Uptake} = (\text{arterial - venous concentration}) \times \text{blood flow}$$

The principal requires three fundamental criteria: [337]

1. The blood flow should be stable
2. The arterial concentration of the substance under investigation should be constant
3. The tissue metabolism of the substance should be constant

Should non-steady-state conditions exist, the Fick principal needs to be modified.[337]

The method has been specifically utilised for the assessment of metabolism of skeletal muscle. In these studies, it is assumed that the arterio-venous difference of amino acids across tissues primarily reflects muscle metabolism, and tissues such as skin, bone, and subcutaneous fat contribute little.[338,339] The method involves administration of a tracer amino acid, and in steady-state conditions samples are taken from both arterial and venous blood. The net balance of the tracee amino acid (NB) is calculated from the formula:

$$NB = (Ca - C v) \times F$$

where Ca is the concentration of the amino acid in arterial blood, and Cv is the concentration in venous blood, and F is the blood flow.

The net balance of the tracer (nb) is calculated from the formula:

$$nb = ((Ca \times Ea) - (Cv \times Ev)) \times F$$

where Ea and Ev are the enrichment of the tracer in arterial and venous blood, Ca and Cv are the concentrations of the amino acid in arterial and venous blood respectively, and F is the blood flow.

For amino acids that are neither (or minimally) transaminated or oxidized in muscle, such as methionine, tyrosine, phenylalanine, threonine, lysine and glycine, synthesis can be calculated from the formula:

$$\text{protein synthesis} = \frac{nb}{Ev}$$

Protein breakdown is calculated from the formula:

$$\text{protein breakdown} = \text{protein synthesis} - NB$$

Protein synthesis and breakdown rates are obtained as μmol tracer amino acid/h and can be converted to g protein/h using the relative occurrence of the amino acid in muscle protein (eg phenylalanine = 3.3 g/100g).

The calculations utilized in the arterio-venous difference methods do not, however, take into account the re-utilization of amino acids derived from protein breakdown for protein synthesis, resulting in underestimations. In order to overcome this limitation, Biolo et al extended the traditional model to a three-compartment model by including the intramuscular pool of free amino acids.[301,340] Use of this model allows, not only an assessment of muscle

protein synthesis, but also calculation of the rate of transmembrane transport (from artery to muscle, and from muscle to vein), and of the direct amino acid flow from artery to vein. Furthermore, the combined use of tracer amino acids not metabolised in muscle (phenylalanine and lysine) and tracers, which are (alanine and glutamine) enables calculation of the de novo synthesis of alanine and glutamine.[301,340]

2.5 MOTIVATION FOR STUDY

Allison has recently defined malnutrition (undernutrition) as:

> "*A state of energy, protein or other specific nutrient deficiency which produces a measurable change in body function, and is associated with a worse outcome from illness as well as being specifically reversible by nutritional support.*"[341]

Normal digestion, and absorption of nutrients requires an adequate supply of digestive (mainly pancreatic) enzymes, and an efficient, absorptive enteric mucosal surface. Synthesis of these enzymes and maintenance of the gut mucosa, in turn, requires an adequate supply of nutrients. Gastric acid also plays a role in the initiation of the digestive process, and prevention of bacterial contamination of the small intestine. In severely malnourished patients, lack of nutrients may adversely affect entero-pancreatic function, resulting in maldigestion, malabsorption and diarrhoea, which may be further aggravated by the presence of co-existent disease. This vicious cycle of events may cause further deterioration in the nutritional state, and may explain the difficulties often experienced when attempting to re-feed severely malnourished patients. Intractable diarrhoea is frequently the terminal condition of famine and malnutrition victims, which can often become exacerbated by food intake.[178] The presence of co-existent disease, in addition to the poor nutritional state, is likely to further aggravate the situation.

Studies in rats have confirmed this interrelationship between nutritional state and digestive function,[342,343] but there have been few studies in human subjects.[344] Particularly in Developing Countries, where the combination of undernutrition and diarrhoea is associated with extremely high mortality rates, an understanding of the relationship between malnutrition and digestive function is not only of academic interest, but should also provide a basis for the development of strategies for nutritional intervention.

The aim of this study was to determine the consequences of severe undernutrition, with and without co-existent disease, on digestive function in human patients, with special reference to gastric acid and pancreatic enzyme secretion, and protein synthesis. In addition, the efficacy of intensive nutritional support in restoring this function was investigated.

Approval for the study was granted by the Research Ethics Committee, University of Cape Town, and informed consent was obtained from all participants prior to study.

CHAPTER 3

GENERAL PATIENT CHARACTERISTICS

3.1 Patients and Methods

Twenty-six severely undernourished hospitalised patients, each of whom had a body mass index (BMI) less than 17 kg/m^2, were studied during the course of their routine clinical management. Patients were recruited simply on the basis of severe malnutrition and the need for nutritional support. Although a number of different parameters have been used in the assessment of nutritional status, the BMI has been shown to be a reliable, and easy to determine index.[345]

Disease patient group: This subgroup consisted of eighteen patients with malnutrition associated with a number of overt disease states. These included patients with Crohn's disease (n=9), tuberculosis (n=3), immunoproliferative small intestinal disease (IPSID) (n=2), carcinoma of the lung (n=1), metastatic melanoma (n=1), disseminated amyloid (n=1), and short bowel following previous trauma (n=1).

The patients with Crohn's disease had relatively inactive disease, and were admitted primarily for nutritional support. One of the patients with tuberculosis had enteric (fistulising) disease. The two patients with IPSID were treated with antibiotics (tetracycline) and subsequent radiotherapy. The patient with metastatic melanoma presented with obstructive disease, and had undergone surgical removal of the melanoma secondary in his small bowel performed prior to entry into the study. The patients with inoperable cancer of the lung, disseminated amyloid, and short bowel following previous trauma had been admitted primarily for investigation, and for nutritional support. These subjects were considered broadly representative of patients admitted to a hospital medical (gastroenterology) ward requiring nutritional support.

Anorexia patient group: This subgroup of eight patients with anorexia nervosa were studied to compare the effects of undernutrition consequent solely to an inadequate intake, with that found in association with the variety disease states. On admission, one of the anorexia

patients had profound hypophosphataemia, requiring admission to the intensive care unit for respiratory support. She was studied once she had been discharged from the intensive care unit and in a more stable condition.

None of the subjects recruited were overtly septic at time of study, and none had evidence of significant cardiac, renal or hepatic disease. Following admission to hospital, studies were commenced once dehydration and electrolyte imbalances, if present, had been corrected.

The malnourished patients were evaluated in comparison to seventeen normal healthy volunteers recruited from medical students and hospital staff.

3.2 Nutritional Support and Repeat Studies

After the initial studies, the malnourished patients received intensive nutritional support as inpatients, aimed at providing 30 kcal/kg ideal body weight/day energy and 1.5 g/kg/day protein. In view of the possibility that these patients may have significant digestive dysfunction, the diet initially consisted of a semi-elemental, hydrolysed protein formulation (Vital or Alitraq, Ross Laboratories, Columbus, Ohio) requiring a minimum of digestion prior to absorption, administered via a fine-bore naso-gastric tube. After one to two weeks, as tolerated, a polymeric feeding formula (Ensure, Ross Laboratories), also administered via a fine-bore naso-gastric tube, was introduced, and subsequently a normal ward diet, in addition to the tube-feeding, permitted. Four patients initially required total parenteral nutrition (30 kcal/kg/day energy and protein/amino acids 1.5 g/kg/day). One patient was profoundly malnourished due to anorexia nervosa; one patient with Crohn's disease, and one with immunoproliferative small intestinal disease experienced exacerbation of their diarrhoea on initiation of enteral feeding; and the fourth had a distal small bowel fistula due to enteric tuberculosis. Enteral feeding was subsequently successfully reintroduced after 2 weeks in the anorexic patient, after 2 weeks in the patient with Crohn's disease, after 3 weeks in the patient with immunoproliferative bowel disease, and after 2 months in the patient with tuberculosis, following closure of the fistula. One patient subsequently died of disseminated tuberculosis and a suspected pulmonary embolus.

Twenty-one patients (*disease patients*, n = 15; *anorexia patients*, n = 6) consented to further study of digestive function and protein synthesis following a period of nutritional support. Studies were performed once the patients were on a normal ward diet and demonstrating sustained weight gain.

3.3 Statistics

Results are given as the mean ± standard error of the mean (SEM). Statistical analysis was by the Statistica statistical package, using analysis of variance (ANOVA), the Student t-test for parametric data, the Mann-Whitney U test for non-parametric data and Pearsons correlation coefficient, whichever was appropriate. A P value of less than 0.05 was considered significant.

3.4 Results

The anthropometrical, haematological, and biochemical characteristics of the subjects on entry into the study are illustrated in Table 3.1.

Table 3.1:

Patients' Characteristics at Entry.

	Controls	All patients	Anorexia patients	Disease patients
Number	17	26	8	18
Age (years)	28.06 (1.19)	34.08 (5.10)	30.50 (4.50)	35.67 (3.07)
BMI (kg/m^2)	23.71 (1.01)	13.39 (0.32)*	12.46 (0.53)*	13.81 (0.36)*
MAC (cm)	[24.15-37.60]	18.88 (0.54)	16.76 (0.73)	19.45 (0.47)
TST (cm)	[0.70-3.05]	0.43 (0.05)	0.27 (0.06)	0.47 (0.08)
AMC (cm)	[20.85-29.95]	17.52 (0.57)	15.91 (0.76)	17.96 (0.64)
AMA (cm^2)	[35.23-71.82]	24.78 (1.85)	20.25 (1.92)	26.02 (1.88)
Albumin (g/l)	48.31 (0.88)	28.77 (1.85)*	38.25 (2.70)*	24.56 (1.60)*#
Glucose (mmol/l)	4.93 (0.12)	5.04 (0.21)	4.60 (0.44)	5.24 (0.23)
Hb (g/dl)	14.58 (0.38)	10.75 (0.46)*	12.27 (0.48)**	10.16 (0.58)*##
Urea (mmol/l)	4.33 (0.31)	4.50 (0.37)	4.64 (0.33)	4.43 (0.52)
Creat (µmol/l)	93.92 (3.31)	68.23 (4.37)*	66.25 (7.05)**	69.11 (5.61)*
AST (U/l)	14.00 (1.00)	17.16 (2.88)	25.29 (7.89)	14.00 (2.24)
ALT (U/l)	13.08 (8.83)	19.85 (4.79)	25.88 (13.76)	12.72 (2.08)
Alk Phos (U/l)	48.54 (2.96)	98.58 (9.87)**	82.75 (13.2)**	105.61 (12.9)**

Results = mean (SEM), [ ] = established normal values.[346] * $P < 0.001$ vs. controls, ** < 0.01 vs. controls, # $P < 0.001$ vs. *anorexia patient* group, ## $P < 0.05$ vs. *anorexia patient* group.

The groups of patients studied were of comparable ages, and there was no significant difference in sex distribution, although the majority of the *anorexia patient* group were female. On admission to the study, the mean body mass index (BMI) of the undernourished patients was 13.39 ± 0.32 kg/m^2, compared to 23.71 ± 1.01 kg/m^2 in the normal healthy control subjects ($P < 0.001$). Anthropometrical assessment of the undernourished group revealed a mean mid arm circumference (MAC) of 18.88 ± 0.54 cm (normal range = 24.15 – 37.60 cm), a mean triceps skin fold thickness (TST) of 0.43 ± 0.05 cm (normal range = 0.70 – 3.05 cm), calculating to a mean arm muscle circumference (AMC) of 17.52 ± 0.57 cm

(normal range = 20.85 – 29.95 cm), and a mean arm muscle area (AMA) of 24.78 ± 1.85 cm^2 (normal range = 35.23 – 71.82 cm^2).

The *anorexia patient* group had a mean BMI of 12.46 ± 0.53 kg/m^2, which was not significantly different to that of the *disease patient* group (13.81 ± 0.36 kg/m^2). The two groups had similar anthropometrical measurements.

None of the patients had evidence of significant renal or hepatic disease, with a mean serum urea concentration of 4.50 ± 0.37 mmol/l (normal range = 1.7-6.7 mmol/l), creatinine 68.23 ± 4.37 μmol/l (normal range = 50-100 μmol/l), aspartate transaminase (AST) 17.16 ± 2.88 U/l (normal range = 7-25 U/l), alanine transaminase (ALT) 19.85 ± 4.79 U/l (normal range = 1-41 U/l), and alkaline phosphatase (ALP) 98.58 ± 9.87 U/l (normal range = 39-117 U/l). The serum levels in the *disease patient* group, and the *anorexia patient* group were similar. Although the levels were within the laboratory range for normal, serum creatinine was significantly lower in the malnourished patients compared to controls (68.23 ± 4.37 vs. 93.92 ± 3.31 μmol/l; $P < 0.001$), and serum alkaline phosphatase was significantly higher (98.58 ± 9.87 vs. 48.54 ± 2.96; $P < 0.01$). Mean blood glucose levels were all within the normal range.

Serum albumin was decreased in the undernourished patients, with a mean concentration of 28.77 ± 1.85 g/l (normal range = 35-55 g/l). Serum albumin levels, however, did not directly relate to severity of undernutrition, with no significant correlation between albumin and BMI in the malnourished patients ($r = -0.23$; P = not significant) (Figure 3.1). The reduction in albumin levels was primarily evident in the *disease patient* group (24.56 ± 1.60 g/l). The *anorexia patient* group had a mean albumin concentration, which, although significantly lower than the control subjects (38.25 ± 2.70 vs. 48.31 ± 0.88; $P < 0.001$), was within the normal range, and significantly higher than that in the *disease patient* group (38.25 ± 2.70 vs. 24.56 ± 1.60 g/l; $p < 0.001$).

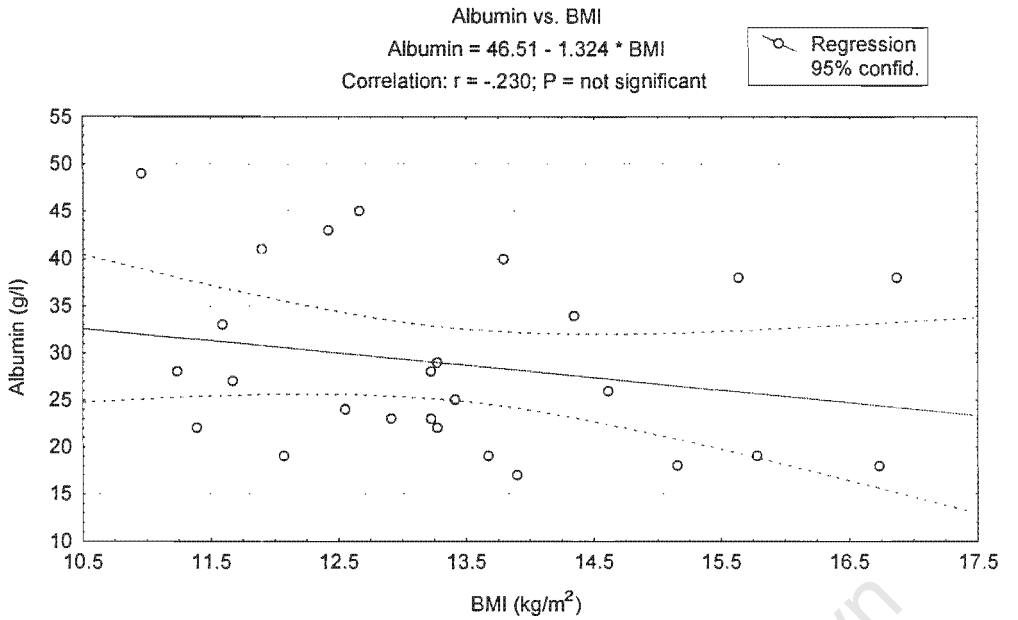


Figure 3.1: Serum albumin vs. body mass index (BMI)

The mean haemoglobin level of the undernourished patients was low at 10.75 ± 0.46 g/dl (normal range = 11.6-15.6 g/dl). This reduction was again most evident in the *disease patient* group (10.16 ± 0.58 g/dl), whereas that of the *anorexia patient* group, although lower than the control subjects (12.27 ± 0.48 vs. 14.58 ± 0.12 g/dl; $P < 0.01$), was within the laboratory normal range (11.6 –15.6 g/dl)).

Following a mean period of feeding of 6.48 ± 0.69 weeks (*anorexia patient* group 6.83 ± 0.75 weeks, *disease patient* group 6.33 ± 0.92 weeks), twenty-one patients (*anorexia patient* group n = 6, *disease patient* group n = 15) consented for further study (Table 3.2). Mean BMI increased to 15.93 ± 0.38 kg/m^2; $P < 0.01$ (*anorexia patient* group 15.02 ± 0.80 kg/m2; $P < 0.02$: *disease patient* group 16.28 ± 0.43 kg/m^2; $P < 0.01$). Mean serum albumin also increased to 35.57 ± 1.65 g/l; $P < 0.01$, (*anorexia patient* group 38.33 ± 3.11 g/l; $P > 0.05$: *disease patient* group 34.47 ± 1.94 g/l; $P < 0.001$), which although still significantly lower than the control subjects, the levels now fell within the laboratory range of normal (35-55 mmol/l). Blood glucose, haemoglobin, renal function and hepatic enzymes remained unchanged compared to levels at entry into the study.

Table 3.2:

Patients' Characteristics Following Nutritional Support

	Controls	All patients	Anorexia patients	Disease patients
Number	17	21	6	15
Feeding (wks)	-	6.47 (0.69)	6.83 (0.75)	6.33 (0.92)
BMI (kg/m^2)	23.71 (1.01)	15.93 (0.38)*#	15.02 (0.80)*#	16.28 (0.43)*#
Albumin (g/l)	48.31 (0.88)	35.57 (1.65)*#	38.33 (3.11)*	34.47 (1.94)*#
Glucose (mmol/l)	4.93 (0.12)	5.56 (0.20)	5.06 (0.38)	5.74 (0.23)
Hb (g/l)	14.58 (0.38)	10.97 (0.36)*	11.30 (0.67)*	10.82 (0.38)*
Urea (mmol/l)	4.33 (0.31)	4.94 (0.38)	5.55 (1.01)	4.68 (0.34)
Creat (µmol/l)	93.92 (3.31)	63.20 (2.65)*	61.00 (6.14)*	64.14 (2.83)*
AST (U/l)	14.00 (1.00)	23.06 (6.23)	29.50 (11.40)	21.21 (6.80)
ALT (U/l)	13.08 (8.83)	27.47 (6.99)	32.20 (8.79)	25.79 (7.94)
Alk Phos (U/l)	48.54 (2.96)	99.20 (9.33)*	102.5 (15.90)*	97.79 (11.62)*

Feeding = period of nutritional support (weeks), Results = mean (SEM). * $P < 0.001$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.02$ vs. patients before nutritional support.

3.5 Discussion

Difficulties are frequently encountered with the re-feeding of severely malnourished patients, with intolerance to meals and diarrhoea. These features indicate significant digestive dysfunction associated with the malnourished state. This study undertook to investigate the consequences of severe undernutrition on digestive function.

Many undernourished patients have disease associated with their malnourished state. The co-existent disease may also affect digestion, and therefore confound assessment of the effects of the nutritional state on digestive function. In order to distinguish the effects of malnutrition due solely to an inadequate intake, from that in association with a variety of disease states, a group of anorexia nervosa patients were studied, and evaluated in comparison to a group of patients with disease.

The degree of wasting in the *anorexic patient* group was similar to that of the *disease patient* group, and the groups were of similar ages. None of the patients had evidence of significant cardiac, renal or hepatic disease.

The finding of normal fasting blood glucose levels suggested preservation of pancreatic endocrine function in the malnourished patients. Previous studies of the effects of malnutrition on pancreatic endocrine function have produced conflicting results. Cases of malnutrition related diabetes mellitus (MRDM) have been reported,[204,205] however this phenomenon has been relatively isolated to certain regions, where it has been referred to as J (Jamaica)-type diabetes. Other studies have also failed to confirm the presence of MRDM.[206-208] Although provocational tests for diabetes (glucose tolerance tests), or plasma insulin levels, were not performed on our patients, the presence of a normal fasting glucose level probably indicates no clinically significant impairment of insulin production.

Although the levels were within the laboratory range for normal, serum creatinine was significantly lower in the malnourished patients compared to controls, probably reflecting their decreased muscle mass. Serum alkaline phosphatase, although also within the normal range, was significantly higher in the malnourished patients, compared to controls. Although acute starvation,[347] and protein energy malnutrition[348] have been associated with reduced levels of alkaline phosphatase, several of our patients had primary disease of the gut, and severe undernutrition (anorexia nervosa) has been associated with osteomalacia,[349,350] which may have increased the levels.

Haemoglobin levels were lower in the *disease patient* group, whereas levels in the *anorexia patient* group, although lower than the control subjects, were within the laboratory range of normal. A similar pattern was evident with the serum albumin, with levels in the *anorexia patient* group within the normal range, whereas those in the *disease patient* group were significantly decreased. These features suggest that both hemoglobin and albumin levels are more affected by the associated disease processes than the malnourished state.

Although serum albumin levels are often considered an index of nutritional status, our results suggest otherwise. Despite profound undernutrition, the levels in the *anorexia patient* group, although significantly lower than those in the control subjects, fell within the laboratory range for normal. The reduction in albumin concentration in these patients may therefore not be

clinically evident. Furthermore, there was no correlation between serum albumin levels, and nutritional status (BMI) of the undernourished patients. Keys et al demonstrated no significant change in total circulating albumin in 24 healthy subjects who underwent semi-starvation for 24 weeks.[17] Malnutriton has been reported to be associated with decreased albumin synthesis rates,[351,352] however, studies in rabbits have demonstrated that although albumin synthesis rates are decreased in response to starvation,[353] serum albumin levels appear to be maintained by a parallel decrease in catabolism.[354,355] A more recent study of patients with anorexia nervosa reported no significant difference in either the catabolism rate or serum level of albumin, whereas there was a significant expansion of the extravascular albumin pool compared to matched controls.[356] During periods of starvation, serum albumin levels appear to be maintained at the expense of other protein sources, predominantly muscle.[357] Although reduction in albumin synthesis may be reflected as a decrease in pre-albumin, redistribution and a reduction in catabolism appear to compensate and prevent a decrease in the serum albumin until the late, pre-terminal phase.[357]

The low levels in the *disease patient* group are probably a result of protein loss (in our patients, most likely through the bowel as a consequence of a protein losing enteropathy), or due to an acute phase response, and are likely to have been aggravated by the malnourished state. Low levels of albumin may also result from increased capillary permeability, decreased lymphatic return, overhydration or fluid retention, due to cardiac, renal or liver failure. Levels may be increased as a consequence of dehydration. O'Keefe and Dicker, in a study of 546 hospitalised patients, showed no significant association between serum albumin concentration and body weight (expressed as a percentage of ideal), or with triceps skin fold thickness.[358] The levels of albumin did, however, relate to the severity of the illness of the patients, and risk of mortality. The use of serum albumin levels as an assessment of nutritional status is therefore inappropriate, however, low levels may, in certain instances, be useful as a prognostic indicator.[357]

Following a mean period of intensive nutritional support of 6.48 ± 0.69 weeks, the malnourished patients demonstrated a significant improvement in nutritional status, although the mean BMI of 15.93 ± 0.38 kg/m^2 indicated that the patients were still significantly undernourished. There were similar improvements evident in the *anorexia patient* and *disease patient* groups. Renal and hepatic function remained normal, and blood glucose

remained within the normal range. Serum albumin improved significantly to 35.57 ± 1.65 g/l, a level within the normal range.

The general characteristics of the patients studied indicated the severity of nutritional impairment evident. Although the patients had a variety of diseases associated with their undernourished state (*disease patient* group), their nutritional status was similar to that of the *anorexia patient* group, and therefore we considered the groups comparable for purposes of further study.

CHAPTER 4

INTESTINAL ABSORPTION AND DUODENAL MUCOSAL MORPHOLOGY

4.1 Introduction

The gut appears particularly vulnerable to the effects of malnutrition, with studies of starvation in rats indicating that in excess of 50% of gut mass may be lost.[212] There have, however, been relatively few studies of the effects of severe malnutrition on gut function in humans.[344] Studies have indicated that the human gut is also adversely affected by a poor nutritional state, with villous atrophy, and impaired absorption noted.[213,229-233] The degree to which gut function in human subjects is affected by malnutrition and/or a lack of enteral stimulation does appear to be somewhat less that that noted in animal studies,[228] and the clinical relevance of these changes remains unclear.

4.2 Aim of Study

To determine the functional consequences of malnutrition on human gut mucosal structure and function, and to investigate the role of nutritional support in correcting this dysfunction.

4.3 Patients and Methods

Standard gut absorption studies were performed on the malnourished patients. These included D-xylose absorption, and faecal fat excretion whilst on a standard diet. In order to assess D-xylose absorption, patients were required to empty their bladder prior to the oral administration of 5 g D-xylose. All urine voided during the next 5 hours was collected, and D-xylose excretion determined. Faecal fat excretion was measured whilst on a standard diet containing 100g fat for a period of 3 days. This diet was administered as a liquid polymeric formula (Ensure, Ross laboratories, with added fat), via a fine bore naso-gastric tube. Stool frequency was documented on the third day whilst on the standard diet.

4.3.1 In-Vivo Measurement of Fat Digestion and Absorption

During the course of the measurement of enteral meal stimulated pancreatic enzyme secretion (Chapter 7.3.2), in-vivo assessment of fat digestion and absorption was performed by means of comparison of the amount of fat and free fatty acids in the feed (Osmolyte HN, Ross Laboratories, Columbus, Ohio) infused into the duodenum, with that in the jejunal contents aspirated from a point 25cm distal to the point of infusion. The formula was constituted with normal saline, and infused at a rate of 300 ml/h, providing 120 mg/kg/h fat, 150 mg/kg/h protein, 500 mg/kg/h carbohydrate with an overall energy infusion of 3.7 kcal/kg/h. A known quantity of polyethylene glycol, molecular weight 4000, (PEG 4000) was included in the infusion solution, determination of the recovery of which in the aspirated juice, allowed assessment, and correction for jejunal content which had escaped aspiration (see below). Jejunal contents were snap-frozen in liquid nitrogen, and both infusate, and jejunal aspirate stored at -80° C until analysed.

Total and free fatty acid concentrations were measured in the infusate and in the jejunal contents aspirated, by means of the method described by van der Kamer et al as modified by Jover and Gordon (Appendix 1).[359,360] PEG was measured by the method of Hyden (Appendix 2).[361]

Fat digestion and absorption were determined by the formulas:

$$\text{Fat (mmol)} = \text{TFA (mmol)} - \text{FFA (mmol)}$$

$$\text{Digestion (\%)} = \frac{\text{Fat infused (mmol)} - \text{Fat aspirated (mmol)}}{\text{Fat infused (mmol)}} \times 100$$

$$\text{Absorption (\%)} = \frac{\text{TFA's infused (mmol)} - \text{TFA's aspirated (mmol)}}{\text{TFA's infused (mmol)}} \times 100$$

where TFA's = total fatty acids, FFA = free fatty acids. Both fat and total fatty acids aspirated were corrected by non-absorbable marker (PEG 4000) recovery (see below).

4.4 Results

The results of the studies of gut absorption, and duodenal mucosal morphology are illustrated in Table 4.1.

Table 4.1:

Gut Absorption and Duodenal Morphology in Malnourished Patients Before Nutritional Support.

Diagnosis	BMI (kg/m^2)	Stool freq. (n/d)	Faecal fat (mmol/d)	D-xylose (g/5h)	Villous architecture
Anorexia	11.24	1	5	0.59	Normal
Anorexia	10.96	2	15	0.89	Normal
Anorexia	11.90	1	15	0.34	Normal
Anorexia	12.42	1	21	0.14	PVA
Anorexia	12.66	1	25	1.30	Normal
Anorexia	15.63	1	NA	1.10	Normal
Anorexia	11.59	2	38	0.31	Normal
Anorexia	13.27	1	7	0.91	Normal
Crohns	13.79	4	20	1.92	Normal
Crohns	14.34	4	9	1.10	Normal
Crohns	16.73	4	31	0.40	NA
Crohns	12.07	4	6	0.60	PVA
Crohns	12.91	6	NA	NA	Normal
Crohns	16.87	Stoma	78	0.52	PVA
Crohns	13.22	5	25	0.60	Normal
Crohns	13.22	6	12	1.80	Normal
Crohns	14.61	5	8	0.83	Normal
Tuberculosis	13.67	6	45	0.42	PVA
Tuberculosis	12.55	3	9	0.43	PVA
Tuberculosis	11.67	2	2	0.17	Normal
IPSID	13.27	7	145	0.44	PVA
IPSID	15.78	6	87	0.02	PVA
Short bowel	15.15	4	95	1.34	Normal
Amyloid	13.89	8	35	0.43	PVA
Melanoma	13.41	5	26	0.02	PVA
Ca lung	11.39	4	NA	NA	Normal

BMI = body mass index, stool freq. = stool frequency, D-xylose = urinary D-xylose excretion, PVA = partial villous atrophy, NA = not available

4.4.1 Gut Absorption Studies

The results of the gut absorption studies are illustrated in Table 4.2. On admission to the study the mean stool frequency of the undernourished patients, whilst on an enteral diet consisting of 100g fat administered via a fine bore naso-gastric tube, was 3.64 ± 0.42/d (range 1-8/d), with a daily stool weight of 418.2 ± 137.5 g (normal < 250 g/d). The faecal fat output was 33.00 ± 7.44 mmol/d (normal < 19 mmol/d). Further evidence of gut absorptive dysfunction was demonstrated by a mean urinary D-xylose excretion of 0.70 ± 0.10 g/5h following a 5 g oral dose (normal > 1.0 g/5h).

In-vivo studies of "fat absorption" demonstrated a mean absorption, over the first 25 cm of small bowel, of 16.80 ± 5.91% in the undernourished patients, compared to 41.63 ± 3.40% in the normal controls ($p < 0.01$). There was no significant difference between the two groups in the assessment of "fat digestion" (58.04 ± 7.68% vs. 65.70 ± 3.31%; P = not significant).

Table 4.2:

Gut Absorption Studies in Control Subjects and Malnourished Patients Before, and After Nutritional Support

	Controls	Patients before	Patients after
Stool frequency (n/d)		3.64 (0.42)	1.47 (0.18)#
Stool weight (g/d)	[< 250]	418.2 (137.5)	207.4 (40.80)
Faecal fat (mmol/d)	[3.9-5.6]	33.00 (7.44)	22.20 (7.29)
Fat "absorption"(%)	41.63 (3.40)	16.80 (5.91)*	39.08 (4.10)##
Fat "digestion" (%)	65.70 (3.31)	58.04 (7.68)	66.08 (4.21)
D-xylose excretion (g/5h)	[> 1.0]	0.70 (0.10)	1.30 (0.11)#

Results = mean (SEM), [ ] = established laboratory normal values. * $P < 0.01$ vs. controls, # $P < 0.01$ vs. patients before nutritional support, ## $P = 0.06$ vs. patients before nutritional support.

In view that several of the patients had primary disease affecting the gut, that may affect gut absorption, the *anorexia patient* group, with no evidence of primary gut pathology, were considered separately, and compared to the group of *disease patient* group (Table 4.3).

Table 4.3:

Gut Absorption Studies in the *Anorexia Patient* and *Disease Patient* Groups Before Nutritional Support.

	Normal values	Anorexia patients	Disease patients
Stool frequency (n/d)		1.25 (0.16)	4.76 (0.38)*
Stool weight (g/d)	[< 250]	119.4 (25.47)	548.9 (189.8)
Faecal fat (mmol/d)	[3.9-5.6]	18.00 (4.27)	39.56 (10.20)
D-xylose excretion (g/5h)	[> 1.0]	0.70 (0.15)	0.70 (0.14)

Results = mean (SEM). * $P < 0.001$ vs. anorexia patients

The *disease patient* group had a significantly higher mean stool frequency than the *anorexia patient* group (4.76 ± 0.38 vs. 1.25 ± 0.16/d; $P < 0.01$). In the *anorexia patient* group, mean stool weight (119.4 ± 25.47 g/d), and faecal fat excretion (18.00 ± 4.27 mmol/d) were within the normal range, whereas in the *disease patient* group, outputs were increased with a mean stool weight of 548.9 ± 189.8 g/d (normal < 250 g/d), and a mean faecal fat excretion of 39.56 ± 10.20 mmol/d (normal < 19 mmol/d). Urinary D-xylose excretion following a 5 g oral dose was similarly decreased in the *anorexia patient* group (0.70 ± 0.15 g/5h), and in the *disease patient* group (0.70 ± 0.14 g/5h) (normal > 1.0 g/5h).

Gut absorption studies and duodenal morphology following the period of nutritional support are illustrated in Table 4.4. Following the period of re-feeding, mean stool frequency in the malnourished patients decreased to 1.47 ± 0.18/d ($P < 0.01$). There was a non-significant fall in daily stool weight to 207.4 ± 40.80 g, and faecal fat excretion remained mildly elevated at 22.20 ± 7.29 g/d. Urinary D-xylose excretion increased significantly to 1.30 ± 0.11 g/5h ($P < 0.01$), a level within the normal range. In vivo "fat absorption" improved to 39.08 ± 4.10

% ($P < 0.06$), a level similar to the control value. "Fat digestion" levels remained similar to control values (66.08 ± 4.21 %).

Table 4.4:

Gut Absorption and Duodenal Morphology in Malnourished Patients After Nutritional Support.

Diagnosis	Feeding (Wks)	BMI (kg/m^2)	Stool freq. (n/d)	Faecal fat (mmol/d)	D-xylose (g/5h)	Villous Architecture
Anorexia	8	14.35	1	3	1.50	Normal
Anorexia	8	11.72	1	2	1.30	Normal
Anorexia	5	17.01	1	25	1.40	Normal
Anorexia	4	16.63	1	2	1.30	Normal
Anorexia	8	16.05	1	NA	1.03	Normal
Anorexia	8	14.34	0	0	1.46	Normal
Crohns	3	18.59	3	29	1.30	NA
Crohns	3	16.49	1	10	1.36	Normal
Crohns	12	16.87	1	7	1.50	Normal
Crohns	12	17.48	1	12	1.50	Normal
Crohns	6	17.50	Stoma	NA	NA	PVA
Crohns	4	13.84	1	NA	0.80	Normal
Crohns	3	15.43	1	2	0.65	Normal
Tuberculosis	4	18.75	1	14	2.68	Normal
Tuberculosis	10	14.61	1	7	1.38	Normal
IPSID	4	16.05	2	53	0.38	PVA
IPSID	4	17.52	3	8	NA	PVA
Short bowel	4	18.31	1	45	1.05	Normal
Amyloid	7	14.20	3	120	0.51	PVA
Melanoma	8	13.96	2	39	0.54	PVA
Ca lung	3	14.61	2	NA	1.93	Normal

Feeding = period of nutritional support (weeks), BMI = body mass index, stool freq. = stool frequency, D-xylose = urinary D-xylose excretion, PVA = partial villous atrophy, NA = not available

In the *anorexia patient* group following re-feeding, mean stool frequency (0.83 ± 0.17 /d), faecal weight (76.00 ± 27.09 g/d), and faecal fat excretion (6.40 ± 4.67 mmol/d) all remained in the normal range (Table 4.5). There was, however, a significant improvement in mean urinary D-xylose excretion (1.33 ± 0.07 vs. 0.70 ± 0.15; g/5h; $P < 0.05$).

Although there was a significant decrease in the mean stool frequency of the *disease patient* group following nutritional support (1.69 ± 0.24 vs. 4.76 ± 0.38; $P < 0.001$), both stool frequency and stool weight remained significantly greater in the *disease patient* group compared to the *anorexia patient* group (stool frequency 1.69 ± 0.24 vs. 0.83 ± 0.17 /d; $P < 0.05$: stool weight 255.2 ± 47.34 vs. 76.00 ± 27.09 g/d; $P < 0.05$). Urinary D-xylose excretion in the *disease patient* group also improved significantly, to a level within the normal range (1.28 ± 0.16 vs. 0.70 ± 0.14 g/5h; $P < 0.02$).

Table 4.5:

Gut Absorption Studies in the *Anorexia Patient* and *Disease Patient* Groups After Nutritional Support.

	Normal values	Anorexia patients	Disease patients
Stool freq. (n/d)		0.83 (0.17)	1.69 (0.24)*
Stool weight (g/d)	[< 250]	76.00 (27.09)	255.2 (47.34)*
Faecal fat (mmol/d)	[3.9-5.6]	6.40 (4.67)	28.79 (9.63)
D-xylose excretion (g/5h)	[> 1.0]	1.33 (0.07)	1.28 (0.16)

Results = mean (SEM). * $P < 0.05$ vs. *anorexia patient* group

Body mass index (BMI) correlated with D-xylose excretion ($r = 0.42$, $P < 0.01$) (Figure 4.1), and with fat absorption ($r = 0.60$, $P < 0.02$) (Figure 4.2).

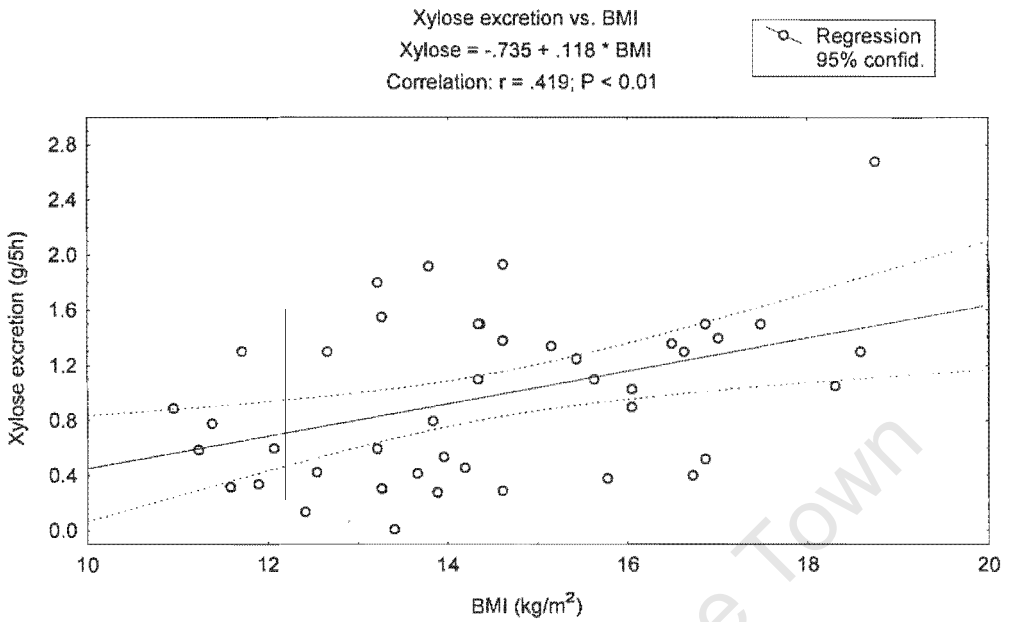


Figure 4.1: Correlation between 5 h urinary xylose excretion and body mass index (BMI).

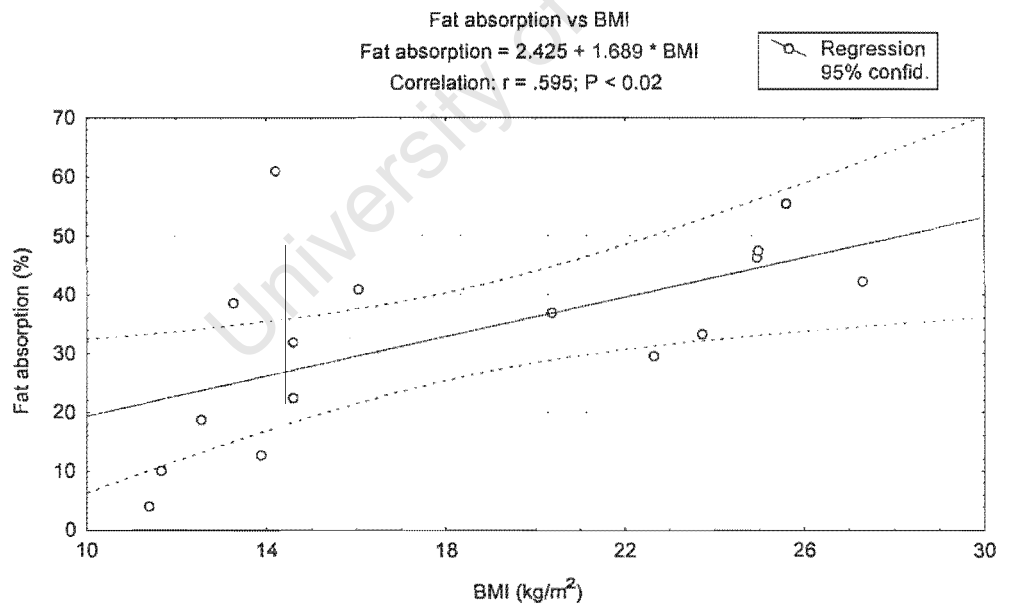


Figure 4.2: Correlation between in-vivo fat absorption and body mass index (BMI)

There was a significant correlation between stool frequency and serum albumin concentration ($r = 0.73$; $P < 0.001$) (Figure 4.3).

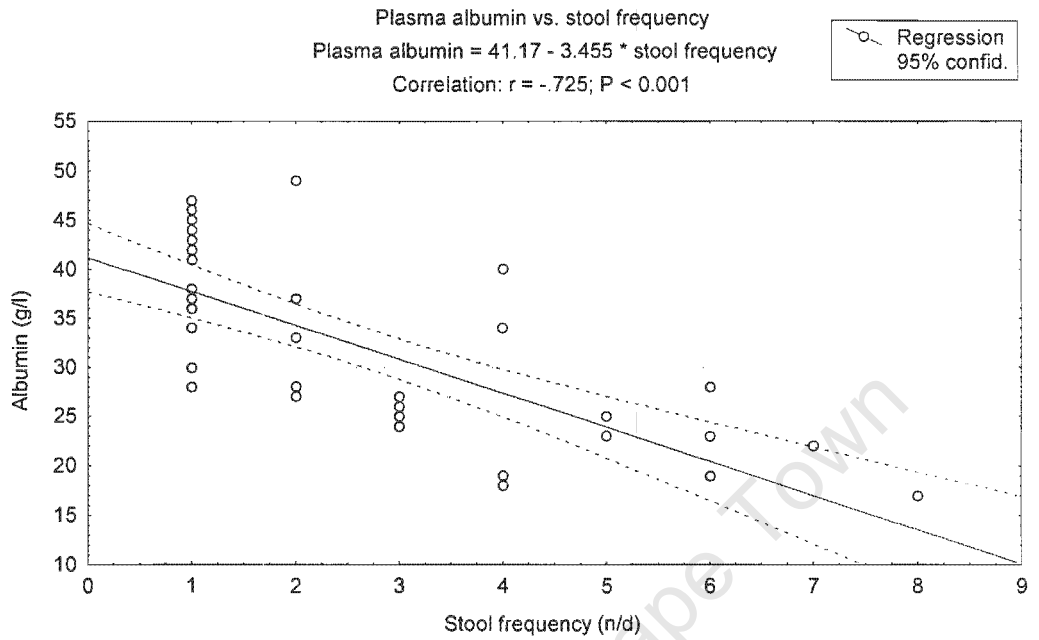


Figure 4.3: Correlation between serum albumin and stool frequency.

4.4.2 Duodenal Morphology

Nine of twenty-five patients studied (36%) had evidence of varying degrees of villous atrophy on duodenal biopsy. These were graded as mild (villous distortion and blunting) in five cases, moderate (partial villous atrophy) in three, and severe (markedly distorted villi with areas of total villous atrophy) in one. Four patients had evidence of involvement of the duodenum by the primary disease process (amyloid, n = 1; immunoproliferative small intestinal disease, n = 2; Crohn's disease, n = 1). Five of the twenty-one remaining patients (23%) who had no evidence of involvement of the duodenum by the primary disease process, had evidence of villous atrophy, which was graded as mild in four cases, and moderate in one (Figure 4.4). Of the eight patients with anorexia nervosa, one (13%) had evidence of villous atrophy, which was graded as mild (villous clubbing).

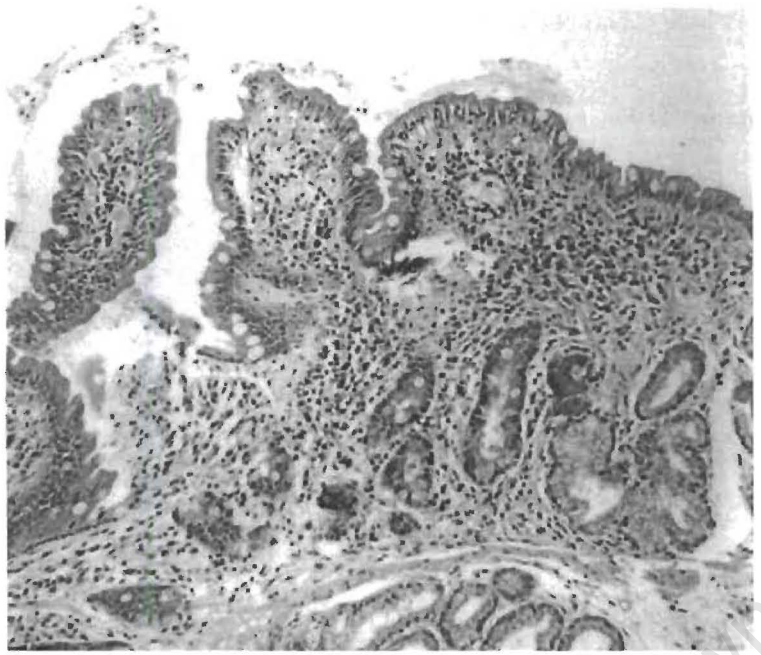


Figure 4.4: Duodenal biopsy from an undernourished patient with Crohns colitis, prior to nutritional support, showing partial villous atrophy, with no evidence of parasites, or increases in inflammatory cells in the lamina propria.

Following the period of nutritional support, duodenal biopsies revealed partial villous atrophy still evident in the four patients who had disease primarily affecting the duodenum, whereas fifteen of sixteen available biopsies (94%) from patients who had no evidence of duodenal involvement by the primary disease process had normal duodenal morphology following feeding (Figure 4.5).

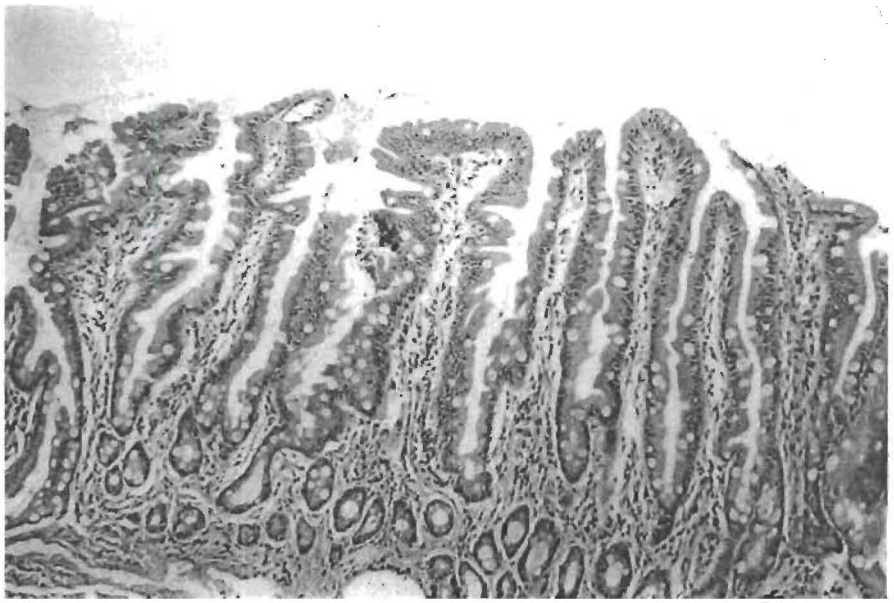


Figure 4.5: Duodenal biopsy from the same patient as Figure 4.4, following intensive enteral nutrional support. Normal villous structure is now evident.

4.5 Discussion

At entry into the study, the malnourished patients had evidence of significant impairment of digestive function, with increased stool frequency, stool weight and faecal fat excretion. The in-vivo tests of fat absorption also indicated impairment, compared to the group of healthy controls. Further evidence of gut mucosal abnormality was demonstrated by the decreased D-xylose absorption, indicated by a decreased urinary excretion following a 5 g oral dose, and several of the patients had evidence of villous atrophy. Although four of the malnourished patients had evidence of primary disease of the gut (IPSID, n = 2; Crohn's disease, n = 1; amyloid, n = 1), five of twenty-one patients (23%), with no evidence of primary disease of the gut, had villous atrophy.

Despite the presence of primary disease of the gut in several of our patients, which may have affected absorption, the direct relationship between the BMI and the parameters of gut absorption (D-xylose excretion and fat absorption) indicates a primary influence of nutritional status on gut mucosal function. This is further supported by the impaired gut absorption (impaired D-xylose excretion) noted in the *anorexia patient* group. The inverse

relationship between stool frequency and serum albumin is likely to reflect the effects of the underlying disease states on both these parameters.

Although the *anorexia patient* group was severely malnourished, and had evidence of impaired gut mucosal function, as determined by the urinary D-xylose excretion, they had normal stool frequency, volume and faecal fat excretion. One of the eight patients had evidence of mild villous atrophy. These features suggest that the presence of co-existent disease plays an important role in the clinical expression of digestive dysfunction in malnourished patients.

Following the period of nutritional support, there was evidence of improved digestive function in the malnourished patients. The *disease patient* group demonstrated a significant fall in stool frequency, and an improvement in in-vivo fat absorption, and both the *anorexia patient* and *disease patient* groups showed an increase in D-xylose absorption, to levels within the normal range. Villous atrophy was still evident in the four patients with disease primarily affecting the gut, whereas fifteen of sixteen biopsies (94%), from patients without primary involvement of the gut, were reported to be normal. Both stool frequency and stool weight remained significantly higher in the *disease patient* group, compared to the *anorexia patient* group, and faecal fat excretion remained slightly increased in the disease patient group compared to normal values. This persistent evidence of digestive dysfunction is likely to represent the consequences of the underlying disease process in these patients.

Studies of acute starvation in human subjects have generally failed to demonstrate significant changes in gut mucosal structure and function. Lee et al studied the effects of food and water deprivation, for a period of 56 days, on two healthy subjects.[362] Despite a weight loss of 30%, xylose absorption and fat excretion remained normal.

On the other hand, chronically malnourished patients have been shown to have significant abnormalities of gut mucosal structure and absorptive function. Although previous studies indicated that the digestion and absorption of protein was not impaired in malnourished children,[243] Duque et al demonstrated impaired D-xylose absorption, and abnormal faecal fat excretion in malnourished adult patients.[363] Jejunal ultrastructural abnormalities of the microvilli, including shortening, diminished numbers, abnormal positioning, branching, and fusion were also noted. Sullivan et al examined jejunal biopsies from forty malnourished

children, and reported a spectrum of mucosal change, ranging from normal, to total villous atrophy.[233] Of seven marasmic children studied by Campos et al, four had evidence of subtotal villous atrophy,[213] and Barbezat et al described varying degrees of mucosal atrophy in seventeen malnourished children.[229] Similar results were reported by Romer et al, in a study of twenty-four children with protein energy malnutrition, although the histological changes did not appear to directly relate to the severity of the malnutrition. [230] On the other hand, Nichols et al found that the degree of villous atrophy present in their malnourished infants did correlate with the severity of their undernutrition. James demonstrated impaired absorption of the sugars glucose, lactose and sucrose in malnourished children, which appeared to relate to the degree of mucosal abnormality evident.[232]

Gut mucosal abnormalities associated with malnutrition not only result in impairment of absorption, but may also result in increased permeability. Reynolds et al described increased antibodies to food antigens in twenty malnourished patients, despite histology revealing no evidence of morphological abnormality of the mucosa, indicating impaired gut barrier function.[240] Increased gut permeability, as assessed by the lactose/manitol test, has been demonstrated by Welsh et al,[238] and van der Hulst et al,[364] also indicating a compromised gut barrier. Although the clinical significance of the findings remains unclear in human subjects,[238] a compromised gut barrier may increase the risk of translocation of bacteria and toxins from the gut lumen, with the potential for septic complications.[365] This would be further aggravated by the impaired intestinal transit,[243] and overgrowth of bacteria in the small intestine,[366] documented in malnourished patients.

The studies of gut mucosal structure and function have indicated significant abnormalities associated with malnutrition. The human gut, however, does appear to have considerable reserve, and clinical expression of these changes does, however, seem to relate to the co-existence of primary disease.

CHAPTER 5

WHOLE-BODY METABOLISM

5.1 Introduction

Prolonged starvation has been reported to result in reduction of both energy expenditure and protein turnover rates in healthy and obese subjects.[17,257,268,269] With acute starvation, this hypometabolism has been shown to be in excess of that anticipated by a reduction of lean body mass, and may be considered as an adaptive mechanism allowing for survival during periods of food shortage.[263] This metabolic adaptation, or increased metabolic efficiency,[275] would have the effect of maintaining fat mass, and reducing the need for essential amino acids.

Although it is generally assumed that in chronic undernutrition the physiological and metabolic responses occur in much the same way as in the studies of experimental or therapeutic semi-starvation,[257] several investigators have failed to demonstrate the phenomenon of metabolic adaptation in chronically undernourished patients, or in those with co-existent disease.[259-264]

In order to extend these findings in chronically malnourished patients, and to investigate the effect of nutritional rehabilitation on metabolic processes, respiratory quotient, resting energy expenditure, and total body protein kinetics were assessed in our subjects. The respiratory quotient (carbon dioxide produced/oxygen consumed) reflects the nature of endogenous fuel being metabolised during the fasting state. Resting energy expenditure indicates the minimum energy required in order to maintain nutritional status, and assessment of protein kinetics reflects the anabolic/catabolic state of the individuals.

5.2 Aim of Study

To determine the functional consequences of malnutrition, and subsequent re-feeding on respiratory quotient, resting energy expenditure, and whole-body protein kinetics.

5.3 Patients and Methods

Indirect calorimetry, and infusion of isotope-labelled leucine (L-[1-^{14}C]leucine) were used to assess respiratory quotient, resting energy expenditure, and whole-body protein kinetics in normal subjects and in malnourished patients before and after nutritional support.

5.3.1 Respiratory Quotient and Resting Energy Expenditure

Total carbon dioxide production and oxygen consumption were measured in the rested, fasted state by means of indirect calorimetry, using a metabolic monitor (MedGraphics CPX/D, St Paul, Minnesota). This allowed determination of the respiratory quotient (RQ), and assessment of the resting energy expenditure (REE).

5.3.2 Isotope Incorporation Studies

In order to assess protein kinetics, a four-hour primed, continuous infusion of L-[1-^{14}C]leucine (0.30 uCi/kg + 0.30 uCi/kg/h) was administered. Venous blood samples were taken at 0, 1, 2.5 and 4 hours for subsequent measure of serum leucine specific activity (Appendices 6,10), and expired breath samples were collected at one-hour intervals for determination of amino acid oxidation rates. Carbon dioxide excretion in the breath (mmol/h) was measured by indirect calorimetry, and 1-mmol samples of expired breath carbon dioxide were trapped in hyamine and counted to calculate the specific activity of ^{14}C-carbon dioxide (dmp/mmol). Total isotope excretion in the breath (dpm/h) was then calculated. Plasma leucine concentrations were measured by high-performance liquid chromatography (HPLC),[24] (Chromjet Integrator, SP8800 Ternary HPLC pump, SpectroSeries UV150 detector; Spectra-Physics, Mountain View, CA). Radioactivity due to ^{14}C was measured by

dual window liquid scintigraphy counting (Tricarb 1500; Packard Instrument, Downers Grove, IL).

5.3.3 Calculations

A stochastic model for whole-body amino acid metabolism was used to calculate rates.[367] During isotopic equilibrium, the rate of entry of leucine into the plasma is equal to the rate of loss from the plasma, and is termed flux (Q). Because the isotope labelled carboxyl group of the amino acid is either irreversibly lost to expired CO_2, or incorporated into protein, the loss of the non-oxidative portion of leucine from the plasma may be considered to represent its incorporation into protein.[263] The leucine flux (Q) in plasma was calculated from the relationship between the rate of isotope infusion and the plateau specific activity of ^{14}C leucine in the blood (specific activity during infusion – basal specific activity).

$$Q\ (\text{mmol/h}) = \frac{\text{Infusion rate of } ^{14}\text{C leucine (dpm/h)}}{\text{Plasma } ^{14}\text{C leucine specific activity (dpm/mmol)}}$$

Leucine oxidation rate (E) was measured from the relationship between the rate of excretion of isotope in the breath carbon dioxide and the specific activity of ^{14}C leucine in the blood.

$$E\ (\text{mmol/h}) = \frac{\text{Excretion rate } ^{14}CO_2 \text{ in breath (dpm/h)}}{0.8 \cdot \text{Plasma } ^{14}\text{C leucine specific activity (dpm/mmol)}}$$

where the excretion rate of ^{14}C in breath is calculated from the specific activity of expired $14CO_2$ (dpm/mmol) and the volume of CO_2 expired in one-hour measured by indirect calorimetry (mmol/h at standard temperature and pressure). The factor of 0.8 takes into account that 80% of metabolic CO_2 is excreted in the breath.[292,294,368]

Consequently the fraction of leucine flux not oxidised, and available for whole-body protein synthesis (S) can be calculated from the equation:

$$Q = S + E$$

In the fasted state, as there is no exogenous input of leucine, from the equation:

$$Q = B + I$$

Intake (I) = 0, and therefore Flux (Q) = Breakdown (B).

The leucine flux, oxidation and synthesis rates are converted to "whole-body protein" rates by assuming that human body mixed protein consists of 8% leucine (by weight).[369-371]

5.4 Results

The results of the studies of respiratory quotient, resting energy expenditure, and whole-body protein kinetics in the control subjects, and the undernourished patients before, and after feeding are illustrated in Table 5.1.

Table 5.1:

Respiratory Quotient (RQ), Resting Energy Expenditure (REE), and Whole-Body Protein Kinetics in Controls, and Malnourished Patients Before, and After Nutritional Support.

	Controls	Patients before	Patients after
RQ	0.89 (0.06)	0.80 (0.03)	0.93 (0.05)##
REE (kcal/d)	1807 (120.8)	1216 (114.9)*	1250 (83.64)*
REE (kcal/kg/d)	25.36 (1.38)	33.02 (2.54)**	28.47 (1.77)
WBP Flux (g/d)	349.1 (22.11)	148.0 (8.74)*	200.6 (15.58)*#
WBP Flux (g/kg/d)	4.88 (0.33)	4.11 (0.32)	4.50 (0.30)
WBP Oxid. (g/d)	44.09 (4.18)	20.70 (2.64)*	28.69 (3.80)**
WBP Oxid. (g/kg/d)	0.61 (0.06)	0.58 (0.08)	0.64 (0.08)
WBP Syn. (g/d)	305.0 (21.64)	127.4 (7.50)*	171.9 (12.92)*#
WBP Syn. (g/kg/d)	4.27 (0.32)	3.56 (0.29)	3.86 (0.25)

WBP Flux = whole-body protein flux, WBP Oxid. = whole-body protein oxidation, WBP Syn. = whole-body protein synthesis. Results = mean (SEM). * $P < 0.01$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.01$ vs. patients before nutritional support, ## $P < 0.05$ vs. patients before nutritional support.

The results of the metabolic studies in *anorexia patient* and *disease patient* groups at entry into the study are illustrated in Table 5.2, and following nutritional support in Table 5.3.

Table 5.2:

Respiratory Quotient (RQ), Resting Energy Expenditure (REE), and Whole-Body Protein Kinetics in Controls, and in the *Anorexia Patient* and *Disease Patient* Groups Before Nutritional Support.

	Controls	Anorexia patients	Disease patients
RQ	0.89 (0.06)	0.85 (0.05)	0.76 (0.02)##
REE (kcal/d)	1807 (120.8)	1058 (134.0)*	1331 (169.7)*
REE (kcal/kg/d)	25.36 (1.38)	32.45 (4.13)	33.43 (3.36)**
WBP Flux (g/d)	349.1 (22.11)	170.6 (13.58)*	139.5 (10.31)*
WBP Flux (g/kg/d)	4.88 (0.33)	5.36 (0.73)	3.65 (0.28)*#
WBP Oxid. (g/d)	44.09 (4.18)	22.99 (4.71)**	19.84 (3.24)**
WBP Oxid. (g/kg/d)	0.61 (0.06)	0.70 (0.14)	0.54 (0.10)
WBP Syn. (g/d)	305.0 (21.64)	147.6 (12.88)*	119.8 (8.57)*
WBP Syn. (g/kg/d)	4.27 (0.32)	4.66 (0.70)	3.14 (0.24)*#

WBP Flux = whole-body protein flux, WBP Oxid. = whole-body protein oxidation, WBP Syn. = whole-body protein synthesis. Results = mean (SEM). * $P < 0.01$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.02$ vs. *anorexia patient* group, ## $P = 0.08$ vs. controls.

Table 5.3:

Respiratory Quotient (RQ), Resting Energy Expenditure (REE), and Whole-Body Protein Kinetics in Controls, and in the *Anorexia Patient* and *Disease Patient* Groups After Nutritional Support.

	Controls	Anorexia patients	Disease patients
RQ	0.89 (0.06)	1.00 (0.07)	0.86 (0.04)
REE (kcal/d)	1807 (120.8)	1133 (94.83)*	1338 (123.9)**
REE (kcal/kg/d)	25.36 (1.38)	27.65 (3.05)	29.09 (2.24)
WBP Flux (g/d)	349.1 (22.11)	201.8 (19.00)*	200.2 (19.88)*
WBP Flux (g/kg/d)	4.88 (0.33)	4.86 (0.21)	4.39 (0.39)
WBP Oxid. (g/d)	44.09 (4.18)	35.67 (6.92)	26.54 (4.46)*
WBP Oxid. (g/kg/d)	0.61 (0.06)	0.84 (0.11)	0.58 (0.09)
WBP Syn. (g/d)	305.0 (21.64)	166.1 (16.75)*	173.6 (16.34)*
WBP Syn. (g/kg/d)	4.27 (0.32)	4.02 (0.29)	3.80 (0.32)

WBP Flux = whole-body protein flux, WBP Oxid. = whole-body protein oxidation, WBP Syn. = whole-body protein synthesis. Results = mean (SEM). * $P < 0.01$ vs. controls, ** $P < 0.05$ vs. controls.

5.4.1 Respiratory Quotient

The mean RQ of the control subjects was 0.89 ± 0.06. The mean RQ of the undernourished subjects was 0.80 ± 0.03, which although lower than that of the controls, the difference did not reach statistical significance. Following re-feeding, the mean RQ of the malnourished patients increased significantly to 0.93 ± 0.05 ($P < 0.05$).

The mean RQ of the *anorexia patient* group was similar to the control values, (0.85 ± 0.05 vs. 0.89 ± 0.06), whereas that of the *disease patient* group tended to be lower (0.76 ± 0.02 vs. 0.89 ± 0.06; $P = 0.08$). Following feeding the mean RQ of the *disease patient* group increased significantly (0.86 ± 0.04 vs. 0.76 ± 0.02; $P < 0.05$), with the mean RQ's of both the *anorexia patient* (1.00 ± 0.07) and *disease patient* (0.86 ± 0.04) groups similar to the controls.

5.4.2 Resting Energy Expenditure

Mean resting energy expenditure (REE) was significantly reduced in the undernourished patients, compared to controls (1216 ± 114.9 vs. 1807 ± 120.8 kcal/d; $P < 0.01$). There was a significant correlation between REE and body weight ($r = 0.65$; $P < 0.001$) (Figure 5.1).

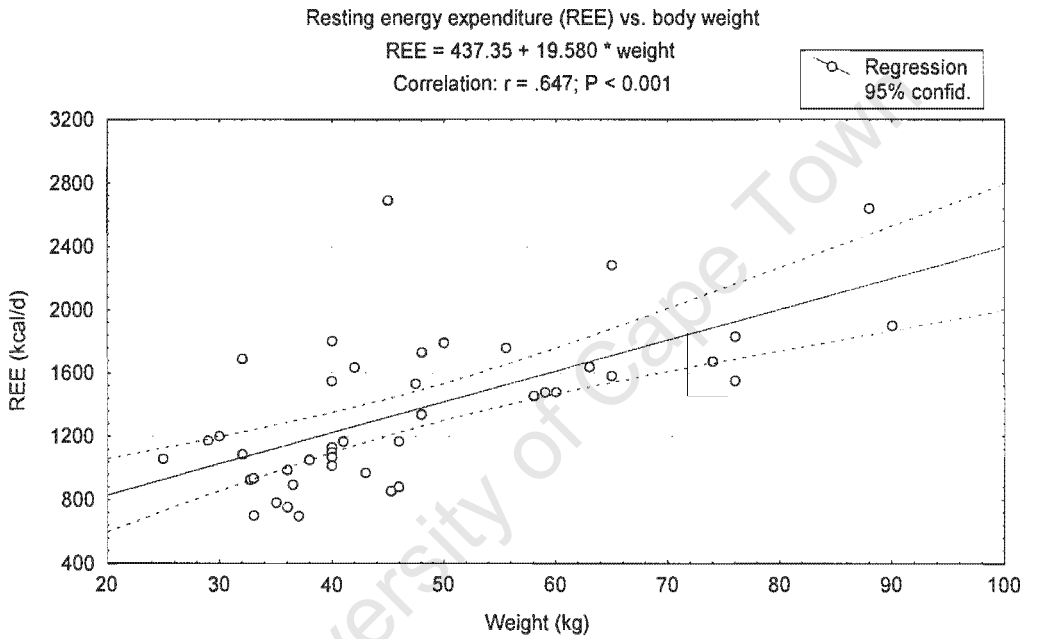


Figure 5.1: Correlation between resting energy expenditure (REE) and body weight.

Although mean REE of the malnourished patients, when expressed in absolute terms (kcal/d), was lower than the control value, when expressed per kg body weight, it was significantly higher (33.02 ± 2.54 vs. 25.36 ± 1.38 kcal/kg/d; $P < 0.05$).

Following feeding, there was no change in the mean REE of the malnourished patients, which remained significantly lower than the control value (1250 ± 83.64 vs. 1807 ± 120.8 kcal/d; $P < 0.01$). However, when expressed per kg body weight, there was now no significant difference between the malnourished patients and the controls (28.47 ± 1.77 vs. 25.36 ± 1.38 kcal/kg/d).

Comparison of the *anorexia patient* and *disease patient* groups revealed no significant difference in mean REE, either before (1058 ± 134.0 vs. 1331 ± 169.7 kcal/d; 32.45 ± 4.13 vs. 33.43 ± 3.36 kcal/kg/d), or after feeding (1133 ± 94.83 vs. 1338 ± 123.9 kcal/d; 27.65 ± 3.05 vs. 29.09 ± 2.24 kcal/d).

5.4.3 Whole-Body Protein Kinetics

Mean whole-body protein flux, oxidation and synthesis were all significantly lower in the malnourished patients before nutritional support, compared to the control group (flux 148.0 ± 8.74 vs. 349,1 ± 22.11 g/d; $P < 0.001$: oxidation 20.70 ± 2.64 vs. 44.09 ± 4.18 g/d; $P < 0.001$: synthesis 127.4 ± 7.50 vs. 305.0 ± 21.64 g/d; $P < 0.001$) (Figure 5.2).

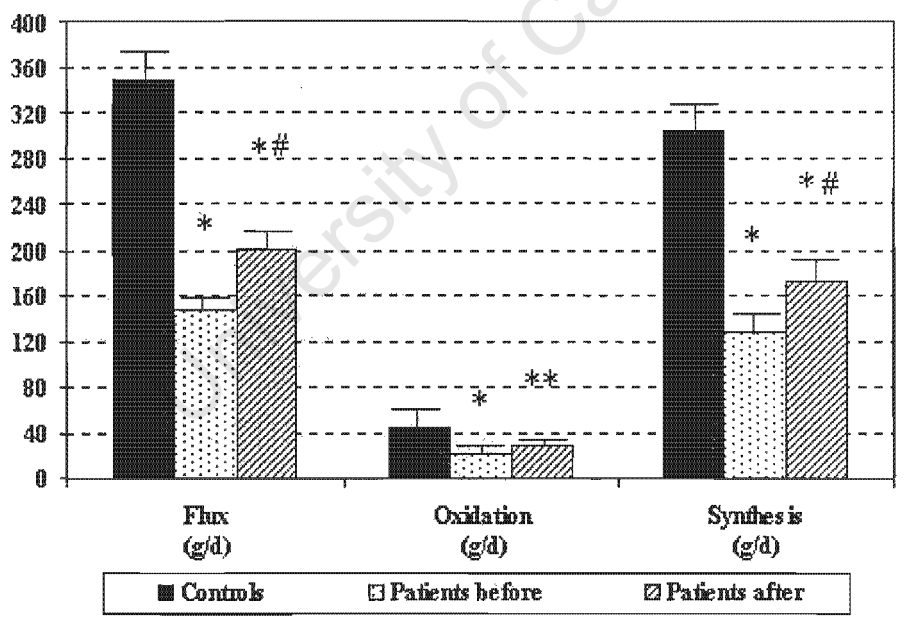


Figure 5.2: Whole-body protein flux, oxidation and synthesis (g/d) in controls, and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.01$ vs. patients before nutritional support.

Following the period of nutritional support, there was a significant increase in both mean whole-body protein flux (200.6 ± 15.58 vs. 148.0 ± 8.74 g/d; $P < 0.01$), and synthesis (171.9 ± 12.92 vs. 127.4 ± 7.50 g/d; $P < 0.01$), with a trend to increased oxidation (28.69 ± 3.80 vs. 20.70 ± 2.64 g/d; $P = 0.08$) in the malnourished patients. Whole-body protein flux, oxidation and synthesis, however, all remained significantly lower than control values (flux 200.6 ± 15.58 vs. 349.1 ± 22.11 g/d; $P < 0.001$: oxidation 28.69 ± 3.80 vs. 44.09 ± 4.18 g/l; $P < 0.02$ g/d: synthesis 171.9 ± 12.92 vs. 305.0 ± 21.64 g/d; $P < 0.001$).

When expressed per kg body weight, whole-body protein flux (4.11 ± 0.32 vs. 4.88 ± 0.33 g/kg/d), oxidation (0.58 ± 0.08 vs. 0.61 ± 0.06 g/kg/d) and synthesis (3.56 ± 0.29 vs. 4.27 ± 0.32 g/kg/d) were, however, not significantly different to the control values (Table 5.1). There was no significant change following the period of nutritional support (flux 4.50 ± 0.30 vs. 4.11 ± 0.32 g/kg/d; oxidation 0.64 ± 0.08 vs. 0.58 ± 0.08 g/kg/d; synthesis 3.86 ± 0.25 vs. 3.56 ± 0.29 g/kg/day).

Body weight correlated significantly with whole-body protein flux rates ($r = 0.74$; $P < 0.001$), protein oxidation rates ($r = 0.58$; $P = 0.001$), and protein synthesis rates ($r = 0.72$; $P < 0.001$) (Figures 5.3-5)

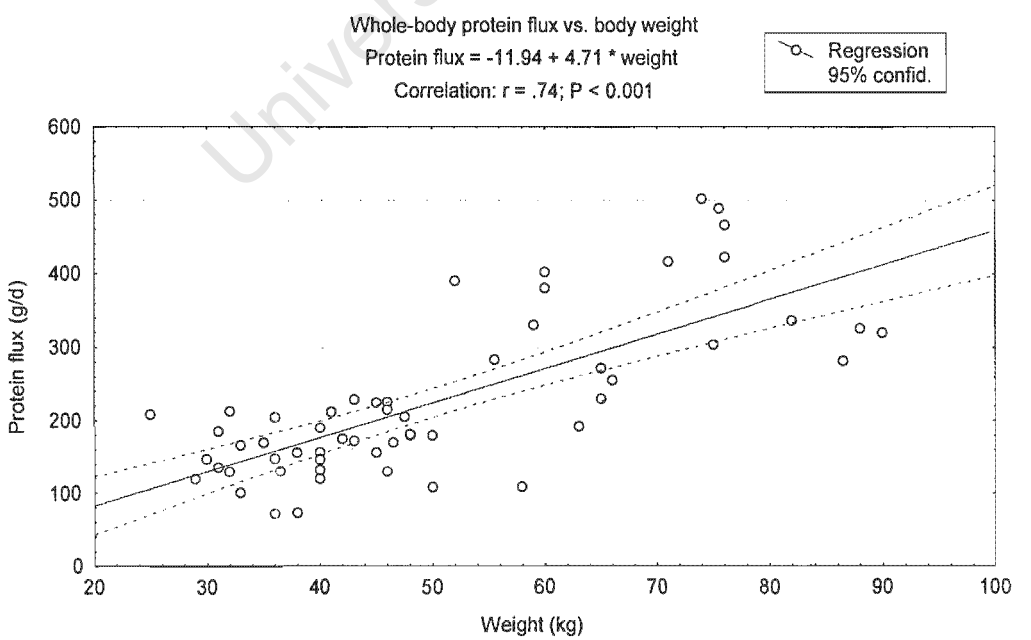


Figure 5.3: Correlation between whole-body protein flux and body weight.

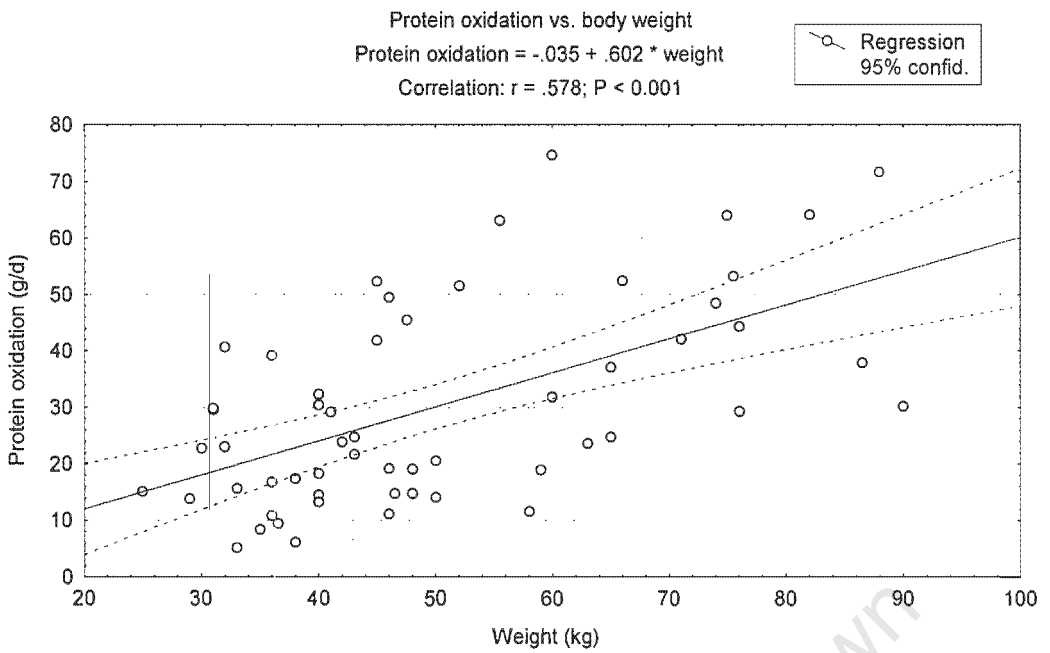


Figure 5.4: Correlation between whole-body protein oxidation and body weight.

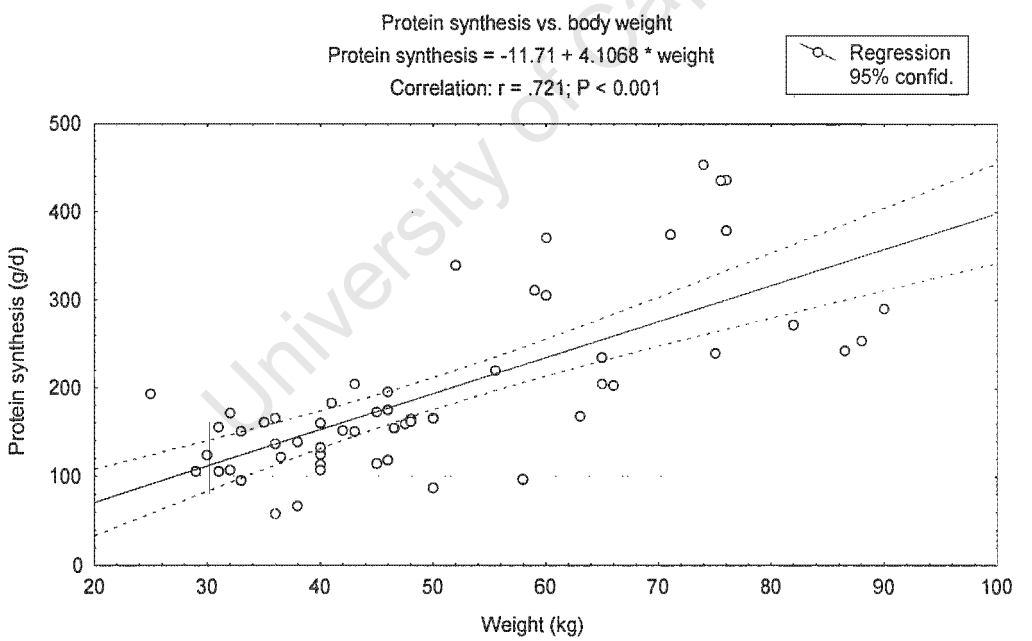


Figure 5.5: Correlation between whole-body protein synthesis and body weight.

Comparison of the *anorexia patient* and *disease patient* groups revealed similar mean whole-body protein flux, oxidation and synthesis, expressed in absolute terms (flux 170.6 ± 13.58 vs. 139.5 ± 10.31 g/d: oxidation 22.99 ± 4.71 vs. 19.84 ± 3.24 g/d: synthesis 147.6 ± 12.88

vs. 119.8 ± 8.57 g/d), which were significantly lower than the control values (flux 349.1 ± 22.11 g/d; $P < 0.001$: oxidation 44.09 ± 4.18 g/kg/d; $P < 0.02$: synthesis 305.0 ± 21.64 g/d; $P < 0.001$).

Expressed per kg body weight, mean whole-body protein flux and synthesis of the *anorexic patient* group was similar to controls (flux 5.36 ± 0.73 vs. 4.88 ± 0.33 g/kg/d: synthesis 4.66 ± 0.70 vs. 4.27 ± 0.32 g/kg/d), whereas the *disease patient* group had significantly lower values than either controls (flux 3.65 ± 0.28 vs. 4.88 ± 0.33 g/kg/d; $P < 0.01$: synthesis 3.14 ± 0.24 vs. 4.27 ± 0.32 g/kg/d; $P < 0.01$) or the *anorexia patient* group (flux 3.65 ± 0.28 vs. 5.36 ± 0.73 g/kg/d; $P < 0.02$: synthesis 3.14 ± 0.24 vs. 4.66 ± 0.70; $P < 0.02$) (Figure 5.6). Mean whole-body protein oxidation rates, expressed per kg body weight were similar in all groups (controls 0.61 ± 0.06 g/kg/d: *anorexia patient* group 0.70 ± 0.14 g/kg/d: *disease patient* group 0.54 ± 0.10 g/kg/d).

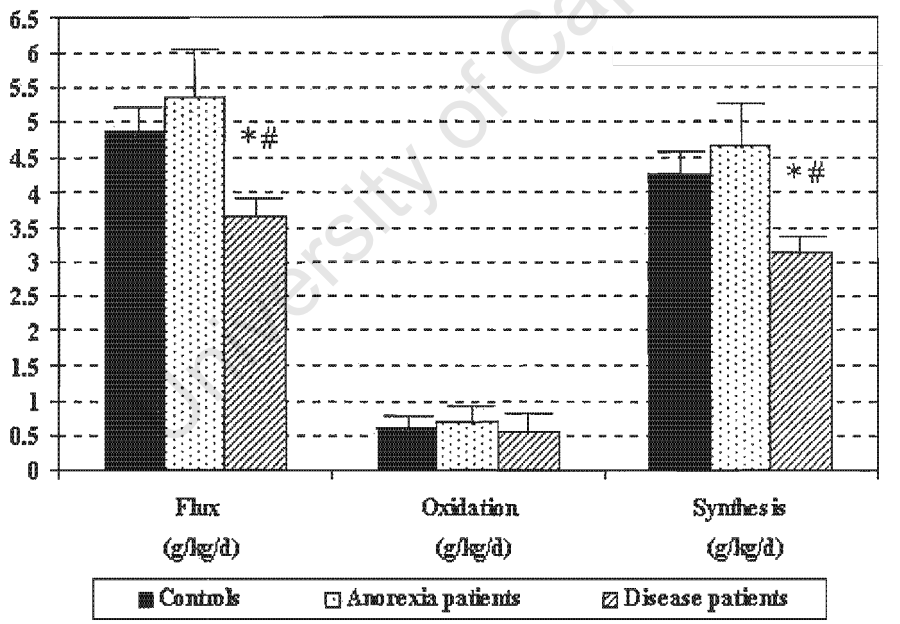


Figure 5.6: Whole-body protein flux, oxidation and synthesis (g/kg/d) in controls, and in the *anorexia patient* and *disease patient* groups before nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. controls, # $P < 0.05$ vs. *anorexia patient* group.

In the *disease patient* group, following the period of nutritional support, there was a significant increase in mean whole-body protein flux (200.2 ± 19.88 vs. 139.5 ± 10.31 g/d; P < 0.01) and synthesis (173.6 ± 16.34 vs. 119.8 ± 8.57 g/d; P < 0.01), whereas the values of the *anorexia patient* group remained similar to the pre-feeding levels (flux 201.8 ± 19.00 vs. 170.6 ± 13.58 g/d: synthesis 166.1 ± 16.75 vs. 147.6 ± 12.88 g/d). The rates remained significantly lower than controls (flux 349.1 ± 22.11 g/d; P < 0.001: synthesis 305.0 ± 21.64 g/d; P < 0.001). Post-feeding, the mean whole-body protein oxidation rate in the *disease patient* group remained significantly lower than the control value (26.54 ± 4.46 vs. 44.09 ± 4.18 g/d; P < 0.01), whereas that of the *anorexia patient* group was similar (35.67 ± 6.92 vs. 44.09 ± 4.18). Expressed per kg body weight mean total body flux, oxidation, and synthesis in the *anorexia patient* and *disease patient* groups were similar to control levels (Table 5.3, Figure 5.7).

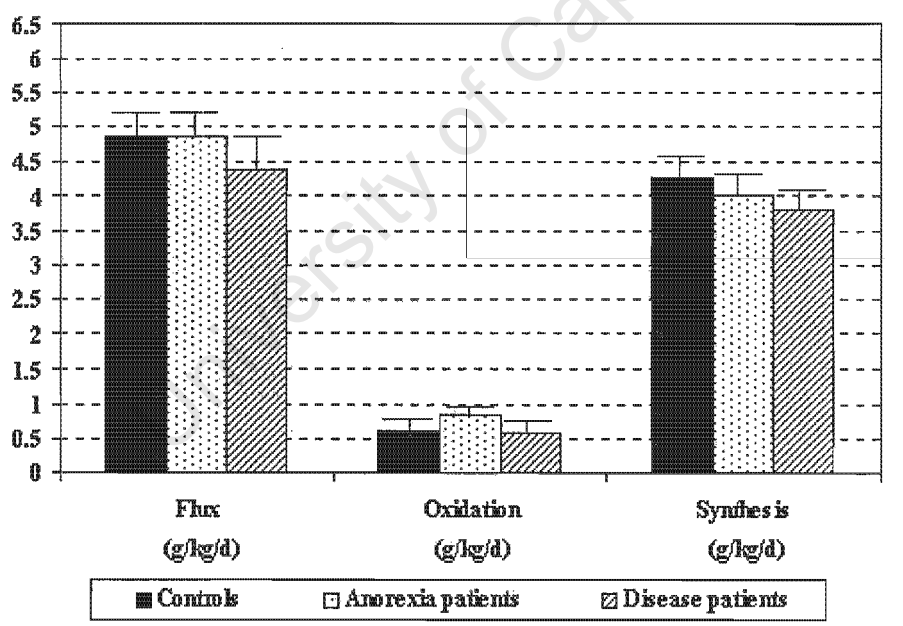


Figure 5.7: Whole-body protein flux, oxidation and synthesis (g/kg/d) in controls, and in the *anorexia patient* and *disease patient* groups after nutritional support. Bars represent means (SEM).

5.5 Discussion

5.5.1 Respiratory Quotient

The fasting respiratory quotient (RQ) reflects the utilisation of endogenous nutrients, which may be affected by available stores. Preferential use of carbohydrate stores (glycogen) will result in a high RQ (≈ 1.0), whereas that of fat will result in lower values (≈ 7.0). Although the malnourished patients, as a group, had a similar mean fasting respiratory quotient (RQ) to the control value, the *disease patient* group tended to have a lower value. This suggests reduced carbohydrate, and increased lipid metabolism in malnourished patients with co-existent disease. This is likely to be the consequence of depleted glycogen stores, which appears to be more pronounced in the patients with disease. Lauvin et al have previously reported that anorexia nervosa patients tended to catabolise carbohydrate, possibly reflecting the nature of their diets, whereas patients with both malignant and non-tumoral disease catabolised lipid.[372]

Following re-feeding, the malnourished patients demonstrated a significant increase in RQ, with the increase principally evident in the *disease patient* group. This indicates replenishment of carbohydrate (glycogen) stores, and a reduction in fat metabolism in these patients.

5.5.2 Resting Energy Expenditure

Although mean resting energy expenditure (REE), considered in absolute terms (g/d), was significantly lower in the malnourished patients compared to controls, when assessed in relation to body mass (kcal/kg/d), it was significantly higher. Mean REE of the *anorexia patient* and *disease patient* groups was similar, both before, and after nutritional support.

These findings contrast with previous studies of semi-starvation in normal individuals. The classic studies of Keys et al demonstrated a 14% reduction of REE per unit fat-free mass (FFM).[17] Shetty demonstrated a similar reduction of REE, related to body mass, and to body surface area, in undernourished, but otherwise healthy Indian labourers,[373] and studies of

therapeutic semi-starvation in obese patients have also produced similar results.[374] Results of these studies suggest metabolic adaptation, or increased metabolic efficiency, associated with the undernourished state.[275]

Other studies have, however, failed to demonstrate evidence of metabolic adaptation in chronically malnourished subjects, or in patients with co-existent disease. Ashworth, although reporting a 12% reduction of REE in malnourished Jamaican subjects, was unable to demonstrate metabolic adatation.[259] Likewise, Lindmark et al did not observe any reduction of REE per unit body cell mass (as assessed using total-body potassium as an index).[375] Other reports by McNeill et al, Carbonnel and a recent study by Ferro-Luzzi et al have also failed to demonstrate metabolic adaptation in stable malnourished patients.[262-264] Similar to our results, a study by Soares and Shetty, demonstrated a tendency to increased REE, expressed per unit active body mass, in undernourished Indian men.[260,261]

Investigating metabolic adaptation, a number of previous studies have related REE to fat free mass (FFM) or active tissue mass (ATM) in order to relate the energy expenditure more directly to the tissues responsible. Ravussin and Bogardus, while confirming the correlation between REE and FFM, have questioned the relationship since the x and y intercept was significantly different to zero.[376]

Our finding of an increased REE, expressed per unit body weight does not necessarily imply reduced metabolic efficiency in the malnourished patients. Shetty has recently pointed out that metabolic rate, expressed per unit body weight, may not reflect true variations in metabolic efficiency, and is likely to be due largely to variations in body composition.[257] Skeletal muscle, although comprising 40-50% of body weight, contributes only 18-22% to the basal metabolic rate.[265,266] Fat, likewise contributes little to metabolic rate. On the other hand, the brain and liver, although comprising only 3-5% of total body weight, utilise 40% of energy.[267] Chronic malnutrition is associated with a disproportionate loss of relatively metabolically inactive fat and muscle tissue, with sparing of the more metabolically active visceral tissue.[257] Our studies related REE to body weight, and as the malnourished patients would be depleted, particularly of fat stores, this would result in an apparent increase in metabolic rate expressed per unit body mass.

There appears, therefore, to be a variable metabolic adaptive response to undernutrition. It has been suggested that the reduction in basal metabolic rate occurs in two phases.[257] Following acute energy restriction, the decrease in metabolic rate is greater than can be attributed to loss of active tissue, providing evidence for increased metabolic efficiency, or metabolic adaptation. After 2-3 weeks, the degree of metabolic adaptation remains constant, and further decrease in metabolic rate is a consequence of loss of active tissue.

Studies of metabolism during recovery from malnutrition have produced conflicting results. High-energy feeding of semi-starved, otherwise healthy, volunteers has been reported as resulting in either a small (± 5%), or no increase in REE per unit surface area, relative to baseline.[17,252] In patients with anorexia nervosa, after 4 weeks of re-feeding, Melchior et al did not observe any change in REE per unit FFM.[377] On the other-hand, Obarzanek, also studying anorexia nervosa patients, reported no significant change in REE per unit FFM after 2 weeks of feeding, whereas after 10 weeks there was a dramatic 37% increase.[378] Piers et al documented a 7% increase of REE per unit FFM in malnourished Indian patients who received energy supplementation for 3 weeks, which increased to 20% after 12 weeks of feeding.[379] REE subsequently decreased to pre-supplementation levels 12 weeks after cessation of supplementation.[380] Conversely, Carbonnel et al recently reported no significant change in REE per unit FFM following a mean gain of 6.5 kg body weight in malnourished patients receiving total parenteral nutrition, although absolute values of REE were increased.[273]

In our patients following the period of nutritional support, although there was no significant change in mean REE expressed in absolute terms, which remained significantly lower than the control value, when expressed per unit body weight, there was now no significant difference compared to controls. This suggests increase in the relatively metabolic inactive fat and protein stores, and a trend to restoration of normal body composition.

5.5.3 Whole-Body Protein Kinetics

The studies demonstrated significantly lower whole-body protein flux, oxidation and synthesis in the malnourished patients, compared to the controls, when considered in absolute terms (g/d). Nutritional support resulted in a significant increase in absolute values of total

body flux and synthesis, however, levels remained significantly lower than control values. Whole-body protein flux, oxidation and synthesis all correlated directly with body weight.

When expressed per kg body weight, however, there was no significant difference in any of the values of the malnourished patients, taken as a group, compared to controls, either before, or after nutritional support.

In comparing the *anorexia patient* group with the *disease patient* group, although there was no significant difference in mean absolute rates of whole-body flux, oxidation and synthesis between the two groups, when expressed per kg body weight the *anorexia patient* group had rates similar to the control subjects, whereas the *disease patient* group had significantly lower protein flux and synthesis rates, than either controls, or the *anorexia patient* group. Following nutritional support, the improvement in total body flux and synthesis was evident principally in the *disease patient* group, with flux and synthesis, expressed per kg body weight, now similar to both the *anorexia patient* group and controls.

Previous studies of the effects of nutritional status on total body protein kinetics have produced apparently conflicting results. Hoffer et al reported a 20-40% reduction in protein flux (expressed per kg body weight per day), as assessed by leucine and alanine tracer infusion, in obese women receiving very low calorie weight reducing diets for three weeks.[269] The same authors also reported a 35% increase in leucine flux, and a 77% increase in leucine oxidation following consumption of a meal in young adult men. A study by Norton et al demonstrated a 23% reduction of whole-body protein synthesis and turnover (g/kg/d) induced by short-term fasting in healthy controls. The authors noted, however, that three malnourished patients without cancer had similar protein synthesis and turnover to the controls, whereas three patients with cancer had marked elevation of synthesis and turnover.

Carbonnel et al also studied a group of malnourished patients with non-neoplastic disease, and although, in comparison to healthy controls, they demonstrated reduced rates of whole-protein synthesis and turnover when expressed in absolute terms (g/d), when expressed in relation to body weight (g/kg/d), the rates were similar.[263] The authors also reported no significant change in whole-body protein kinetics, expressed per kg body weight, following nutritional recovery with total parenteral nutrition, although there was a slight increase when expressed per kg fat free mass.[273] Conversely, Golden, Waterlow and Picou studied five

children with protein-energy malnutrition, and reported whole-body protein turnover, studied with ^{15}H-glycine, to increase 140% during nutritional recovery.[268] It should be noted, however, that these children gained 45% of body weight during 54 ±14 d of feeding, as compared to 18% in the Carbonnel study .

Holt et al reported similar whole-body protein synthesis rates in malnourished cystic fibrosis patients with stable pulmonary disease, compared to normal healthy children.[272] However, patients with active infectious disease had markedly reduced protein synthesis. These results indicated that increased metabolic demands during acute disease, in the absence of sufficient energy, resulted in reduced protein synthesis.

Our study demonstrated significant reduction in whole-body protein turnover and synthesis (g/kg/d) only in patients with co-existent disease (*disease patient* group), whereas chronically malnourished patients without overt disease (*anorexia patient* group) had values similar to healthy controls. Thus, the presence of disease in association with the malnutrition appears to have a significant adverse affect on whole-body flux and synthesis.

CHAPTER 6

GUT MUCOSAL PROTEIN FRACTIONAL SYNTHESIS

6.1 Introduction

> "*... (body) tissues may be divided into three groups. First, one in which they continue to multiply throughout the life of an individual; to this class belong ... epithelial coverings and their glandular prolongations, etc. These are tissues with transient elements (elementi labile).*"

Giulio Bizzozero, Professor of General Pathology, Turin (1894)[381]

The gastro-intestinal epithelium is a prime example of *elementi labile*. Studies in rats, and in man have demonstrated that the entire mucosal lining of the gut is replaced every two to eight days.[382] The rapid cell turnover associated with the continual replenishment of the mucosal surface is likely to impose a considerable demand on the bodies resources,[383] and is therefore likely to be adversely affected by a poor nutritional state. In health, the overall mass of the intestinal mucosa remains remarkably constant, and therefore equilibrium must exist between cell birth in the crypts of Lieberkuhn and cell loss into the lumen.[383] Impaired protein synthesis, and a disproportionate loss of enteric cells may therefore result in villous atrophy, often documented in malnourished patients.

The fractional rates of synthesis (turnover) of body tissue protein, or individual specific proteins, can be assessed from the measurement of the change in incorporation of a labelled amino acid into the protein over time, in relation to the labelling of the precursor pool from which the amino acid is derived. As previously discussed, constant intravenous infusion of an isotope labelled amino acid results in the specific activity of that amino acid in plasma rising rapidly to a plateau (Figure 6.1). The specific activity of the free amino acid in the tissue intracellular pool also rises to a plateau, the level of which is, however, lower than that in the plasma due to dilution of the intracellular pool with amino acid released by protein degradation (Figure 6.1). From the intracellular pool, labelled amino acid is incorporated into tissue protein.

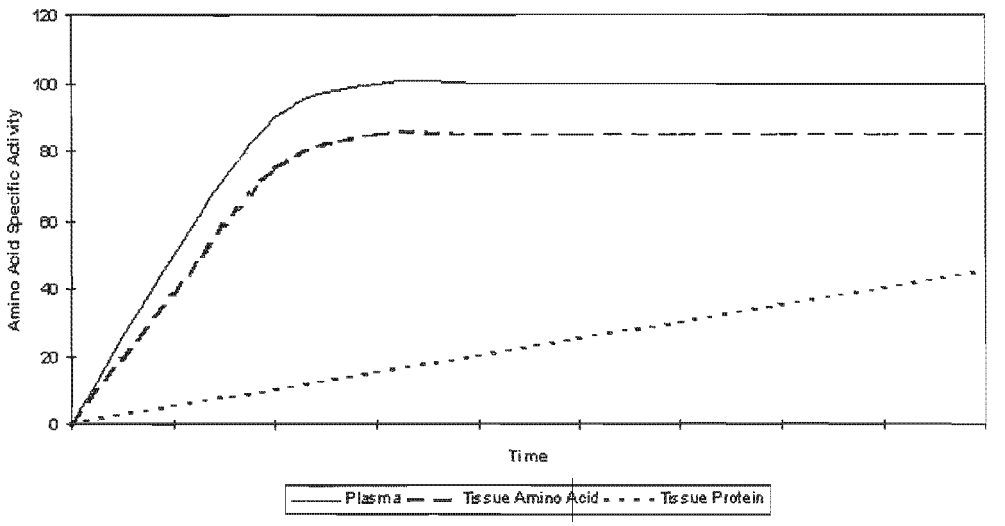


Figure 6.1: The rise of amino acid specific activity in plasma and tissues during constant infusion of radiolabelled amino acid. For amino acids such as leucine, tyrosine and phenylalanine, plateau is reached within 2 hours. Dilution of the tissue amino acid pool with amino acid released from intracellular protein degradation reduces the specific activity. Incorporation of labelled amino acid into tissue protein is linear, allowing for assessment of the fractional synthetic rate.

Following a period of time, incorporation of the amino acid tracer into the tissue protein can be assessed following biopsy, or sampling of the tissue under investigation, precipitation of the protein, and hydrolysis of the protein into its constituent amino acids. The rate of incorporation of amino acids into tissue protein is linear,[318,384] and therefore the fractional synthetic rate of the protein can be determined by directly relating the specific activity of labelled amino acid in the tissue protein with that in the precursor pool and with the duration of the infusion:

$$MPFS = \frac{S_b}{S_p} \times \frac{24}{T} \times 100$$

where MPFS is the mucosal protein fractional synthetic rate (%/day), S_b is the specific activity of protein bound amino acid, S_p is the specific activity of the precursor amino acid pool, and T is the duration of the isotope infusion (hours).

The technique depends on the sensitivity of the methods in accurately measuring the specific activity of the tracer amino acid in the tissue protein. The amount of incorporation of which depends on the rate of turnover of the protein. The faster the turnover, the greater the incorporation, and therefore the shorter duration of infusion required. Studies have reported the measurement of skeletal muscle turnover in man with the investigators performing muscle biopsy following a 6-8 hour intravenous infusion of labelled amino acid.[277,385] Gut mucosal protein has a turnover rate sufficiently fast (approximately 30%/d) to enable measurements to be taken in biopsies obtained following a 4-hour infusion of isotope.[386]

As discussed in Chapter 2.4.1.2, definition of the appropriate precursor amino acid pool for protein synthesis is critical for the accurate assessment of protein turnover. The intracellular aminoacyl-tRNA pool is likely to be the best reflector of the precursor pool, however technical difficulties in measuring this pool have generally precluded its use. Surrogates for this pool have included plasma enrichment (both arterial and venous), plasma α-KIC (in the case of L-[1-^{13}C]leucine tracer, and intracellular enrichment. As discussed previously, the plasma α-KIC is a good surrogate for the intracellular leucine pool. However, a variety of experiments in cultured cells, in perfused or incubated tissues, and in vivo have indicated that the specific activity of the amino acid that is incorporated into tissue protein is not, in general, the same as that in either the plasma or tissue pool.[283] Precursor specific activities have been reported as high as in the plasma,[387] to below the intracellular specific activity.[325,388] Moreover, the enrichment of the direct precursor might be different, not only for different tissues, but also for individual proteins in the same tissue.[389] The situation is further complicated in the gut, where there is evidence that amino acids are efficiently taken up into mucosal protein directly from the gut lumen.[390] However, in the fasted, post absorptive state, this is unlikely to be a significant source of precursor amino acids. Another possible source of inaccuracy is from the recycling of labelled amino acids released from protein degradation following initial synthesis. The length of the infusion of the tracer should only be as long as that required to obtain a reliably measured increase in enrichment of the protein under investigation, and should not be prolonged to the extent that tracer recycling occurs after breakdown of the protein.[283] The uncertainties regarding the appropriate precursor pool for amino acid incorporation into protein do create some ambiguity regarding the measurement of the actual rate of protein synthesis in tissue.

Despite the uncertainties surrounding the absolute values of protein synthesis, as measured by the various methods, there appears to be a remarkably constant relationship between the specific activities of the various pools, which permits some confidence in the interpretation of results when doing comparative work between groups in response to various physiological and pathophysiological conditions, regardless of which pool is measured.[304]

6.2 Aim of Study

To assess the effects of malnutrition, and subsequent re-feeding on gastric and duodenal mucosal protein fractional synthesis.

6.3 Patients and Methods

In order to assess the fractional synthesis (turnover) of mucosal protein in the control subjects and malnourished patients during primed/constant infusion of L-[1-^{14}C]leucine (0.30 uCi/kg + 0.30 uCi/kg/h, an endoscope (Olympus PQ20, Tokyo, Japan) was passed and biopsies taken from the second part of the duodenum, gastric antrum, and gastric body (four biopsies, with approximately 50 mg of tissue obtained from each site). Biopsies were taken prior to initiation of the labelled amino acid infusion, as well as following four hours of the infusion, with the time of infusion carefully noted. The biopsies were immediately washed in normal saline, homogenized, and the protein precipitated in 5 ml 10% trichloroacetic acid (Appendix 7). The precipitated protein was then stored at –80° C for further analysis. The protein was subsequently hydrolysed to the constituent free amino acids, and leucine concentrations measured by high-performance liquid chromatography (HPLC),[24] (Chromjet Integrator, SP8800 Ternary HPLC pump, SpectroSeries UV150 detector; Spectra-Physics, Mountain View, CA) (Appendix 10). Radioactivity due to ^{14}C was measured by dual window liquid scintigraphy counting (Tricarb 1500; Packard Instrument, Downers Grove, IL). The plasma leucine SA was measured as an index the SA of the precursor pool.

6.3.1 Calculations

In view of the linear nature of the uptake of labelled amino acids into proteins, the mucosal protein fractional synthesis (MPFS) or "turnover" rate can be calculated from the relationship between the uptake of isotope into the mucosal protein and the specific activity of the plasma ^{14}C leucine, representing the precursor pool:

$$\text{MPFS (\%/day)} = \frac{\text{SA protein (dpm/mmol)} \times 24 \times 100}{\text{Plasma } ^{14}\text{C leucine SA (dpm/mmol)} \times \text{T (h)}}$$

where MPFS is the mucosal protein fractional synthesis rate (%/d), SA protein is the specific activity of the mucosal protein following the infusion of isotope, less the specific activity prior to infusion, and T is the duration of infusion received prior to the biopsy being taken.

6.4 Results

The results of the gut mucosal protein fractional synthesis (MPFS) studies at entry into the study are illustrated in Table 6.1. The MPFS rates of the malnourished patients were similar to those of the healthy controls (gastric body 33.80 ± 3.53 vs. 34.09 ± 3.18 %/d; gastric antrum 25.27 ± 2.57 vs. 25.52 ± 2.75 %/d; duodenum 26.64 ± 2.08 vs. 25.34 ± 3.24 %/d) (Figure 6.2).

Table 6.1:

Mucosal Protein Fractional Synthesis (MPFS) Rates of Gastric Body, Gastric Antrum, and Duodenum in Controls and in Malnourished Patients Before, and After Nutritional Support.

	Controls	Patients before	Patients after
Gastric Body (%/d)	34.09 (3.18)	33.80 (3.53)	47.51 (7.60)
Gastric Antrum (%/d)	25.52 (2.75)	25.27 (2.57)	31.75 (3.45)
Duodenum (%/d)	25.34 (3.24)	26.64 (2.08)	43.05 (8.41)*#

Results = mean (SEM). * $P < 0.05$ vs. controls, # $P < 0.05$ vs. patients before.

Following nutritional support, there was a significant increase in the mean MPFS rate of the duodenum (43.05 ± 8.41 vs. 26.64 ± 2.08 %/d; $P < 0.05$), to a rate significantly greater than the control value (43.05 ± 8.41 vs. 25.34 ± 3.24 %/d; $P < 0.05$). The same trend was noted in the MPFS rates in the gastric body (47.51 ± 7.60 vs. 33.80 ± 3.53 %/d; $P = 0.08$) and in the gastric antrum (31.75 ± 3.45 vs. 25.27 ± 2.57 %/d), but statistical significance was not achieved (Figure 6.2).

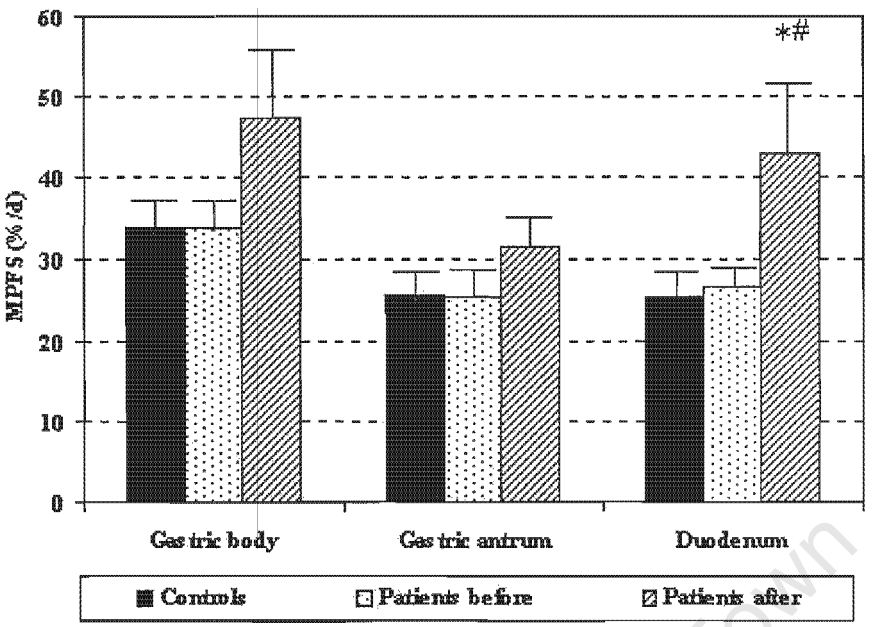


Figure 6.2: Mucosal protein fractional synthesis (MPFS) rates of gastric body, gastric antrum and duodenum in controls, and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.05$ vs. controls, # $P < 0.05$ vs. patients before feeding.

The results of the MPFS studies performed on patients who, at entry into the study, were shown to have normal duodenal morphology with no evidence of disease involvement of the upper gastro-intestinal tract, and those with disease involvement and/or villous atrophy are illustrated in Table 6.2.

Table 6.2:

Mucosal Protein Fractional Synthesis (MPFS) Rates of Gastric Body, Gastric Antrum, and Duodenum in Controls, and Malnourished Patients with Normal Duodenal Morphology and those with Villous Atrophy, Before Nutritional Support.

	Controls	Malnourished Patients	
		Normal morphology	Villous atrophy
Gastric Body (%/d)	34.09 (3.18)	37.85 (4.12)	25.03 (5.54)#
Gastric Antrum (%/d)	25.52 (2.75)	26.45 (3.34)	23.07 (4.14)
Duodenum (%/d)	25.34 (3.24)	28.18 (2.30)	23.30 (6.29)

Results = mean (SEM). # P = 0.09 vs. malnourished patients with normal histology.

Although there was no significant difference in the mean MPFS rates between the two groups prior to nutritional support, there was a trend to lower synthesis rates in the gastric body in patients with villous atrophy (25.03 ± 5.54 vs. 37.85 ± 4.12 %/d; P = 0.09).

Following nutritional support, the group of patient with no evidence of upper gastro-intestinal tract mucosal involvement by disease, and/or villous atrophy, demonstrated a significant increase of MPFS rates in the gastric body (63.55 ± 8.73 vs. 37.85 ± 4.12 %/d; P < 0.01), and in the duodenum (52.46 ±13.16 vs. 28.18 ± 2.30 %/d; P < 0.05), with a trend to an increase in the gastric antrum (36.45 ± 3.65 vs. 26.45 ± 3.34 %/d; P = 0.06) (Table 6.3, Figure 6.3). The post-feeding MPFS rates were all significantly greater than the control values (gastric body 63.55 ± 8.73 vs. 34.09 ± 3.18 %/d; P < 0.001: gastric antrum 36.45 ± 3.65 vs. 25.52 ± 2.75 %/d; P < 0.05: duodenum 52.46 ± 13.16 vs. 25.34 ± 3.24 %/d; P < 0.02). The patients, who, at entry into the study had involvement of the upper gastro-intestinal tract by the primary disease process and/or villous atrophy, did not show any change in MPFS rates following nutritional support.

Table 6.3:

Mucosal Protein Fractional Synthesis (MPFS) Rates of Gastric Body, Gastric Antrum, and Duodenum in Controls, and Malnourished Patients with Normal Duodenal Morphology and those with Villous Atrophy at Entry, After Nutritional Support.

	Controls	Malnourished Patients	
		Normal morphology	Villous atrophy
Gastric Body (%/d)	34.09 (3.18)	63.55 (8.73)*	26.12 (6.97)#
Gastric Antrum (%/d)	25.52 (2.75)	36.45 (3.65)**	24.70 (5.90)
Duodenum (%/d)	25.34 (3.24)	52.46 (13.16)**	28.95 (3.65)

Results = mean (SEM). * P = 0.01 vs. controls, ** P < 0.05 vs. controls, # P < 0.01 vs. malnourished patients with normal histology.

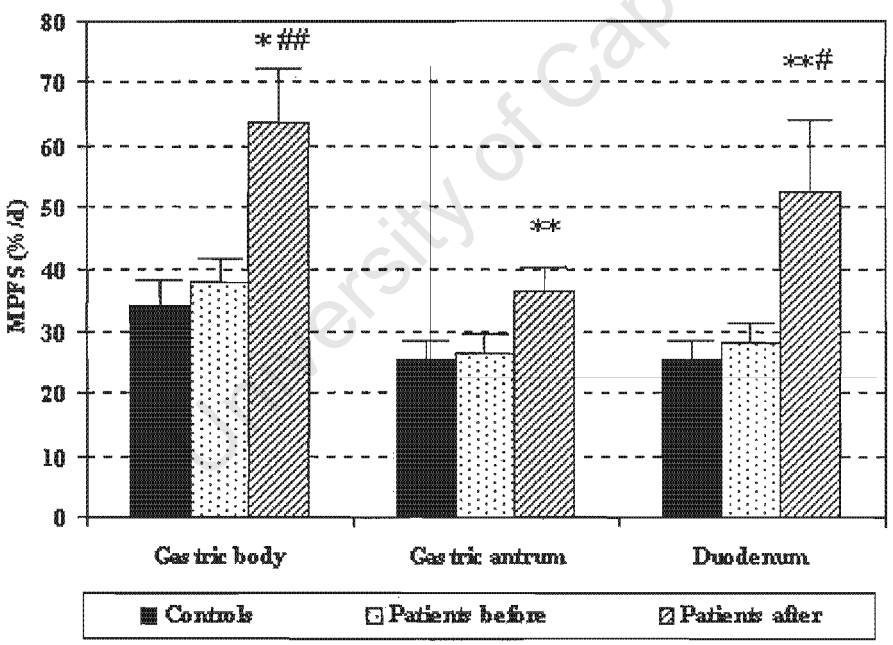


Figure 6.3: Mucosal protein fractional synthesis (MPFS) rates of gastric body, gastric antrum and duodenum in controls, and malnourished patients without evidence of upper gastro-intestinal tract disease and/or villous atrophy, before, and after nutritional support. Bars represent means (SEM). * P < 0.01 vs. controls, ** P < 0.05 vs. controls, # P < 0.05 vs. patients before feeding, ## P < 0.01 vs. patients before feeding.

Comparison of the MPFS rates of the *anorexia patient* group with the *disease patient* group showed the rates in the gastric antrum and duodenum to be significantly less in the *disease patient* group (gastric antrum, 21.26 ± 2.25 vs. 34.62 ± 5.28 %/d; $P < 0.02$: duodenum, 23.05 ± 1.59 vs. 34.41 ± 4.30 %/d; $P < 0.01$), although the rates in both groups were not significantly different to the control values (Table 6.4).

Table 6.4:

Mucosal Protein Fractional Synthesis (MPFS) Rates of Gastric Body, Gastric Antrum, and Duodenum in Controls, *Anorexia Patient* Group, and *Disease Patient* Group Before Nutritional Support.

	Controls	Anorexia patients	Disease patients
Gastric Body (%/d)	34.09 (3.18)	37.34 (2.29)	32.17 (5.05)
Gastric Antrum (%/d)	25.52 (2.75)	34.62 (5.28)	21.26 (2.25)*
Duodenum (%/d)	25.34 (3.24)	34.41 (4.30)	23.05 (1.59)*

Results = mean (SEM). * $P < 0.05$ vs. *anorexia patient* group.

Following nutritional support, the MPFS rates in the *anorexia patient* and *disease patient* groups were similar (Table 6.5).

Table 6.5:

Mucosal Protein Fractional Synthesis (MPFS) Rates of Gastric Body, Gastric Antrum, and Duodenum in Controls, *Anorexia Patient* Group, and *Disease Patient* Group After Nutritional Support.

	Controls	Anorexia patients	Disease patients
Gastric Body (%/d)	34.09 (3.18)	54.05 (4.55)*	44.89 (10.56)
Gastric Antrum (%/d)	25.52 (2.75)	36.03 (6.59)	30.20 (4.13)
Duodenum (%/d)	25.34 (3.24)	36.65 (1.30)	45.38 (11.53)**

Results = mean (SEM). * $P = 0.01$ vs. controls, ** $P = 0.06$ vs. controls.

6.5 Discussion

There are relatively few studies of gastro-intestinal mucosal protein synthesis in human subjects. Recent studies by Rittler et al, using stable isotope (^{13}C-leucine) infusion techniques, demonstrated an ileal mucosal protein fractional synthesis (MPFS) rate of 14 %/d,[391] and a colonic MPFS rate of 18 %/d in normal healthy controls.[392] Using intracellular leucine enrichment as an index of the precursor pool, Nakshabendi reported a duodenal MPFS rate of 62 %/h. Bouteloup-Demange et al reported a rate of 48 %/d using intracellular leucine enrichment as an index of the precursor pool, and 24.0 %/d if plasma KIC was used.[393] A recent study by Charlton, Ahlman and Nair reported a rate of 32 %/d using plasma KIC.[394] O'Keefe demonstrated a MPFS rate of 39 %/d in duodenal mucosa, and a rate of 57 %/d in gastric antral musosa.[386] There does appear to be considerable variation in the rates of fractional synthesis of mucosal protein reported in the various studies, and Bouteloup-Demange and colleagues have commented that the absolute rates of fractional synthetic rates of gut mucosal protein should be interpreted with caution, as they do appear to depend on the precursor pool chosen for the analysis, the route of the tracer administration, and the tracer itself.[393] The situation may be complicated by the direct uptake of luminal amino acids for protein synthesis by the enteric mucosa.[395] However, in the fasted state, the major source of precursor amino acids is the plasma, and a recent study by Bouteloup-Delange reported no significant effect of feeding on gut mucosal protein synthesis, as assessed by an intravenous tracer.[393]

In our study of normal healthy control subjects, we demonstrated a mean MPFS rate of 34.09 %/d in the gastric body, 25.52 %/d in the gastric antrum, and 25.34 %/d in the duodenum. These figures indicate that the gastric and duodenal mucosa is being replaced, or "turned-over" every 3-4 days. In the malnourished patients, prior to nutritional support, despite nine of the twenty-five patients studied having histological evidence of mucosal villous atrophy, MPFS rates were similar to the controls. There was no significant difference between the rates in those malnourished patients with normal duodenal morphology, and those with villous atrophy.

Although the study did not demonstrate a significant difference to controls, the *disease patient* group had significantly lower MPFS rates of the duodenum and antrum, compared to

the *anorexia patient* group, suggesting that the co-existence of disease states with malnutrition may have an adverse effect on the protein synthesis in the tissues.

Following nutritional support, there was a significant increase in the mean MPFS rate in the duodenum, to a level significantly greater than the control value, with a trend to an increase of that of the gastric body. These changes were evident primarily in those patients who were shown not to have involvement of the upper gastro-intestinal tract by disease and/or villous atrophy at entry into the study. In this group of patients, nutritional support resulted in a marked increase in mucosal protein fractional synthesis rates, with that of the duodenum increasing 86% from pre-feeding values, and that of the gastric fundus increasing 68%. These levels were significantly greater than the control values. These changes are likely to reflect the trophic effects on the gastro-intestinal mucosa of enteral feeding received during the period of nutritional support. A previous study by Bouteloup-Demange failed to demonstrate any effect of feeding on duodenal MPFS in normal healthy individuals.[393] The subjects in this study, however, were studied after only ten days of a "standardised diet", and therefore reflect only short-term variations in nutritional state. Furthermore their subjects were of normal nutritional status.

The studies in malnourished patients with villous atrophy, four of whom had primary disease involving the duodenum, which was still evident following nutritional support, demonstrated no change from the pre-feeding values. The trophic effect of the enteral nutrition on mucosal protein synthesis was, therefore, only evident in those patients with normal enteric morphology.

These studies indicate that in humans, despite severe malnutrition, gut mucosal protein fractional synthesis remains essentially normal, although there was a suggestion that the presence of co-existent disease may have an adverse effect on protein synthesis. Investigations following the period of nutritional support of malnourished patients indicate that, providing the upper gastro-intestinal tract is free of disease, a period of intensive enteral feeding results in a potent trophic effect on mucosal protein synthesis.

CHAPTER 7

GASTRIC ACID AND PANCREATIC ENZYME SECRETION

7.1 Introduction

Initiation of the digestive process, and prevention of bacterial contamination of the small bowel is dependent on the secretion of gastric acid, and completion of digestion within the lumen of the bowel requires the adequate production of enzymes by the pancreas. Malnutrition is associated with villous atrophy and impaired gut mucosal function, and may therefore result in reduced gastric and pancreatic secretion, subsequent to an impaired mucosal response to nutrients within the lumen. In addition, severe malnutrition may have a direct effect on gastric parietal and pancreatic acinar cell synthetic function. Impaired gastro-pancreatic secretion associated with undernutrition may therefore adversely affect digestive function.

7.2 Aim of Study

To determine the functional consequences of malnutrition, and subsequent re-feeding, on gastric acid and pancreatic enzyme secretion, and to relate these to digestive function.

7.3 Patients and Methods

Following an overnight fast, subjects were provided with light sedation with midazolam 2.5 mg, and underwent intubation with a 3-lumen oro-enteric tube using endoscopic guidewire techniques and fluoroscopic control. The multi-lumen tube was specifically constructed for the tests, and consisted of two 14 FG radio-opaque aspiration tubes, and an 8 FG radio-opaque infusion tube bonded together. The distal port of the tube was positioned in the jejunum approximately 25 cm distal to the ampulla of Vater, the middle port at the level of

the ampulla, and the proximal port in the gastric antrum. (Figure 7.1) Successful intubation was achieved in all subjects.

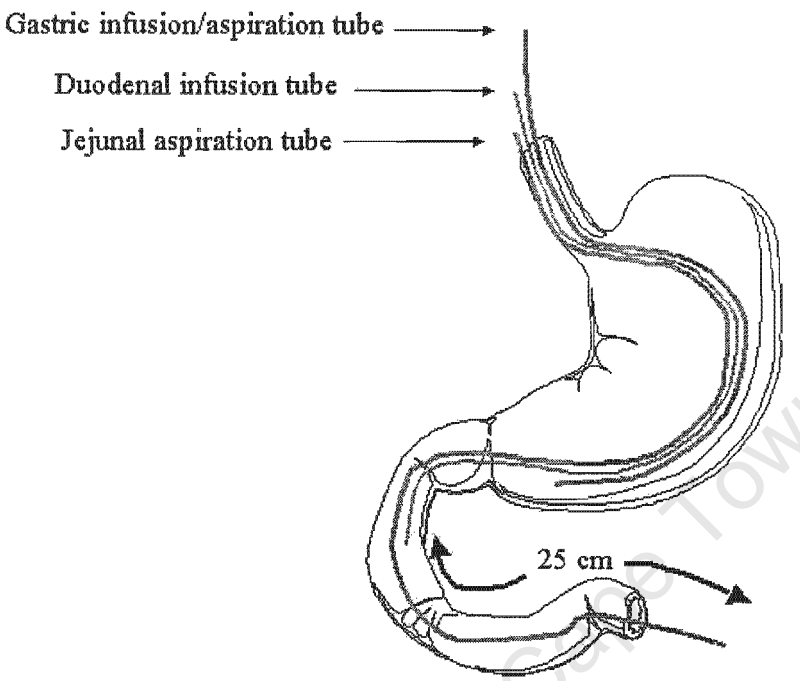


Figure 7.1: Positioning of the multi-lumen oro-enteric tube, with one lumen positioned in the gastric antrum, one at the level of the ampula (mid-duodenum), and one in the proximal jejunum.

Assessment of gastric acid and pancreatic enzyme secretion was performed under conditions of direct hormonal (pentagastrin and cholecystokinin) stimulation. In order to investigate the relative contribution of gut mucosal response to gastric acid and pancreatic enzyme secretion, enteral meal stimulation tests of gastro-pancreatic secretion were also performed.

7.3.1 Hormone Stimulation of Gastric Acid and Pancreatic Enzyme Secretion

Following a 30-minute baseline collection, stimulation of gastric acid secretion was provided by a 1-hour intravenous infusion of pentagastrin 6 μg/kg. Pancreatic exocrine secretion was stimulated by a continuous infusion of CCK-8 at a rate of 40 ng/kg/h. Continuous aspiration of the gastric port enabled collection of gastric acid secretion during pentagastrin

administration, and also prevented contamination of duodenal secretion with acid for the remainder of the study. Low-pressure suction (-60 to -80mmHg) was applied to the jejunal port for a period of 4 hours, with the secretions collected on ice and aliquots collected every 30 minutes. In order to assess, and correct for completeness of collection of the duodenal juice, PEG 4000 5g/l in normal saline was infused at a rate of 300 ml/h though the port adjacent to the ampulla.

The procedure was generally well tolerated, but had to be discontinued prematurely in 3 subjects (1 control subject and 2 undernourished patients) due to nausea and retching induced by the presence of the oro-enteric tubes. One was discontinued after 2 hours, one after 3 hours, and the third after 3.5 hours. All provided sufficient samples for analysis.

7.3.2 Enteral Meal Stimulation of Gastric Acid and Pancreatic Enzyme Secretion

Following a 30-minute baseline collection, a 200 ml bolus of a polymeric feeding formula (Osmolyte HN, Ross Laboratories, Columbus, Ohio) was infused via the gastric port into the stomach. The formula was constituted with normal saline and provided 80 mg/kg fat, 100 mg/kg protein, and 330 mg/kg carbohydrate. In order to measure, and correct for losses through the pylorus, the non-absorbable marker polyethylene glycol, molecular weight 4000 (PEG 4000) 5g/l was added to the infusion solution. Gastric aspiration was discontinued for a period of 15 minutes, and then recommenced with gastric contents separated into four 15-minute collections. Gastric aspirations were then assessed for acid and PEG content.

Enteral pancreatic stimulation was provided by a continuous infusion of Osmolyte HN at a rate of 300 ml/hr via the duodenal port adjacent to the ampulla. The formula was constituted with normal saline and provided 120 mg/kg/h fat, 150 mg/kg/h protein, 500 mg/kg/h carbohydrate with an overall energy infusion of 3.7 kcal/kg/h. To assess and correct for loss of duodenal content beyond the duodenal aspiration port, PEG 4000 at a concentration of 5 g/l was also included in the enteral feed solution. Low-pressure suction (-60 to -80mmHg) was applied to the jejunal port for a period of 4 hours, with the secretions collected on ice and aliquots collected every 30 minutes. Continuous gastric aspiration was continued throughout the study to prevent contamination of the duodenal juice with gastric secretions.

7.3.3 Measurement of Gastric Acid and Pancreatic Enzyme Secretion

Gastric acid output was determined by titrametric analysis of the gastric juice. Pancreatic enzyme activities in the duodenal juice were assessed on the day of study, using standard laboratory techniques.[396] Amylase activity was determined according to the method of Pimstone (Appendix 3),[397] using soluble starch as substrate; lipase by the method of Weber,(Appendix 4)[398] using olive oil as substrate; and trypsin by that of Schwert and Takenaka (Appendix 5),[399] using N-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) as substrate. Concentrations of the polyethylene glycol (PEG) in the gastric and duodenal juice were measured according to the method of Hyden (Appendix 2).[361] PEG is not absorbed from the gastro-intestinal tract. This feature makes the compound useful for accurately determining fractional collection of gastro-intestinal secretions. Following infusion of a known quantity of PEG into the upper gastro-intestinal tract, determination of the recovery of the PEG in the aspirated juice allowed assessment, and correction for gastric and duodenal secretions which had escaped aspiration. Gastric acid and pancreatic enzyme output was calculated according to the formula:

$$\text{Acid/Enzyme output}_{\text{corrected}} = \frac{\text{PEG}_{\text{In}}}{[\text{PEG}]_{\text{Out}}} \times [\text{Acid/Enzyme}]_{\text{Out}}$$

where acid/enzyme $\text{output}_{\text{corrected}}$ is the corrected acid output (mEq/h) or enzyme output (U/h), PEG_{in} is the quantity of PEG infused (g/h), $[\text{PEG}]_{\text{out}}$ is the concentration of PEG in the aspirated gastric or duodenal juice (g/l), and $[\text{acid/enzyme}]_{\text{Out}}$ is the concentration of acid (mEq/l) or enzyme (g/l) in the aspirated gastric or duodenal juice.

7.4 Results

7.4.1 Gastric Acid Secretion

7.4.1.1 Basal Acid Output

Gastric acid studies were performed in twenty-three malnourished patients, and in fifteen normal healthy controls. The malnourished patients had evidence of basal hypochlorhydria, with a mean basal acid output (BAO) of 0.45 ± 0.21 mEq/h compared to 3.33 ±0.76 mEq/h in the healthy controls ($P < 0.001$). The BAO of the *disease patient* group was similar to that of the *anorexia patient* group (0.52 ± 0.20 vs. 0.20 ± 0.20 mEq/h).

Following the period of re-feeding, the mean BAO of the undernourished patients was 0.82 ± 0.33 mEq/h, which was not significantly different to the level before nutritional support, and remained significantly lower than the control value ($P < 0.01$) (Figure 7.2). The mean post-feeding BAO of the *disease patient* group was similar to that of the *anorexia patient* group (0.91 ± 0.39 vs. 0.30 ± 0.30 mEq/h).

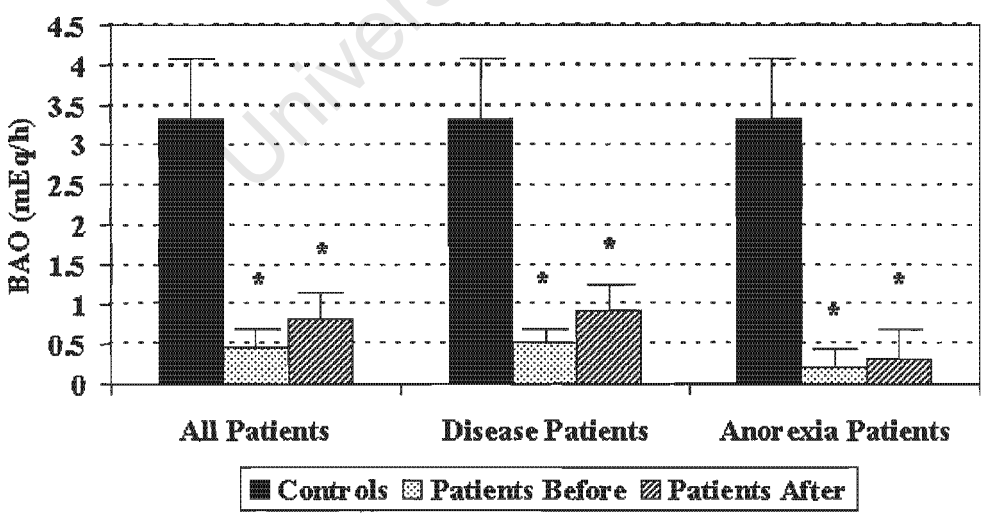


Figure 7.2: Basal acid output (BAO) in controls, and in malnourished patients (all patients, *disease patient* group, and *anorexia patient* group) before, and after nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. controls.

7.4.1.2 Hormone (Pentagastrin)-Stimulated Acid Output

Pentagastrin-stimulated maximum acid output (MAO) was determined in sixteen malnourished patients (*anorexia patients* n = 5, *disease patients* n = 11), and in eight controls. The mean MAO of the undernourished patients was 6.66 ± 1.21 mEq/h, and was significantly impaired compared to the mean control value of 25.53 ± 4.58 ($P < 0.001$) (Figure 7.3). The mean MAO of the *disease patient* group was significantly lower than that of the *anorexia patient* group (5.04 ± 1.31 vs. 10.24 ± 1.83 mEq/h; $P < 0.05$), and both were significantly lower than the control values (*disease patient* group 5.04 ± 1.31 vs. 25.53 ± 4.58 mEq/h; $P < 0.001$: *anorexia patient* group 10.24 ±1.83 vs. 25.53 ± 4.58 mEq/h; $P < 0.03$)

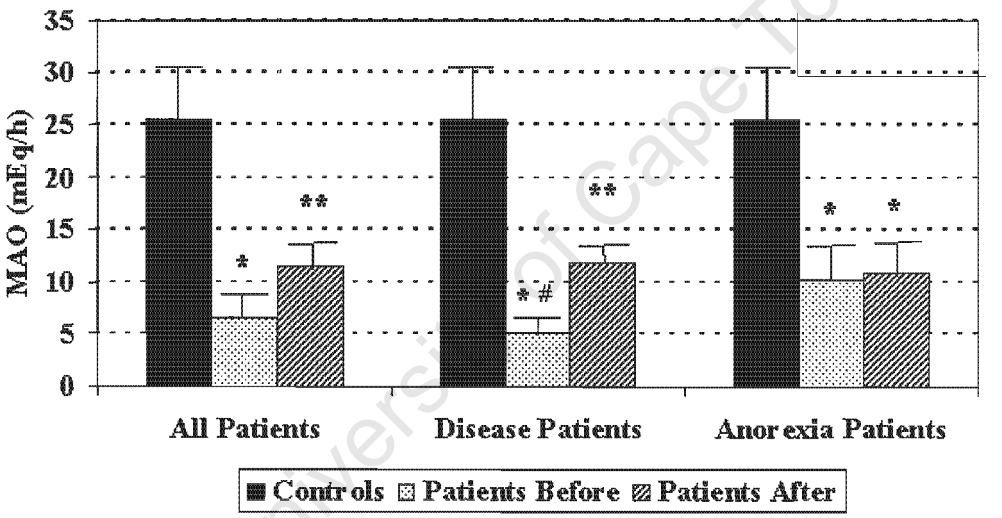


Figure 7.3: Pentagastrin stimulated maximum acid output (MAO) in controls, and in malnourished patients (all patients, *disease patient* group and *anorexia patient* group) before, and after nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. controls; # $P < 0.05$ vs. *anorexia patient* group before nutritional support; ** $P < 0.02$ vs. patients before nutritional support, and controls

There was a direct correlation between nutritional status (BMI) and both BAO and pentagastrin stimulated MAO ($P < 0.001$) (Figures 7.4-5).

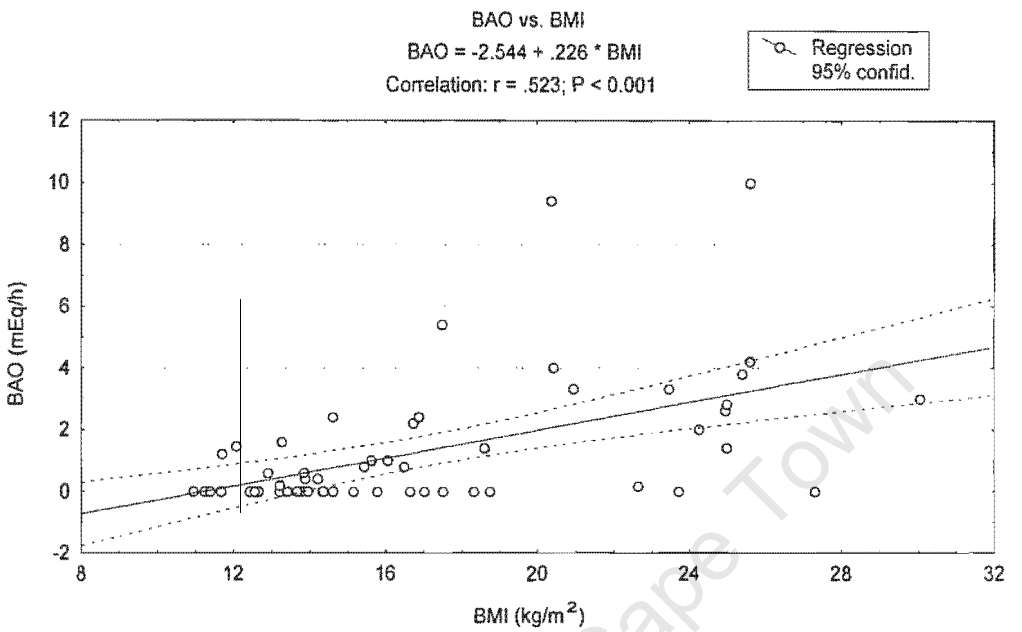


Figure 7.4: Correlation between basal acid output (BAO) and body mass index (BMI).

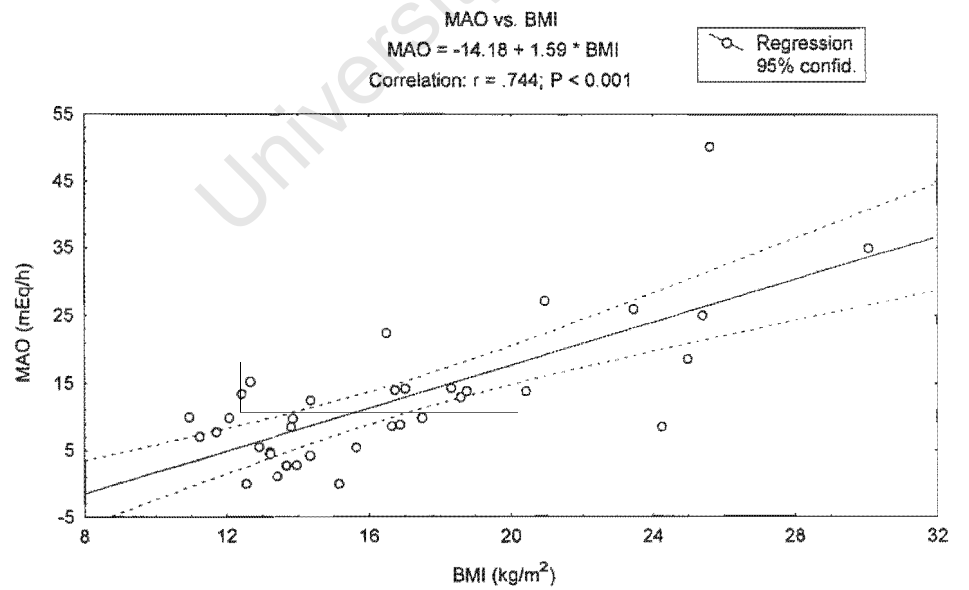


Figure 7.5: Correlation between maximum acid output (MAO) and body mass index (BMI).

Following the period of nutritional support, the mean MAO of the malnourished patients improved significantly to 11.49 ± 1.38 mEq/h; $P < 0.02$, but remained significantly impaired compared to the control values (11.49 ± 1.38 vs. 25.53 ± 4.58 mEq/h; $P < 0.01$) (Figure 7.3). The post-feeding MAO of the *disease patient* group was similar to that of the *anorexia patient* group (11.85 ±1.98 vs. 10.78 ±1.54 mEq/h).

7.4.1.3 Enteral Meal-Stimulated Acid Output

In order to investigate the contribution of an impaired gastric mucosal response to luminal nutrients to the impaired gastric acid secretion in the subjects, enteral meal-stimulated gastric acid studies (ES) were performed in seven healthy controls, and in seven malnourished patients. The results were evaluated in comparison with controls and the malnourished patients who received hormone stimulation (HS) with pentagastrin.

The group of control subjects who received ES were matched to the group, which received hormone stimulation with pentagastrin (HS) as regards age (28,7 ± 1.92 vs. 27.6 ± 1.57 y) and body mass index (24.22 ± 0.85 vs. 23.36 ± 1.09 kg/m^2). Basal acid output was similar in the two groups (2.73 ± 1.28 vs. 3.85 ± 0.93 mEq/h). However, acid secretion stimulated by pentagastrin was significantly greater than that stimulated by the enteral meal (25.53 ± 4.58 vs. 10.90 ± 1.33 mEq/h)

None of the patients in the *anorexia patient* group received enteral meal-stimulation, and therefore the primary comparison between enteral meal-stimulation (ES) and hormone (pentagastrin)-stimulation (HS) was performed in patients from the *disease patient* group. The mean BMI of the undernourished patients who received ES was similar to that of the HS group (13.31 ± 0.60 vs. 13.99 ± 0.44 kg/m^2). The ES group consisted of patients with immunoproliferative small intestinal disease (n = 2), tuberculosis (n=2), amyloidosis (n = 1), carcinoma of the lung (n = 1), and Crohn's disease (n = 1). The HS group consisted of patients with Crohns disease (n = 8), tuberculosis (n = 2), short bowel after trauma (n = 1), and metastatic melanoma (n = 1). None of the patients had evidence of primary disease of the stomach.

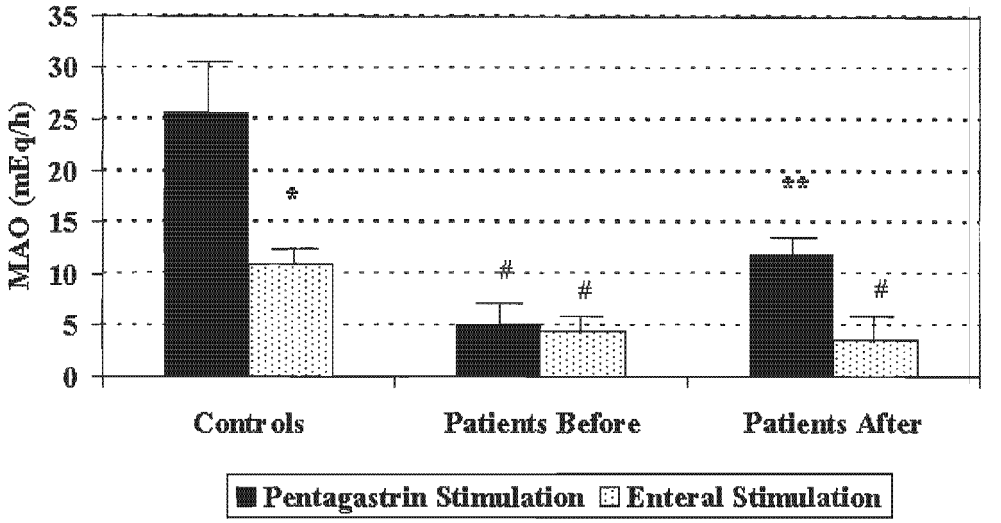


Figure 7.6: Hormone (pentagastrin)- versus enteral meal-stimulation of gastric acid secretion in controls, and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. pentagastrin stimulation; # $P < 0.01$ vs. controls; ** $P < 0.02$ vs. patients before nutritional support, and vs. controls.

Acid output in the ES malnourished group was similar to that in the HS malnourished group (4.39 ± 0.72 vs. 5.04 ± 1.31 mEq/h), and was significantly impaired compared to the ES control group (4.39 ± 0.72 vs. 10.90 ± 1.32 mEq/h; $P < 0.01$) (Figure 7.6). The ES and HS groups received similar periods of re-feeding, with a mean of 6.6 ± 1.03 weeks (range 2 weeks to 3 months). Following the period of nutritional support, there was no change in the acid output of the ES group, which remained significantly impaired compared to the control group (3.52 ± 1.60 vs. 10.90 ± 1.32 mEq/h; $P < 0.01$). There was a significant improvement in acid output in the HS group (11.85 ± 1.98 vs. 5.04 ± 1.31 mEq/h; $P < 0.02$), but the level remained lower than the control value (11.85 ± 1.98 vs. 25.53 ± 4.58 mEq/h; $P < 0.01$).

7.4.2 Pancreatic Enzyme Secretion

Cholecystokinin (CCK-8)-stimulated pancreatic function tests were performed on eighteen undernourished patients. The group consisted of patients with anorexia (n = 6), Crohns disease (n = 8), tuberculosis (n =2), short bowel after trauma (n = 1), and metastatic melanoma (n = 1). In addition, enteral meal-stimulated pancreatic function tests were

performed on the seven malnourished patients who underwent enteral meal-stimulated gastric acid studies. Results were evaluated in comparison to ten normal healthy controls who underwent CCK-8 stimulation, and seven who received enteral meal stimulation.

The profile of secretion of amylase in normal healthy controls over the four-hour study period is illustrated in Figure 7.7.

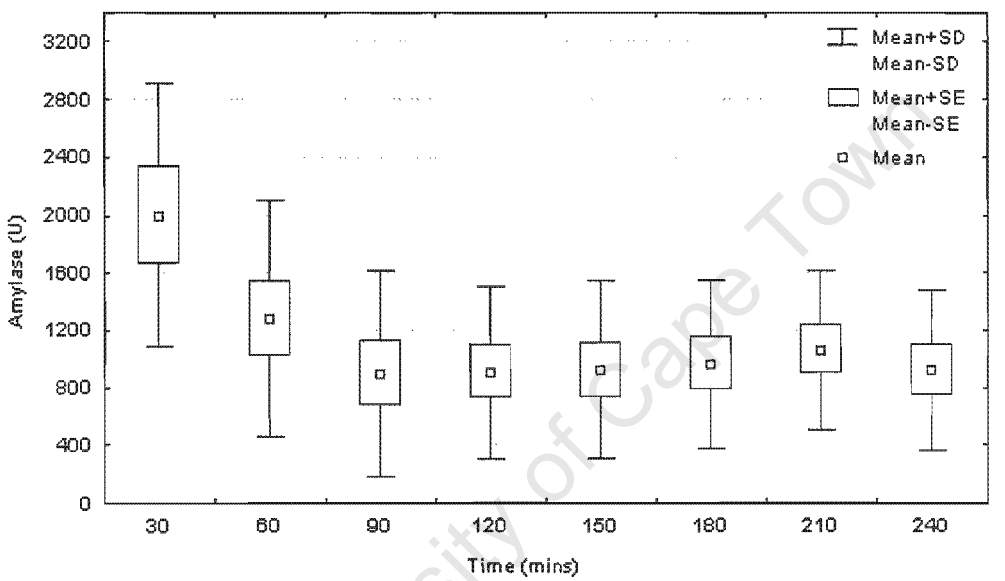


Figure 7.7: Amylase output (U/30mins) in normal healthy controls.

Secretion of the enzyme was higher during the first 30-60 minutes of stimulation ($P < 0.02$), with the output during the remainder of the study remaining constant.

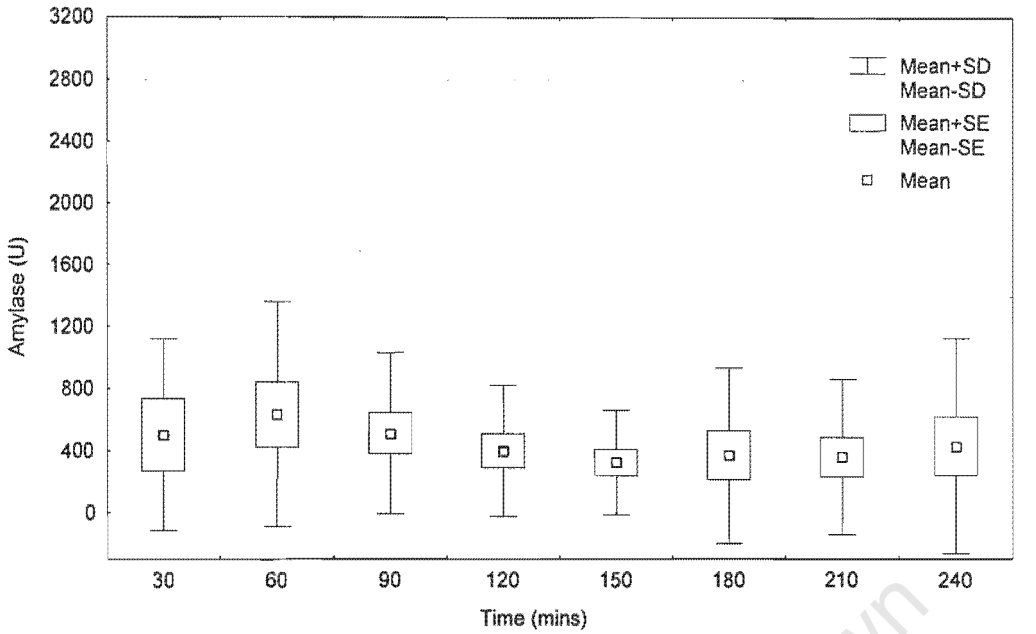


Figure 7.8: Amylase output (U/30mins) in malnourished patients before nutritional support.

The malnourished patients, prior to nutritional support, had a constant output of enzyme throughout the course of the study (Figure 7.8). Following nutritional support, there was a suggestion of an increased secretion during the first 30 minutes of the study, however the difference did not reach significance (P = 0.06) (Figure 7.9).

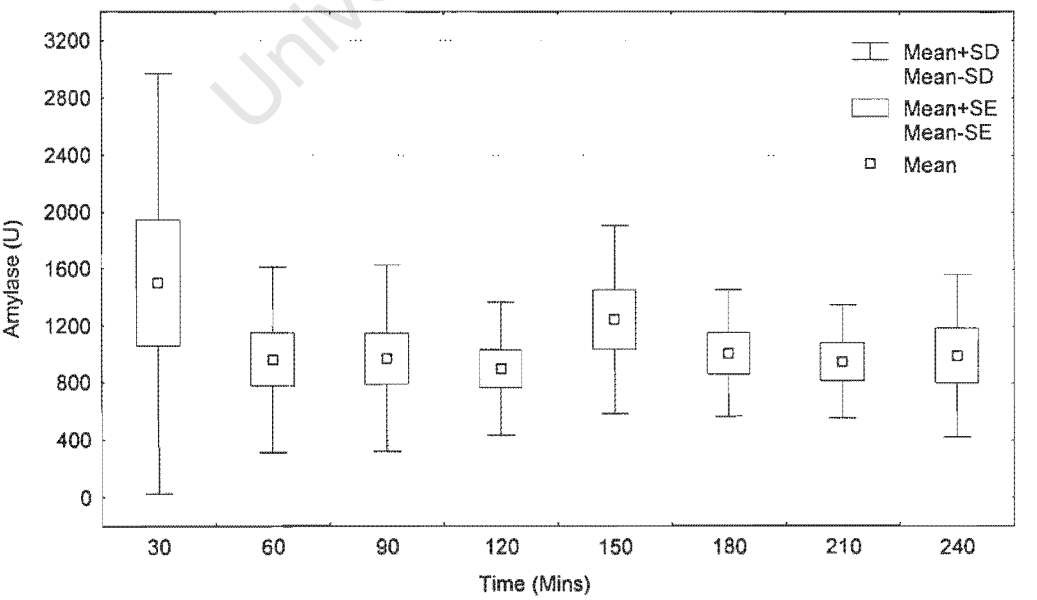


Figure 7.9: Amylase output (U/30mins) in malnourished patients after nutritional support.

Lipase and trypsin secretion profiles in the controls demonstrated similar patterns to that of amylase, with significantly higher outputs during the first 30-60 minutes ($P < 0.05$), and a constant secretion thereafter (Figures 7.10-11).

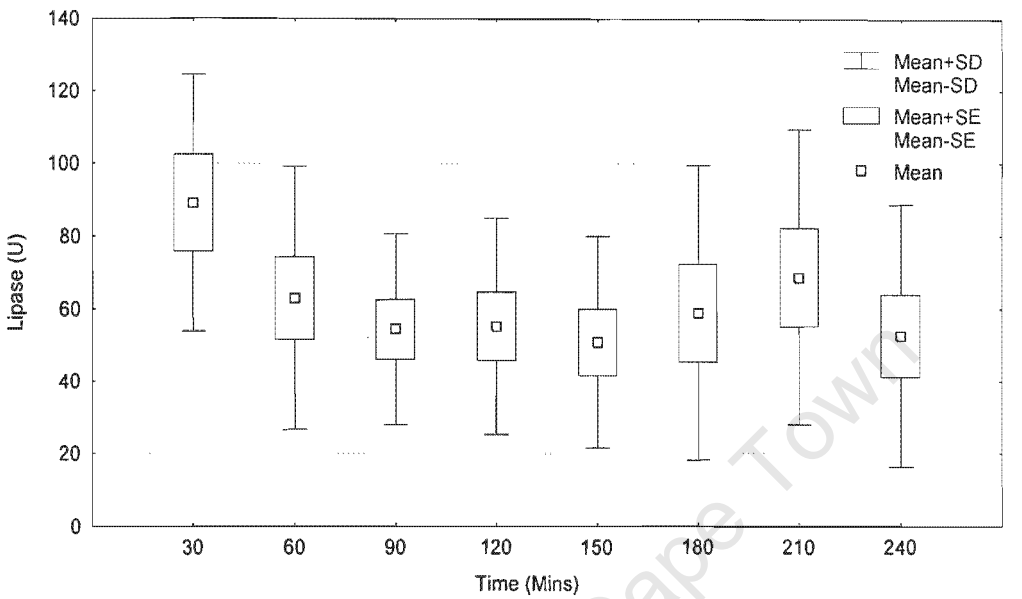


Figure 7.10: Lipase output (U/30mins) in normal healthy controls.

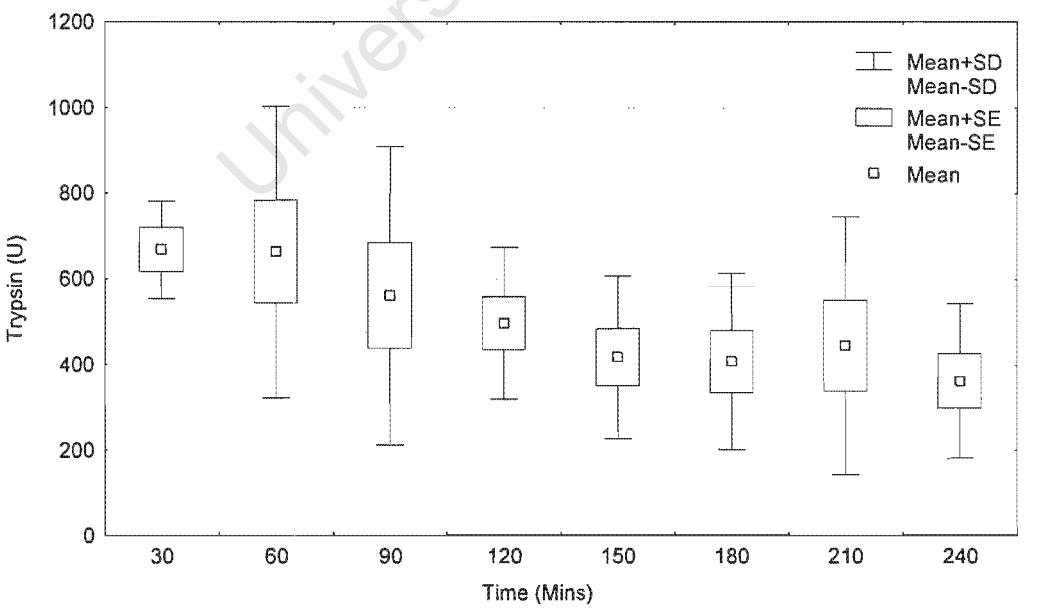


Figure 7.11: Trypsin output (U/30mins) in normal healthy controls.

The malnourished patients, both before, and after feeding, demonstrated a relatively constant secretion of lipase and trypsin throughout the course of the study (Figures 7.12-15).

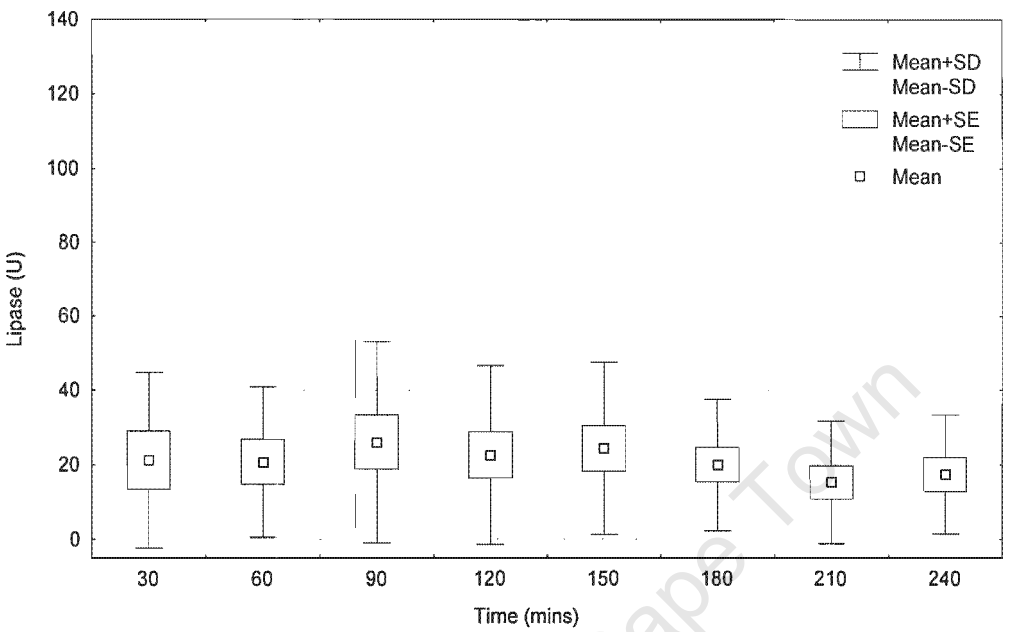


Figure 7.12: Lipase output (U/30mins) in malnourished patients before nutritional support.

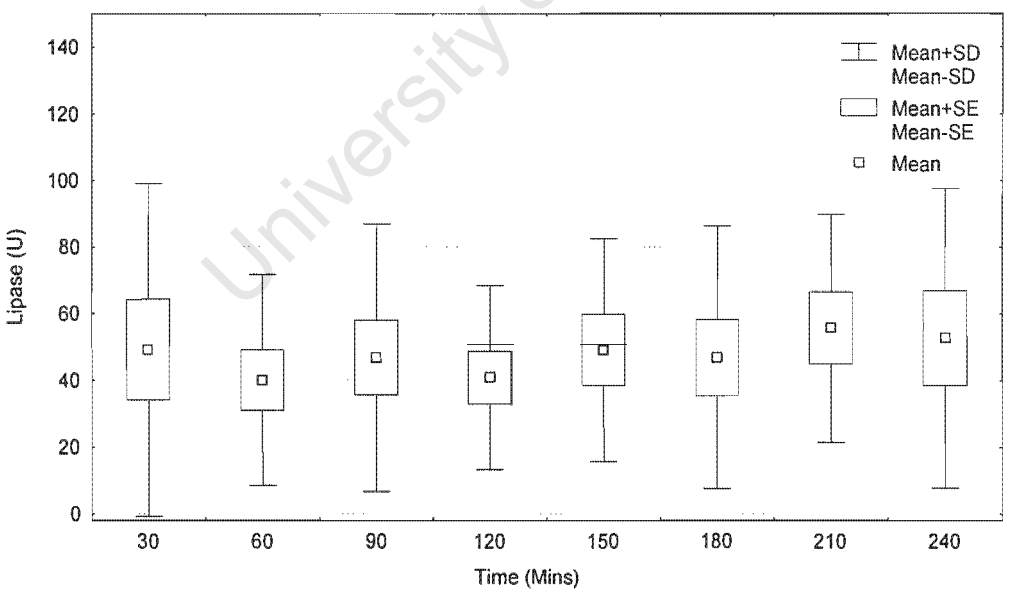


Figure 7.13: Lipase output (U/30mins) in malnourished patients after nutritional support.

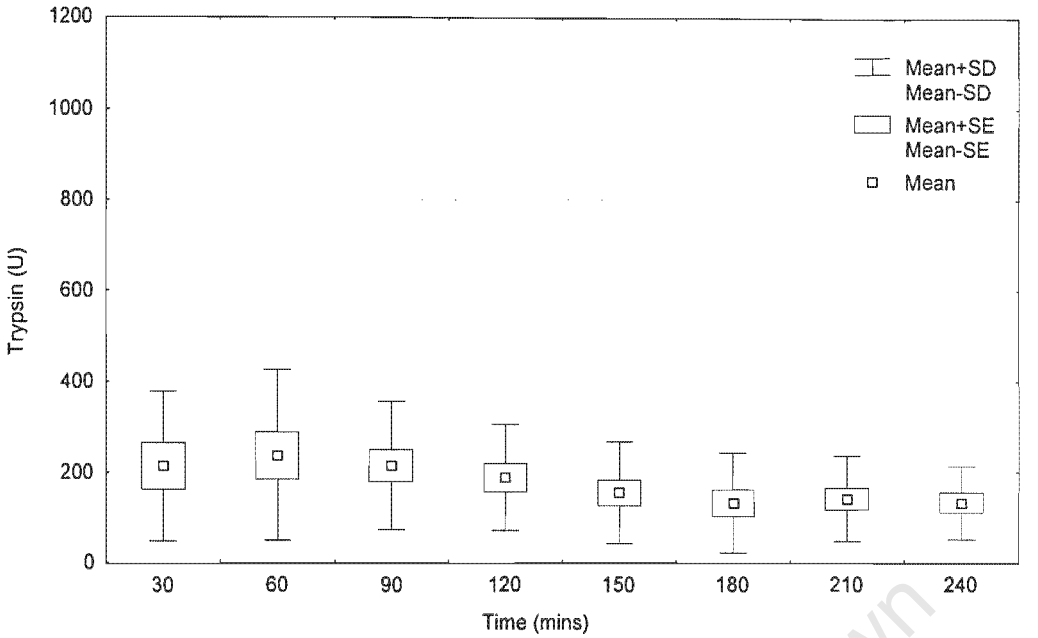


Figure 7.14: Trypsin output (U/30mins) in malnourished patients before nutritional support.

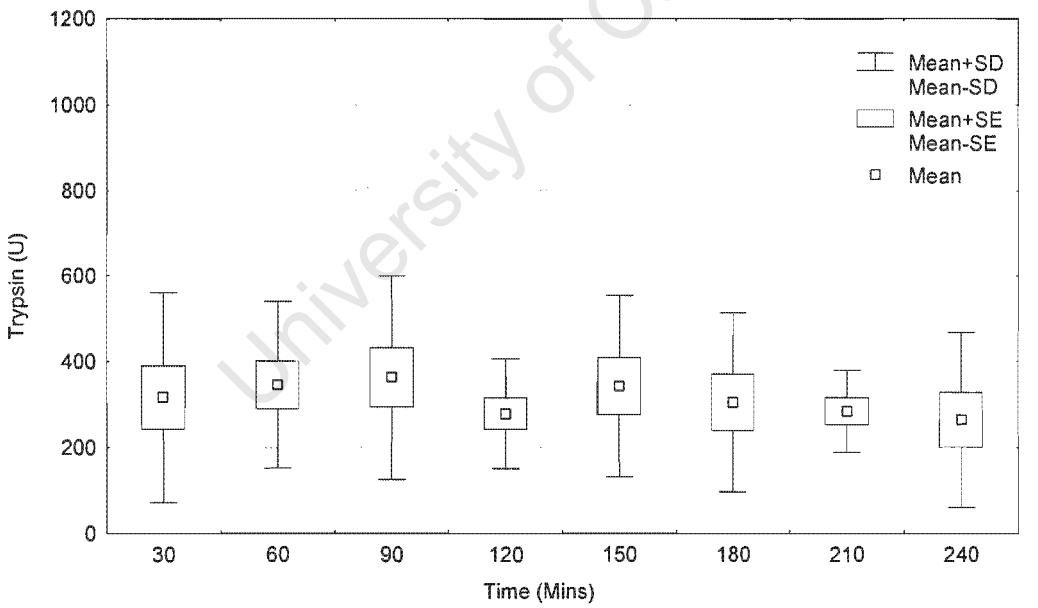


Figure 7.15: Trypsin output (U/30mins) in malnourished patients after nutritional support.

7.4.2.1 Cholecystokinin (CCK-8)-Stimulated Pancreatic Enzyme Secretion

The results of the CCK-8-stimulated pancreatic function tests performed in the eighteen malnourished patients are illustrated in Figure 7.16. Pancreatic enzyme secretion was significantly reduced compared to the healthy controls. Mean amylase output was 827.8 ± 165.0 vs. 2305 ± 346.4 U/h; $P < 0.001$, lipase 41.46 ± 9.21 vs. 118.6 ± 18.89 U/h; $P < 0.001$, and trypsin 122.7 ± 16.39 vs. 341.4 ± 40.80 U/h; $P < 0.001$.

Fourteen patients consented to further study following the period of intensive nutritional support. Mean amylase output in the malnourished group increased significantly to 2091 ± 263.8 U/h; $P < 0.001$, and the lipase to 101.5 ±19.81 U/h; $P < 0.01$. These levels were similar to the control values. There was a significant increase in the mean trypsin output to 208.7 ± 26.09 U/h; $P < 0.01$, however this output remained significantly lower than the control value of 341.4 ± 40.80 U/h; $P < 0.01$.

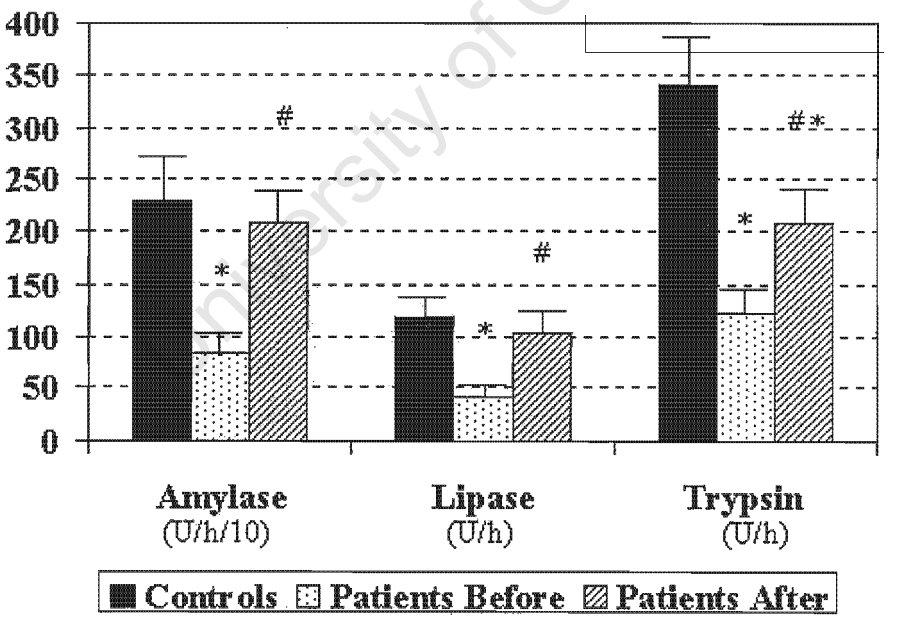


Figure 7.16: Cholecystokinin (CCK-8)-stimulated pancreatic amylase, lipase and trypsin outputs of controls, and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. controls; # $P < 0.01$ vs. patients before nutritional support.

There was a direct correlation between the outputs of the pancreatic enzymes amylase, lipase, and trypsin in the malnourished patients before, and after nutritional support (Figures 7.17-19).

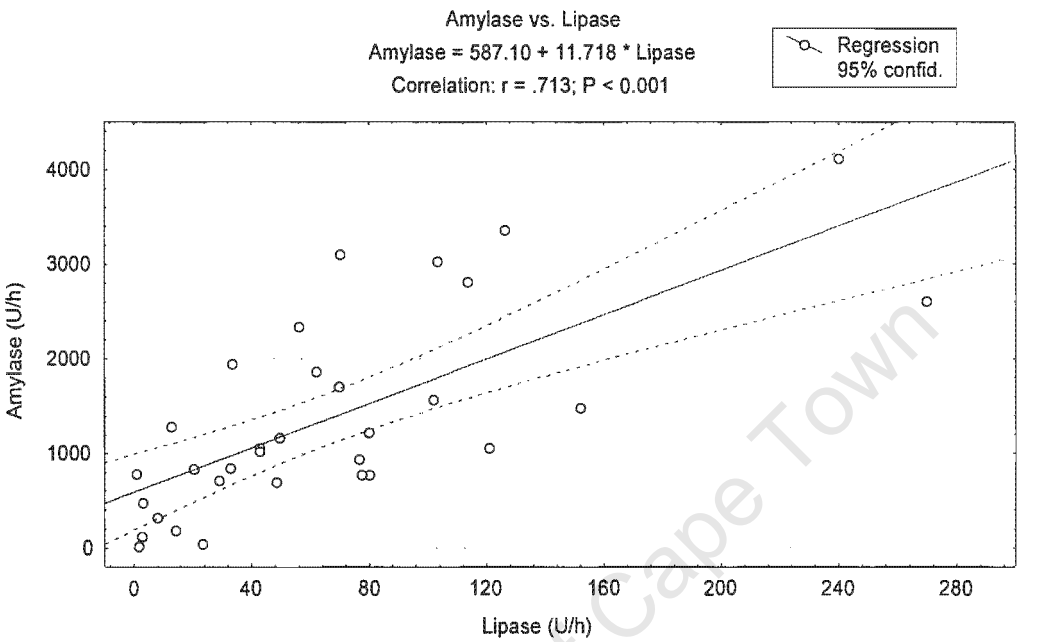


Figure 7.17: Correlation between the output of amylase and lipase in malnourished patients.

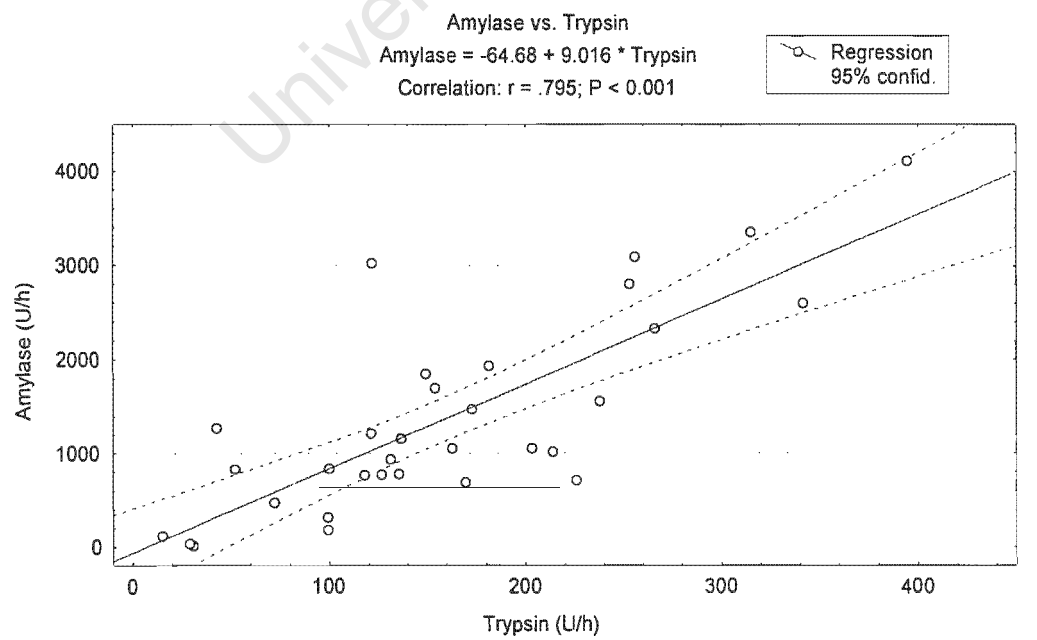


Figure 7.18: Correlation between the output of amylase and trypsin in malnourished patients.

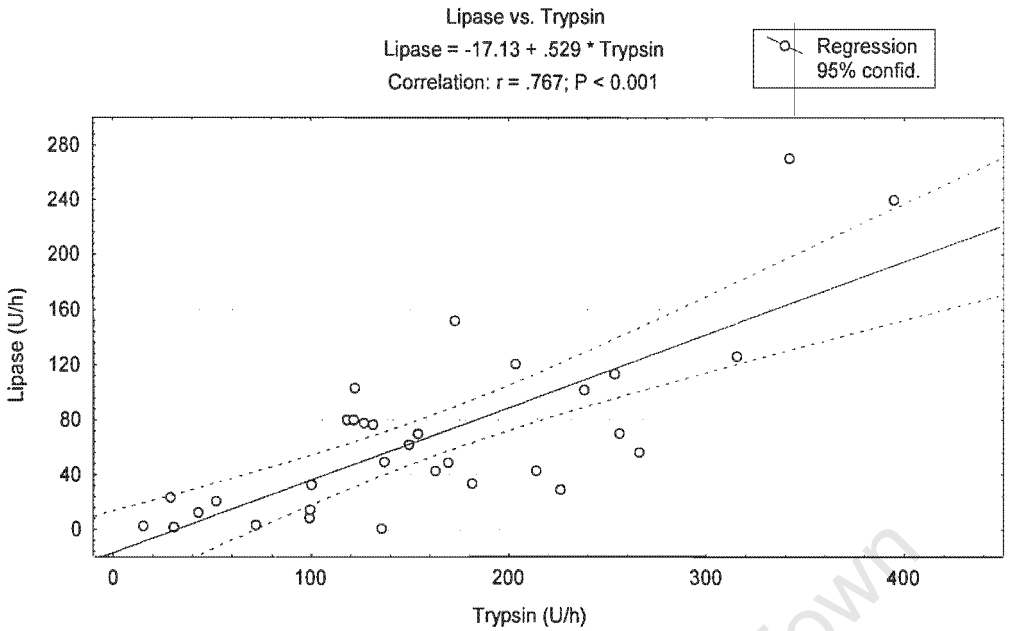


Figure 7.19: Correlation between the output of lipase and trypsin in malnourished patients.

7.4.2.2 Enteral Meal-Stimulated Pancreatic Enzyme Secretion

In order to investigate the contribution of an impaired gut mosal response to impaired pancreatic enzyme secretion in the malnourished patients, the seven patients who had enteral meal-stimulation of gastric acid secretion performed, also underwent enteral meal-stimulated tests of pancreatic secretion. None of the patients in the *anorexia patient* group received enteral meal-stimulation, and therefore the results were evaluated in comparison to the twelve patients from the *disease patient* group, who underwent hormone (CCK-8)-stimulation. Seven normal healthy controls received enteral-stimulation, and ten, CCK-8. Six of the enteral meal-stimulated malnourished patients, and ten of the CCK-8-stimulated group consented to further study following the period of nutritional support. The results are shown in Figure 7.20, and Figure 7.21.

In the control subjects, pancreatic stimulation with either enteral feed (ES), or with intravenous CCK-8 (HS), resulted in similar outputs of the enzymes amylase (2213 ± 302.9 vs. 2305 ± 346.4 U/h), and lipase (84.93 ± 8.56 vs. 118.6 ± 18.89 U/h). There was a trend to increased trypsin output in the ES group, but this difference did not reach statistical significance (498.9 ± 67.89 vs. 341.4 ± 40.80 U/h; $P > 0.05$).

In the malnourished patients, stimulation with enteral feeding (ES) or with CCK-8 (HS) resulted in similar mean outputs of the enzymes amylase (870.1 ± 373.1 vs. 686.5 ± 233.9 U/h; $P > 0.05$), lipase (30.68 ± 17.54 vs. 25.96 ± 8.51 U/h; $P > 0.05$), and trypsin (175.6 ± 70.08 vs. 109.3 ± 19.88 U/h; $P > 0.05$). However, outputs of these enzymes were all significantly impaired compared with control values ($P < 0.02$). After nutritional support, both ES and HS groups demonstrated significant improvement in amylase output (ES 2351 ± 567.7 vs. 870.1 ± 373.1 U/h, $P < 0.05$; HS 2228 ± 355.3 vs. 686.5 ± 233.9 U/h, $P < 0.01$) to levels similar to those in the control subjects. Lipase output also was significantly improved in the HS group (84.91 ± 20.07 vs. 25.96 ± 8.51 U/h, $P < 0.01$). Post-feeding levels in both groups did not differ significantly from control values. Although there was a significant improvement in the output of trypsin in the HS group (213.1 ± 32.32 vs. 109.3 ± 19.88 U/h, $P < 0.01$), the levels in both groups remained significantly impaired compared with control values (ES 226.4 ± 75.06 vs. 498.9 ± 67.89 U/h $P < 0.03$; HS 213.1 ± 32.32 vs. 341.4 ± 40.80 U/h, $P < 0.03$).

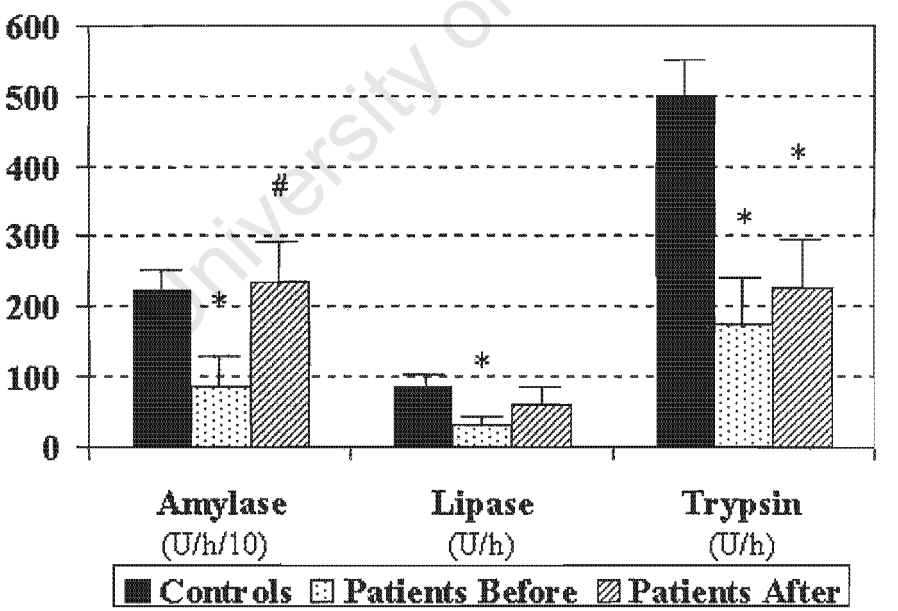


Figure 7.20: Enteral feed-stimulated pancreatic enzyme outputs of controls and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.03$ vs. controls. # $P < 0.05$ vs. before nutritional support.

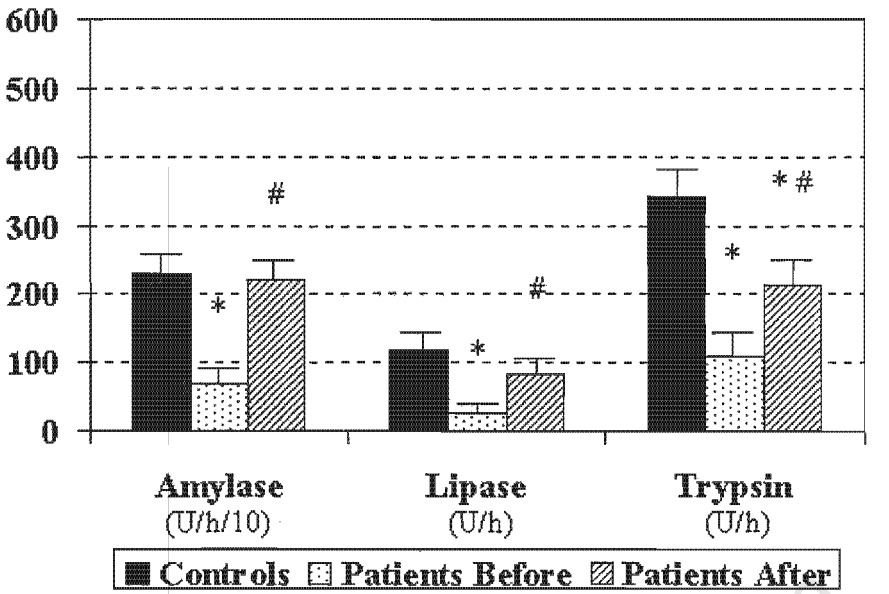


Figure 7.21: Hormone (CCK-8)-stimulated pancreatic enzyme outputs of controls and malnourished patients before, and after nutritional support. Bars represent means (SEM). $P < 0.01$ vs. controls. # $P < 0.02$ vs. before nutritional support.

7.4.2.3 Pancreatic Enzyme Secretion and Nutritional State

Output of the pancreatic enzymes correlated directly with nutritional state (BMI) ($P < 0.001$). This correlation was particularly evident in control subjects, and undernourished patients prior to nutritional support. (Figures 7.22-24)

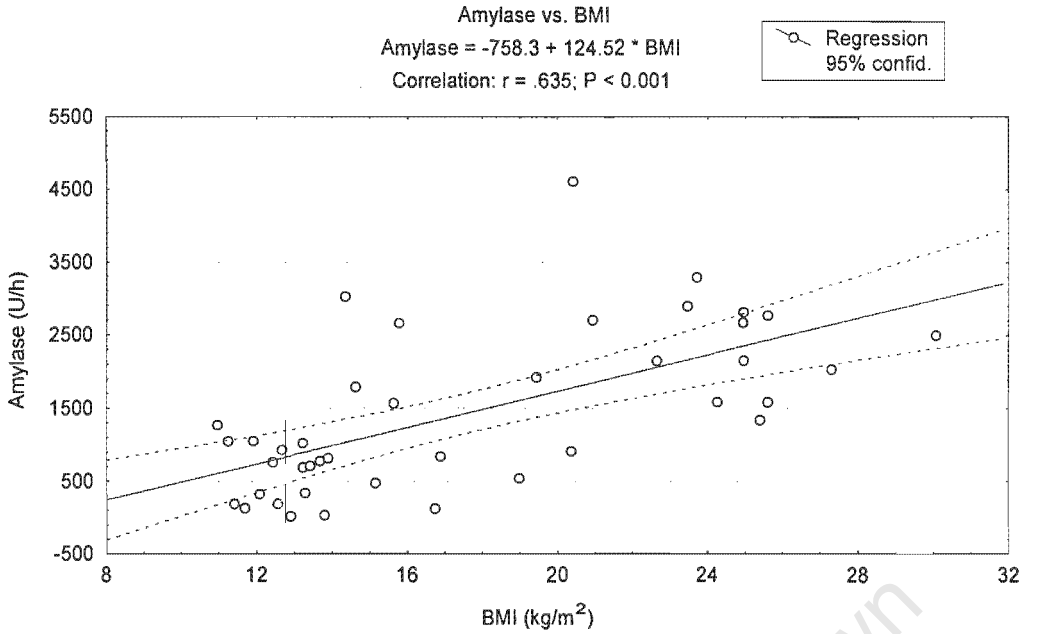


Figure 7.22: Correlation between amylase output and body mass index (BMI) in controls and malnourished patients before nutritional support.

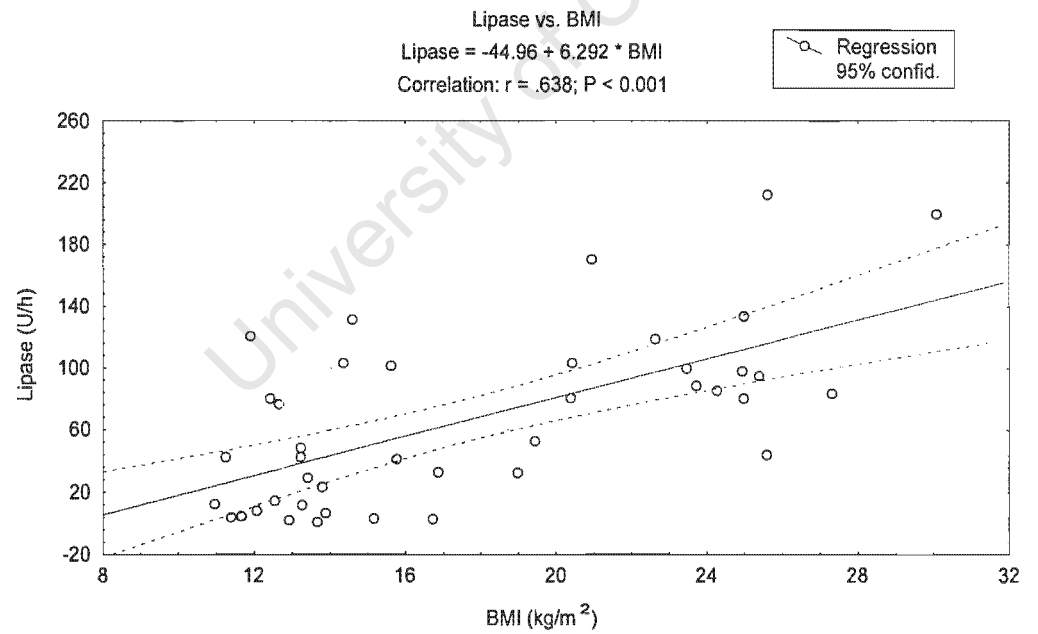


Figure 7.23: Correlation between lipase output and body mass index (BMI) in controls and malnourished patients before nutritional support.

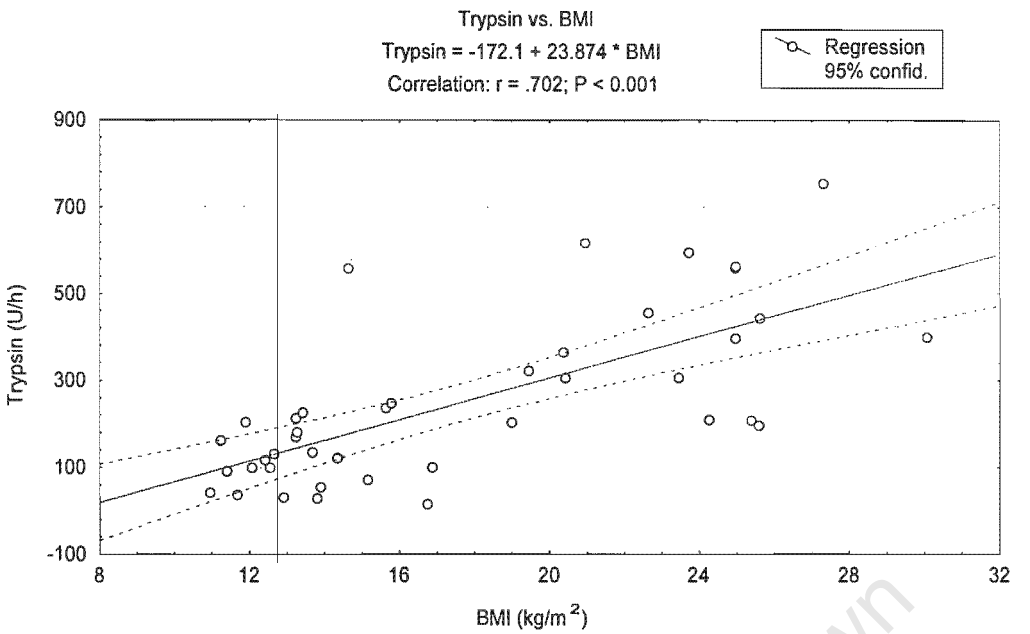


Figure 7.24: Correlation between trypsin output and body mass index (BMI) in controls and malnourished patients before nutritional support.

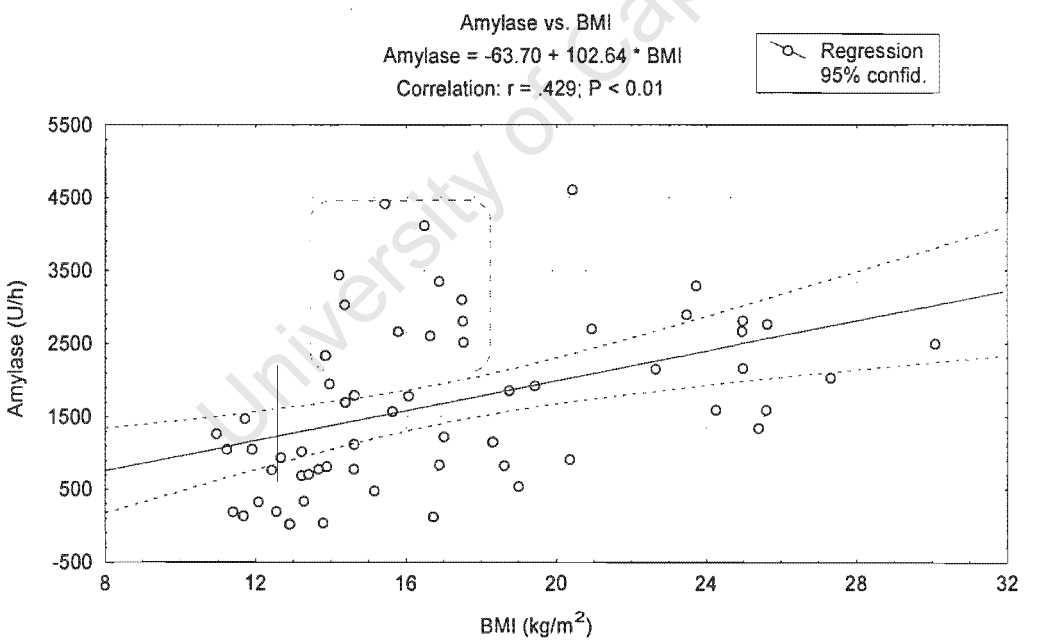


Figure 7.25: Correlation between amylase output and body mass index (BMI) in controls and malnourished patients, including those after nutritional support. The highlighted group identifies patients in whom amylase secretion was induced by nutritional support.

Inclusion of the patients following nutritional support, resulted in weakening of the correlation, particularly that between BMI and amylase and lipase, although the relation was still significant, (amylase, r = 0.43; P < 0.01: lipase, r = 0.33; P < 0.01) (Figure 7.25). Trypsin output still strongly correlated with BMI (r= 0.63; P < 0.001) (Figure 7.26).

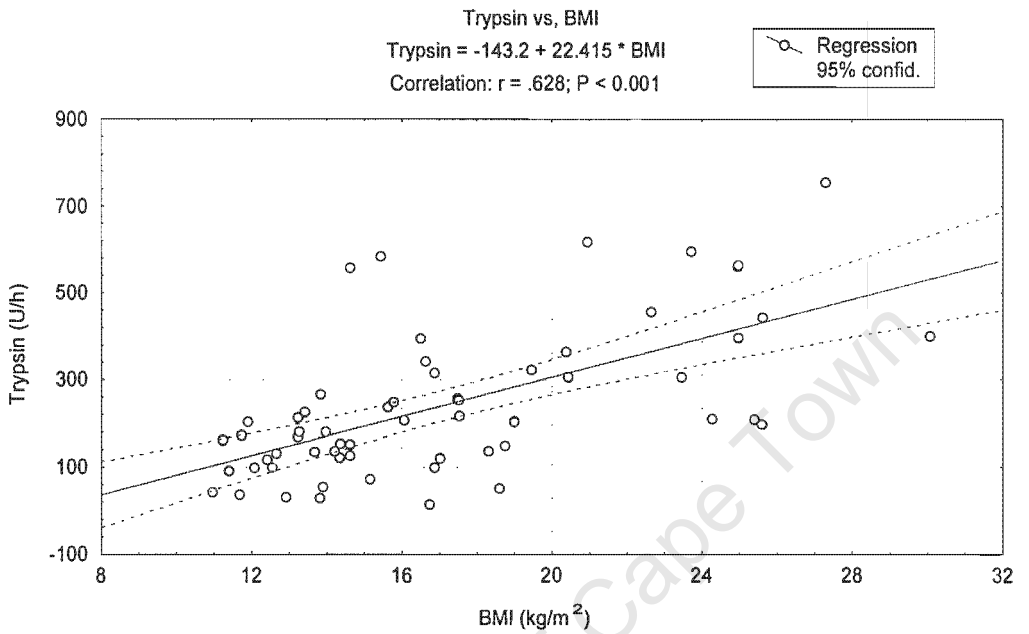


Figure 7.26: Correlation between trypsin output and body mass index (BMI) in controls and malnourished patients, including those after nutritional support.

Comparison of pancreatic secretion related to nutritional state (ie U/h/BMI) in the malnourished patients with that in controls, revealed significant impairment of the outputs of trypsin (7.84 ± 1.09 vs. 17.16 ± 1.62 U/kg/m^2; P < 0.001) and amylase (61.49 ± 10.57 vs. 96.39 ± 11.01 U/kg/m^2; P < 0.05) and a trend to a decreased lipase secretion (2.86 ± 0.60 vs. 4.38 ± 0.46 U/kg/m^2; P = 0.07) (Figure 7.27). Nutritional support resulted in a significant increase in the secretion of amylase (137.2 ± 15.51 vs. 61.49 ± 10.57 U/kg/m^2; P < 0.001) and lipase (5.63 ± 0.97 vs. 2.86 ± 0.60 U/kg/m^2; P < 0.02), with the amylase output now significantly higher than the control value (137.2 ± 15.51 vs. 96.39 ± 11.01 U /kg/m^2; P < 0.05). Trypsin secretion, related to nutritional status, was not significantly changed following refeeding 10.66 ± 1.49 vs. 7.84 ± 1.09 U/kg/m^2; P = not significant), with the output remaining significantly impaired compared to the controls (10.66 ± 1.49 vs. 17.16 ± 1.62 U/kg/m^2; P < 0.01).

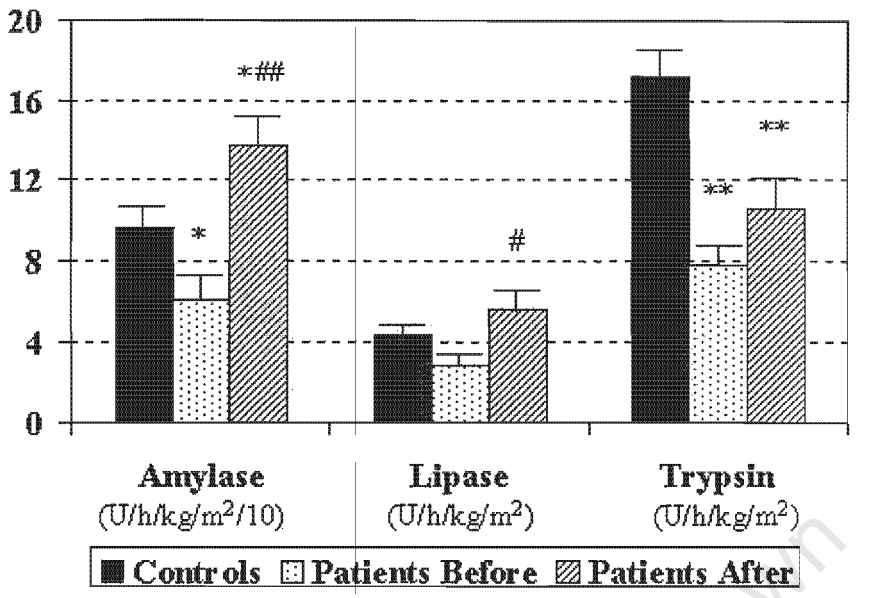


Figure 7.27: Pancreatic enzyme secretion, related to body mass index, in controls and malnourished patients before, and after nutritional support. * P = 0.07 vs. controls, ** P < 0.02 vs. controls, # P < 0.01 vs. patients before nutritional support, ## P < 0.05 vs. patients before nutritional support.

7.4.2.4 Pancreatic Enzyme Secretion and Digestive Function

Faecal fat excretion correlated directly with lipase secretion (r = 0.37; P < 0.02) (Figure 7.28), and stool frequency correlated with both lipase secretion (r = 0.51; P < 0.001) (Figure 7.29) and amylase secretion (0.35; P < 0.05) (Figure 7.30), but not with trypsin secretion (r = 0.24; P = not significant) (Figure 7.31).

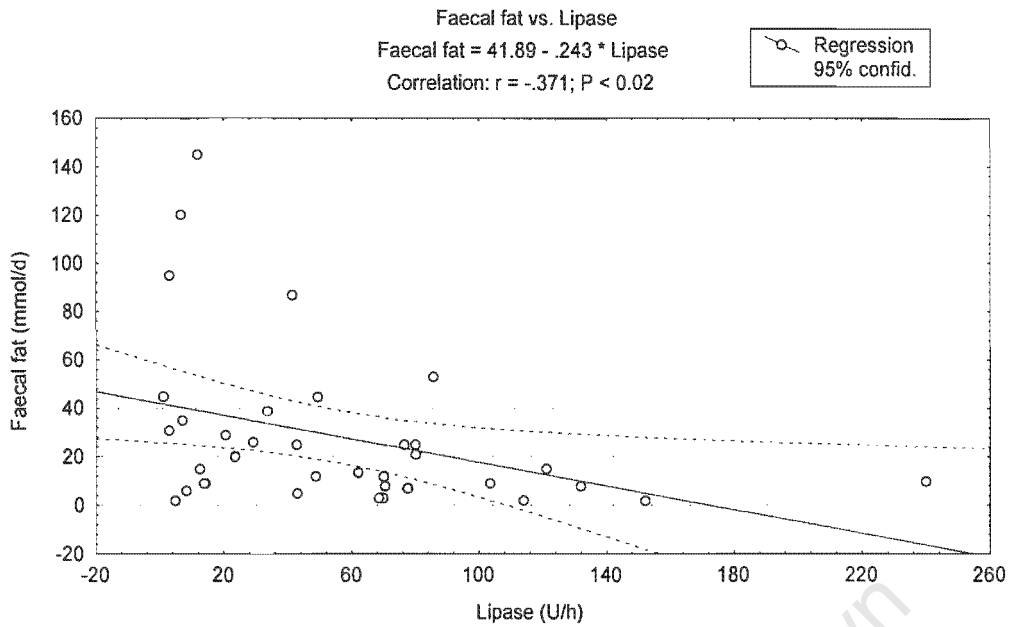


Figure 7.28: Correlation between faecal fat excretion and lipase output.

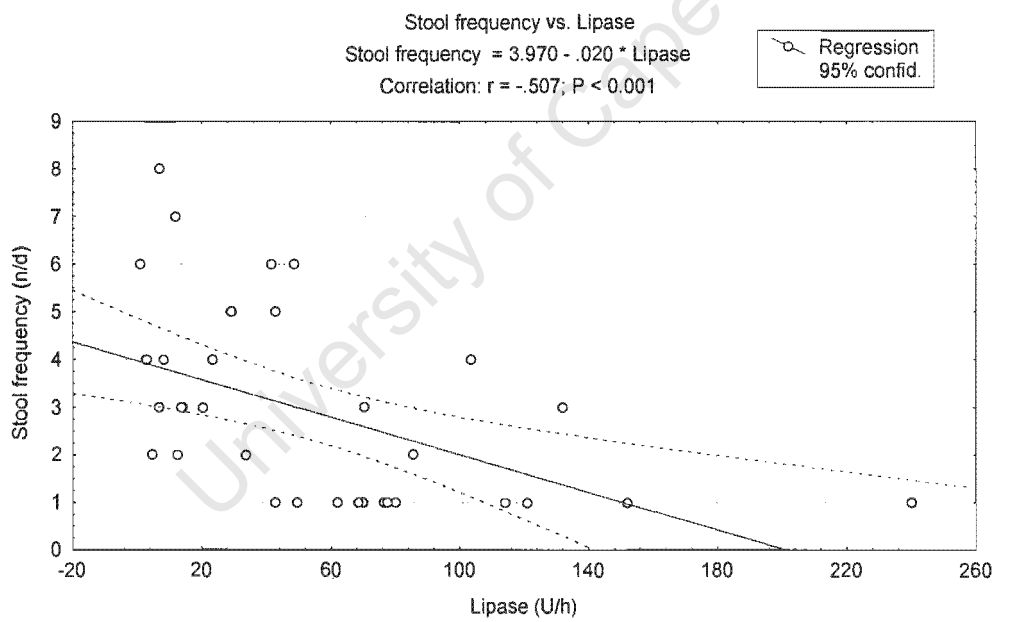


Figure 7.29: Correlation between stool frequency and lipase output.

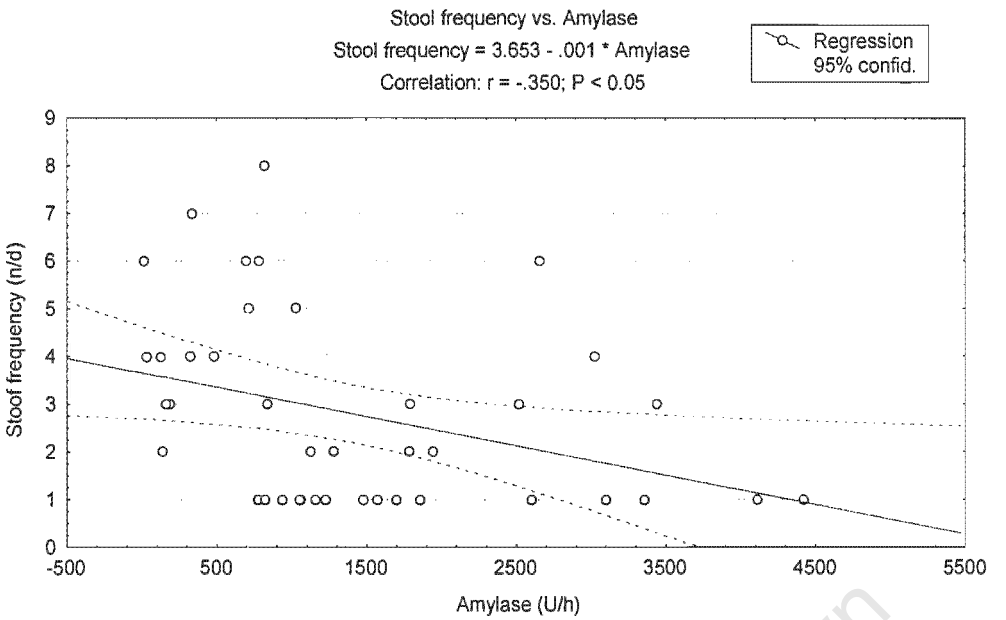


Figure 7.30: Correlation between stool frequency and amylase output.

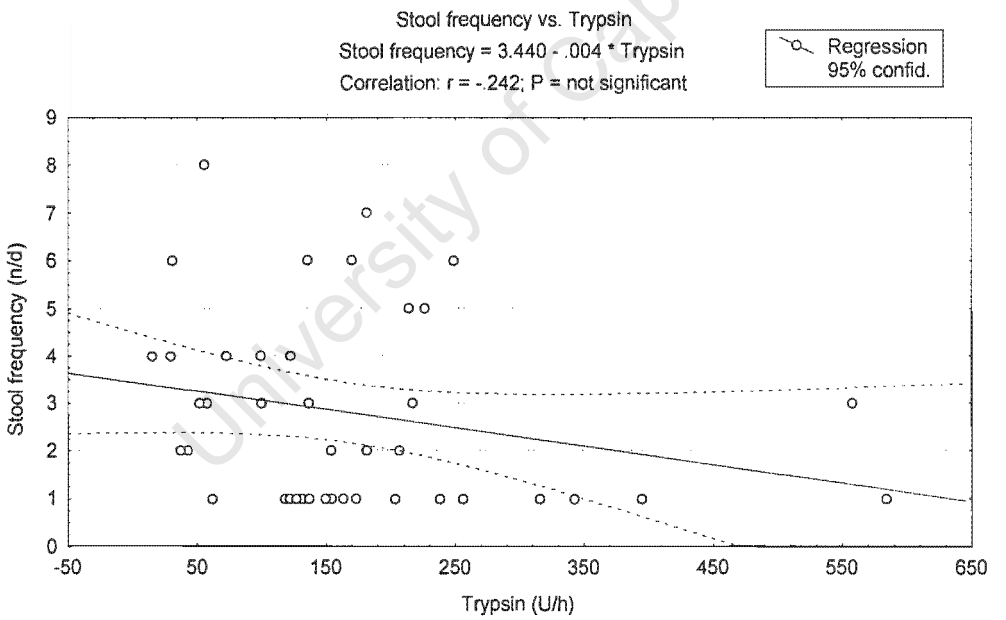


Figure 7.31: Correlation between stool frequency and trypsin output.

7.4.2.5 Pancreatic Enzyme Secretion of Anorexia Patients and Disease Patients

Results of the studies of CCK-8-stimulated pancreatic enzyme secretion in controls, and in the *anorexia patient* and *disease patient* groups prior to nutritional support are illustrated in Figure 7.32. The *anorexia patient* group had significant impairment of secretion of amylase and trypsin compared to control values (amylase 1111 ± 114.4 vs. 2305 ± 346.4 U/h; $P < 0.05$: trypsin 149.4 ± 28.03 vs. 341.4 ± 40.80 U/h; $P < 0.01$), whereas lipase, although lower than the control value, the difference did not achieve statistical significance (72.45 ± 16.08 vs. 118.6 ± 18.89 U/h; $P > 0.05$). In the *disease patient* group, secretion of all the enzymes were significantly reduced compared to control values (amylase 686.5 ± 233.9 vs. 2305 ± 346.4 U/h; $P < 0.01$: lipase 25.96 ± 8.51 vs. 118.6 ± 18.89 U/h; $P < 0.01$: trypsin 109.3 ± 19.88 vs. 341.4 ± 40.80 U/h; $P < 0.01$). Comparison of the *anorexia patient* and *disease patient* groups revealed that although the secretion of amylase and trypsin was lower in the disease patient group, the difference was not statistically different (amylase 1110 ± 114.4 vs. 686.5 ± 233.9 U/h; trypsin 149.4 ± 28.03 vs. 109.3 ± 19.88 U/h). Lipase secretion, however, was significantly reduced in the *disease patient* group (25.96 ± 8.51 vs. 72.45 ± 16.08 U/h; $P < 0.02$).

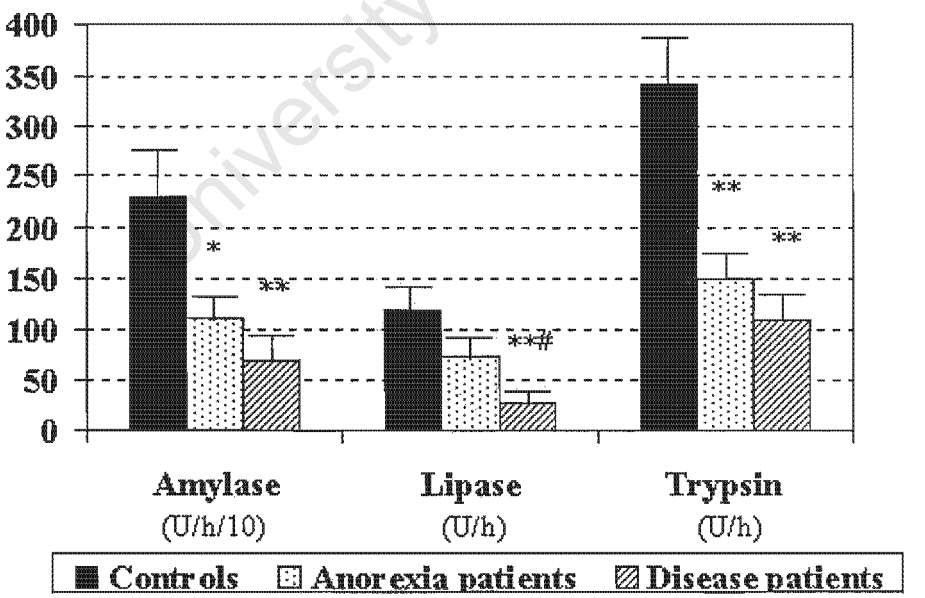


Figure 7.32: Cholecystokinin (CCK-8)-stimulated pancreatic amylase, lipase and trypsin outputs of controls, and of the *anorexia patient* and *disease patient* groups before, nutritional

support. Bars represent means (SEM). * $P < 0.05$ vs. controls, ** $P < 0.01$ vs. controls, # $P < 0.02$ vs. *anorexia patient* group.

Pancreatic enzyme outputs following nutritional support are illustrated in Figures 7.34 and 7.35. There was a significant increase in amylase secretion in both groups, to a level similar to control values (*anorexia patient* group 1750 ± 300.2 vs. 1111 ± 114.4 U/h; $P < 0.05$: *disease patient* group 2228 ± 355.3 vs. 686.5 ± 233.9 U/h; $P < 0.01$). Outputs of lipase and trypsin were significantly increased in the *disease patient* group (lipase 84.91 ± 20.07 vs. 25.96 ±8.51 U/h; $P < 0.01$: trypsin 213.1 ± 32.32 vs. 109.3 ± 19.88 U/h; $P < 0.05$), however, the secretion of trypsin remained significantly impaired compared to controls in both the *anorexia patient* and *disease patient* groups (*anorexia patient* group 197.5 ± 49.32 vs. 341.4 ± 40.80 U/h; $P < 0.05$: *disease patient* group 213.1 ± 32.32 vs. 341.4 ± 40.80 U/h; $P < 0.01$).

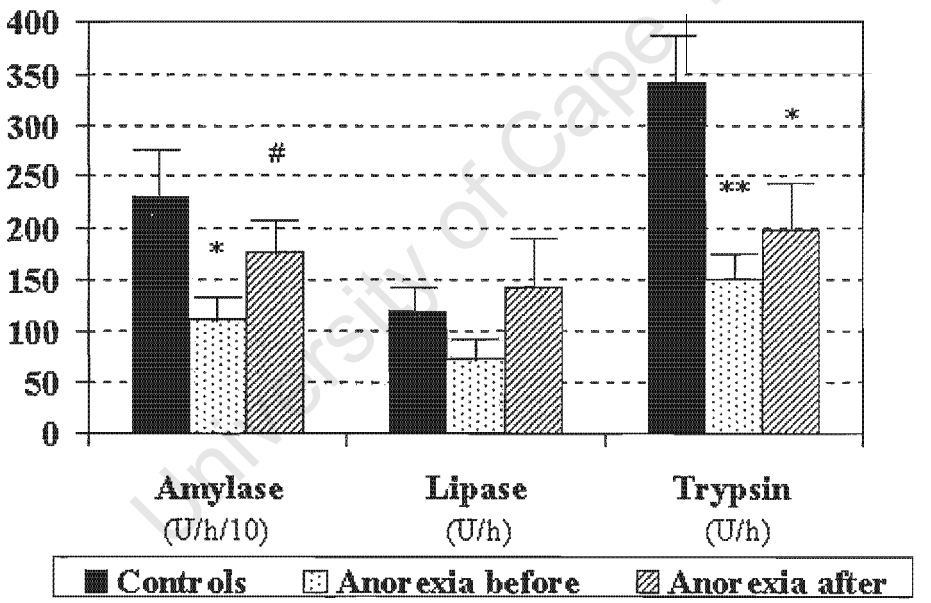


Figure 7.33: Cholecystokinin (CCK-8)-stimulated pancreatic amylase, lipase and trypsin outputs of controls, and of the *anorexia patient* group before, and after nutritional support. Bars represent means (SEM). * $P < 0.05$ vs. controls, ** $P < 0.001$ vs. controls, # $P < 0.05$ vs. *anorexia patient* group before nutritional support.

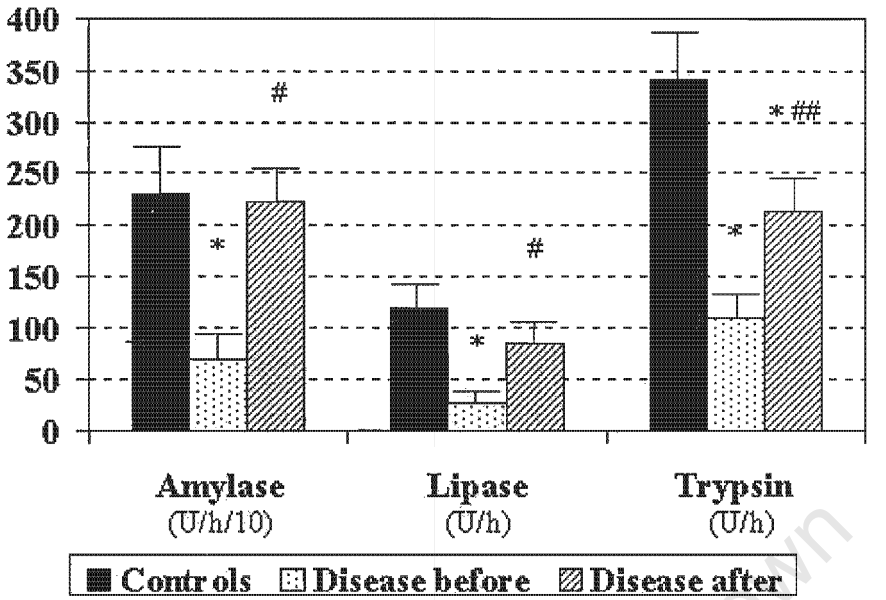


Figure 7.34: Cholecystokinin (CCK-8) stimulated pancreatic amylase, lipase and trypsin outputs of controls, and of the *disease patient* group before, and after nutritional support. Bars represent means (SEM). * $P < 0.01$ vs. controls, # $P < 0.01$ vs. *disease patient* group before nutritional support, ## $P < 0.05$ vs. *disease patient* group before nutritional support.

7.5 Discussion

7.5.1 Gastric Acid Secretion

The malnourished patients had evidence of significant impairment of acid secretion, with 70% of the patients having basal achlorhydria. Marked impairment of both pentagastrin- and enteral meal-stimulated acid output was also demonstrated. Basal acid output (BAO), and maximal acid output (MAO) correlated directly with nutritional status as assessed by the BMI. Following the period of nutritional support, there was no significant change in the BAO of the malnourished patients, which remained significantly less than the control value. There was significant improvement in pentagastrin stimulated output, but not in the enteral meal stimulated output, although the post-feeding level remained impaired compared to the control value.

Comparison of the *anorexia patient* group with the *disease patient* group indicated that although the BAO of both groups were similarly impaired, both before, and after nutritional support, pentagastrin stimulated MAO was significantly more impaired in the *disease patient* group prior to re-feeding. Post feeding levels were similar in the two groups, and remained significantly lower than the control value.

In order to assess the relative contributions of primary parietal cell failure, and gastric mucosal dysfunction, induced by the malnourished state, both hormone (pentagastrin)-stimulated, and enteral meal-stimulated tests of gastric acid secretion were performed. Studies in the normal healthy control group, however, demonstrated a significant difference between meal- and pentagastrin-stimulated acid secretion, and it was therefore difficult to fully assess the contribution of an impaired gastric mucosal response in our malnourished patients. In our study, the acid output in control subjects stimulated by meal infusion was 43% of that stimulated by pentagastrin. Lam et al also noted that a meal induces less than maximal stimulation of gastric acid, with an 8% peptone meal infused into the stomach resulting in an acid secretion 65% of that stimulated by pentagastrin.[400]

The studies in the malnourished patients demonstrated similar impairment in acid secretion induced by either pentagastrin or enteral meal. However, in view of the studies in normal healthy subjects indicating unequal stimulation of acid secretion by the two methods, direct comparison of the two methods was problematic. However, as enteral meal stimulation of acid secretion was less efficient than with pentagastrin, the finding of similarly impaired responses in the malnourished patients to the two forms of stimulation suggests that the principal defect was that of parietal cell response, with little further contribution from mucosal abnormality.

Following nutritional support, the pentagastrin-stimulated malnourished group demonstrated significant improvement in acid secretion, although the output remained lower than the control value, whereas the meal-stimulated group did not. This indicates that gastric mucosal dysfunction was present, which persisted despite improvements in nutritional state.

Nutritional support was associated with improvement in CCK-8-stimulated acid secretion. However the output remained significantly impaired compared to controls, indicating persistent parietal cell dysfunction. Although the overall mucosal turnover (fractional

synthesis) rate in the stomach is rapid (approximately 3 days), there is a wide variation between the various cell types. Gastric parietal cells do not divide, and have to be replaced from precursor stem cells within the gastric pits. This results in a rather slow turnover rate of these cells (approximately 54 days) in the mucosa,[401,402] and the slow improvement in restitution of gastric acid secretion may relate to the time taken to regenerate the parietal cell mass in malnourished patients. Further study is required to determine the time and degree of nutritional repletion required before gastric secretion returns to normal.

The role of *Helicobacter pylori* as a factor in the impairment of acid secretion was not investigated. Although chronic *H. pylori* infection has been associated with an increased secretion of acid, this appears to be in those patients with antral predominant infection.[403] Malnourished patients may be susceptible to diffuse *H. pylori* gastritis, which has been associated with a reduction in acid secretion.[403] Mucosal dysfunction, as a result of a persistent *H. pylori* gastritis also might have prevented nutritional support from normalising enteral meal stimulated acid output, and further study is required.

7.5.2 Pancreatic Enzyme Secretion

In normal healthy control subjects, the profile of pancreatic enzyme secretion demonstrated a significantly higher output of enzymes over the initial 30-60 minutes of stimulation, with a constant secretion thereafter. This pattern was particularly evident with the secretion of amylase, and suggests initial release of enzymes from preformed zymogen stores (zymogen pool), with subsequent synthesis and release of newly synthesised enzymes at a more constant rate. This feature, however, was not evident in the malnourished patients, suggesting possible depletion of zymogen stores. Following the period of nutritional support, the profile of secretion of amylase in the malnourished patients did suggest an increased output during the first 30 minutes, although this did not achieve statistical significance ($P = 0.06$), suggesting restitution of amylase stores.

Assessment of CCK-8-stimulated pancreatic enzyme output of the malnourished patients demonstrated significant impairment of all three enzymes studied compared to control values. The outputs of the individual enzymes correlated with each other, indicating that the secretions of the three enzymes were proportionately reduced. Following the period of

intensive nutritional support there was a significant improvement in the secretion of the pancreatic enzymes. The secretion of amylase and trypsin returned to normal values, whereas that of trypsin, althought significantly increased from baseline, remained significantly impaired compared to controls.

The impaired pancreatic secretory response to CCK-8 indicates pancreatic acinar cell dysfunction in malnourished patients. An impaired gut mucosal response to luminal nutrients, due to mucosal dysfunction consequent to either the malnourished state or the primary disease process, may also contribute to a deficient pancreatic secretory response. Reduced secretion of the gastro-intestinal hormone cholecystokinin (CCK) in response to a test meal has been demonstrated in patients with villous atrophy due to coeliac sprue, with subsequent impairment of pancreatic function and maldigestion of fat.[404,405] Restoration of intestinal mucosal integrity reverses this abnormality.[406,407] In order to determine the relative contributions of primary pancreatic acinar cell dysfunction, and gut mucosal dysfunction to the impaired pancreatic exocrine response in malnourished patients, meal-stimulated tests of pancreatic secretion were also performed.

The studies in normal healthy controls demonstrated that intraduodenal infusion of a polymeric feeding formula resulted in a pancreatic enzyme output comparable to that stimulated by 40 ng/kg/h (35 pmol/kg/h) of CCK-8. This dose of CCK-8 has been shown to result in maximal stimulation of pancreatic enzyme secretion.[408,409] Previous studies in dogs[410] and humans[411,412] reported that a normal meal does not maximally stimulate exocrine pancreatic secretion. However, our use of a constant intraduodenal infusion of a defined formula diet may have resulted in more efficient stimulation of pancreatic secretion, and gave a more reliable assessment of maximal enzyme output.

Although our patients were heterogenous, with a variety of primary diseases associated with their malnutrition, the group which received CCK-8 stimulation was similar to that which received enteral meal as regards severity of malnutrition, as determined by BMI. Plasma albumin levels, which may be affected by both protein synthesis as well as inflammation, were also similar at the beginning of the study. As we were concerned primarily with the effects of malnutrition, rather than the effects of specific disease processes, on gastro-pancreatic function, we considered the groups comparable for the purposes of this study. Several of the patients had evidence of gut mucosal abnormality, with villous atrophy, and

impaired xylose and fat absorption as a result of the primary disease process or as a consequence of the malnourished state. The degree of abnormality was similar in the two groups.

Comparison of the results in the two groups demonstrated that the degree of impairment of pancreatic secretion in response to the enteral meal was similar to that in response to direct CCK-8 stimulation. This finding indicated that the reduction in pancreatic enzyme secretion in malnourished patients was primarily consequent to pancreatic acinar cell failure, and any dysfunction of the gut mucosal response induced by the malnourished state, or the primary disease process, did not contribute further to the impairment in exocrine pancreatic function.

Both groups of malnourished patients received similar periods of nutritional support, and had comparative improvements in BMI, urinary xylose excretion, stool frequency, and faecal fat excretion. After nutritional support, there were similar improvements in enteral- and CCK-8-stimulated enzyme secretion, with amylase and lipase outputs returning to levels similar to control values. Trypsin secretion, although significantly improved in the CCK-8-stimulated group, remained significantly impaired compared with control values.

The enzyme outputs correlated directly with nutritional state, as assessed by the body mass index (BMI). The significant correlation between pancreatic enzyme output, and nutritional status suggested that pancreatic enzyme secretion was directly related to body mass. Gaia et al have previously demonstrated that in normal control subjects, pancreatic enzyme output was positively related to body surface area, and suggested that assessment of pancreatic secretion should always be made in relation to body size.[413] Wisen and Johansson, however, demonstrated decreased secretion of pancreatic enzymes, in response to a test meal, in obese patients,[414] indicating that the association is not linear. In our study, expression of the outputs of the pancreatic enzymes in relation to their nutritional state (BMI) indicated a disproportionate decrease, particularly in trypsin and amylase secretion, in malnourished patients. Re-feeding subsequently resulted in a disproportionate increase in the secretion of amylase and lipase, with the amylase levels now significantly higher than the control values. There was, however, no significant change in the secretion of trypsin (expressed in relation to nutritional status), with the post feeding levels remaining significantly impaired compared to controls. These features indicated that in malnourished patients, reduction in secretion, particularly of trypsin and amylase, was in excess of that possibly predicted by their reduced

body size. Re-feeding positively induces secretion, particularly that of amylase but also lipase, but not of trypsin. Increase in the secretion of trypsin appears to more directly relate to the increase in body mass.

Comparison of the *anorexia patient* group with the *disease patient* group revealed similar reductions in the secretion of amylase and trypsin, which were significantly impaired compared to control values. Lipase output, however, was significantly lower in the *disease patient* group, compared to both controls and the *anorexia patient* group. Although the lipase output in the *anorexia group* was lower than controls, the difference did not achieve significance. These results indicate that lipase secretion in the *anorexia patient* group was less affected by the nutritional state than the other two enzymes, and this may possibly relate to the relative absence of symptoms of bowel dysfunction such as diarrhoea, and increased faecal fat excretion in these patients, as compared to the *disease patient* group.

There is generally a surplus of digestive enzyme secretion from the pancreas, and DiMagno and colleagues have noted that a suppression of lipase greater than 90% was required before fat malabsorption would occur.[415] Ten of twenty-four patients studied (42%) had lipase outputs impaired to this degree. However, in patients with co-existent gut mucosal dysfunction, more modest impairment of pancreatic enzyme secretion may be sufficient to result in maldigestion and malabsorption. This gut dysfunction may be the result of the primary disease process, or may be secondary to the malnourished state. The inverse relationship between lipase output, and faecal fat excretion, and between both lipase and amylase output and stool frequency indicate that pancreatic enzyme insufficiency was contributing significantly to the maldigestion and malabsorption in these patients. It is noteworthy, however, that the anorexic patient group, despite having impairment of pancreatic enzyme secretion, particularly that of amylase and trypsin, and reduced xylose absorption, did not have diarrhoea. As noted previously, however, their lipase output was relatively preserved, and in the malnourished patients, lipase output correlated most strongly with stool frequency and faecal fat excretion.

The period of nutritional support resulted in a significant increase in the secretion of pancreatic enzymes, with the amylase and lipase outputs returning to levels similar to the control values. Although there was a significant increase in the secretion of trypsin, the output remained significantly impaired compared to controls. These results indicate that the

restitution of pancreatic enzyme secretion following nutritional support in malnourished patients occurs in a non-parallel fashion, with amylase and lipase generally quickly restored. Barbezat and Hanson, using secretin/pancreazymin stimulation also noted a dramatic increase in amylase following treatment of children who suffered from kwashiorkor, and showed that lipase levels returned to the normal range.[189] In contrast to our results, however, they found trypsin to be least affected by protein-calorie malnutrition, and following treatment, the children had a normal trypsin output. Other studies from India have demonstrated early recovery of exocrine pancreatic function in patients with protein-calorie malnutrition when they were fed a high protein diet (50 kcal/kg/d).[196,197] However, these patients may have been less severely malnourished, did not have co-existant disease, and pancreatic secretion was only reduced 30-40%.

In summary, severely malnourished patients have significant impairment of gastric acid and pancreatic enzyme secretion, which is likely to adversely affect the digestive process. Our studies indicated that, although an impaired gut mucosal response may play a role, there is primary gastric parietal cell and pancreatic acinar cell failure that, at least in the case of pancreatic secretion, appears to be the determining factor controlling enzyme output in these patients. Improvement after feeding indicated that the dysfunction was nutritionally mediated.

CHAPTER 8

PANCREATIC ENZYME SYNTHESIS

8.1 Introduction

The studies of exocrine pancreatic function in malnourished patients demonstrated marked impairment of secretion of pancreatic enzymes, with significant improvement following nutritional support. There is, therefore an intimate relationship between nutrition and pancreatic function. Digestion and absorption of nutrients in the gut is dependent on an adequate supply of digestive (pancreatic) enzymes, and an efficient, absorptive enteric mucosa. Synthesis of these pancreatic enzymes is, in turn, dependent on an adequate supply of substrate amino acids in the bloodstream. For its size, the pancreas is an extremely active organ producing 2-8 g enzyme protein per day.[416] The demand for amino acids for the synthesis of pancreatic enzymes is clearly demonstrated by the marked reduction in plasma amino acid concentrations following pancreatic stimulation, a feature that has been exploited as a test of pancreatic function.[417]

Conventional studies of exocrine pancreatic function, such as cholecystokinin-stimulation of enzyme secretion, are somewhat limited, as the enzymes secreted are generally derived from pre-formed stores (the zymogen pool). Acute changes of enzyme synthesis may therefore not be apparent.[418] Consequent to earlier work by Boyd et al,[419] our Unit has previously developed a method of directly investigating the incorporation of isotope labelled amino acid (^{14}C-leucine) into the pancreatic enzymes amylase and trypsin.[420] With this method, considerable information regarding enzyme synthesis, zymogen pool turnover rate, and zymogen pool size can be obtained.

8.2 Aim of Study

To determine the consequences of malnutrition, and subsequent re-feeding on pancreatic enzyme synthesis, and zymogen pool stores.

8.3 Patients and Methods

In order to further assess the relationship between nutritional status and exocrine pancreatic function, a four-hour primed/constant intravenous infusion of L-[1-^{14}C]leucine was administered during the CCK-8 stimulated pancreatic secretion tests. Aliquots of duodenal secretions were collected at 30-minute intervals. Amylase and trypsin were then isolated from the duodenal juice samples by affinity chromatography (Appendix 8). Amylase was extracted using the affinity ligand acarbose,[421] and trypsin using antitrypsin antibody.[422] The enzymes were hydrolysed to their constituent free amino acids, and leucine concentrations and radioactivity due to ^{14}C measured (Appendix 9). The specific activity of free ^{14}C leucine in plasma samples was measured (Appendix 6). This value was used as an index of the specific activity of leucine in the precursor pool for protein synthesis.[418]

8.3.1 Calculations

Assessment of enzyme production time and zymogen pool turnover can be determined from the characteristics of the graphs of the specific activities of ^{14}C leucine incorporated into enzyme protein versus time (Figure 8.1).[418,420]

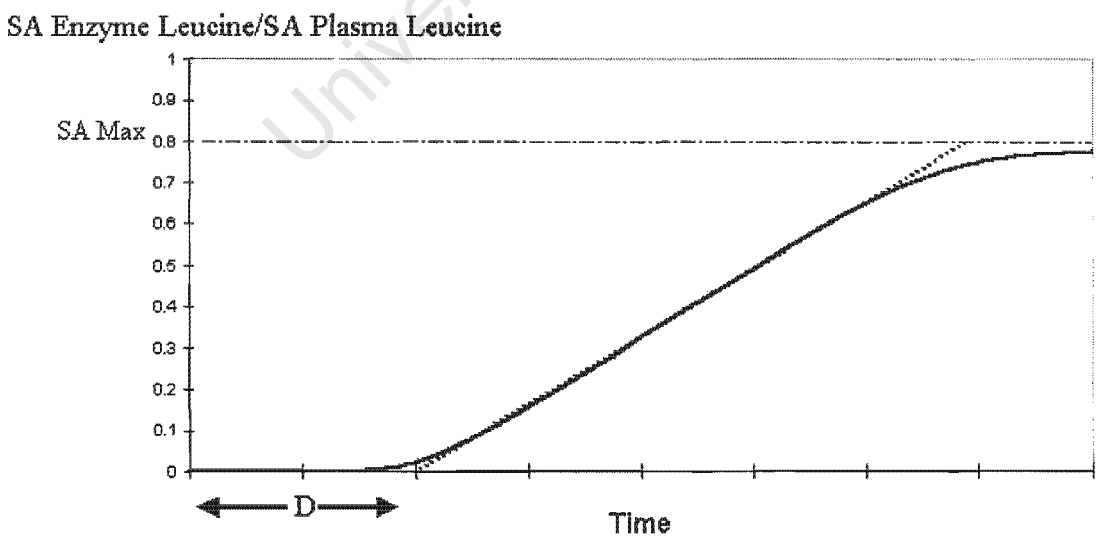


Figure 8.1: Incorporation of isotope-labelled leucine into pancreatic enzyme protein

In order to standardize the graphs, the specific activities of the enzyme protein ^{14}C-leucine were expressed as a fraction of the plasma ^{14}C leucine for the individual subjects. The graphs follow a curvi-linear plot with a delay parameter D (Figure 8.1). The time taken for labelled enzyme to appear in the duodenal secretions (D) represents the minimum time taken for the synthesis and secretion of the enzyme (enzyme production time). The rate of increase of labelling thereafter represents the rate of turnover, or replacement, of zymogen stores (zymogen pool turnover, %/h). This part of the graph fitted well with a simple linear regression model (with a mean r-value of 0.97 ± 0.004), and extrapolation of this regression line to the x-axis provided an objective estimate of the parameter D. The graph plateaus at the maximum specific activity (SA Max), a level representing the specific activity of the precursor pool for the amino acid. Previous work has shown that this level is approximately 80% of the value of the plasma leucine specific activity,[418,420,423] From the assessment of zymogen pool turnover rates of amylase and trypsin (%/h), and the direct measurement of the outputs of the enzymes during this period (U/h), the pool sizes of the two enzymes could be calculated:

$$\text{Zymogen pool size (U)} = \frac{\text{Enzyme output (U/h)}}{\text{Zymogen pool turnover (\%/h)}} \times 100$$

8.4 Results

8.4.1 Pancreatic Enzyme Synthesis in Normal Controls and in Malnourished Patients

The results of the composite plots of pancreatic enzyme (amylase and trypsin) leucine specific activity (SA), extracted from the aspirated duodenal juice, expressed in relation to the individual plasma leucine SA's, are illustrated in Figures 8.2-7. The initial secretions were unlabelled, indicating release of enzyme from preformed stores. The graphs then followed a curvi-linear pattern, with the increase in SA of the enzyme protein fitting well with a simple linear regression model (r-value = 0.97 ± 0.004).

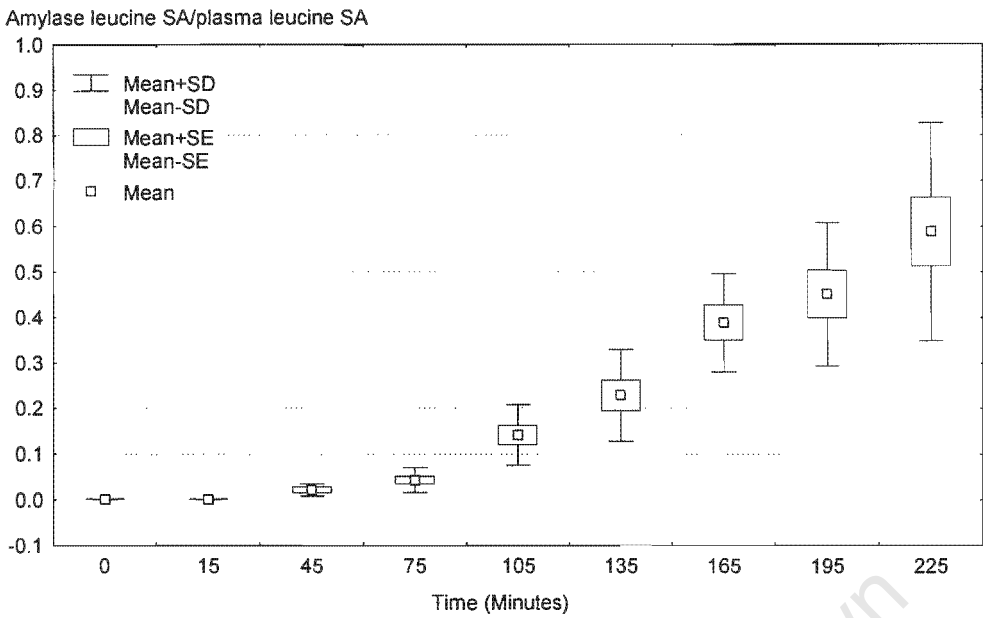


Figure 8.2: Box and whisker graph of pancreatic amylase leucine specific activity (enzyme leucine SA/plasma leucine SA) in aspirated jejunal juice during stimulation with CCK-8, plotted against time, in control subjects.

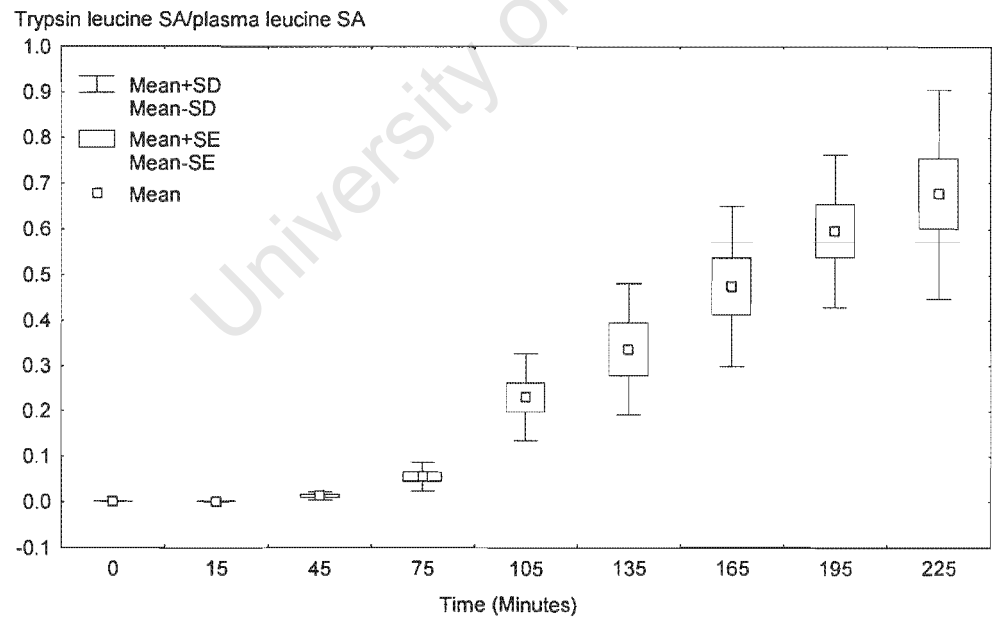


Figure 8.3: Box and whisker graph of pancreatic trypsin leucine specific activity (enzyme leucine SA/plasma leucine SA) in aspirated jejunal juice during stimulation with CCK-8, plotted against time, in control subjects.

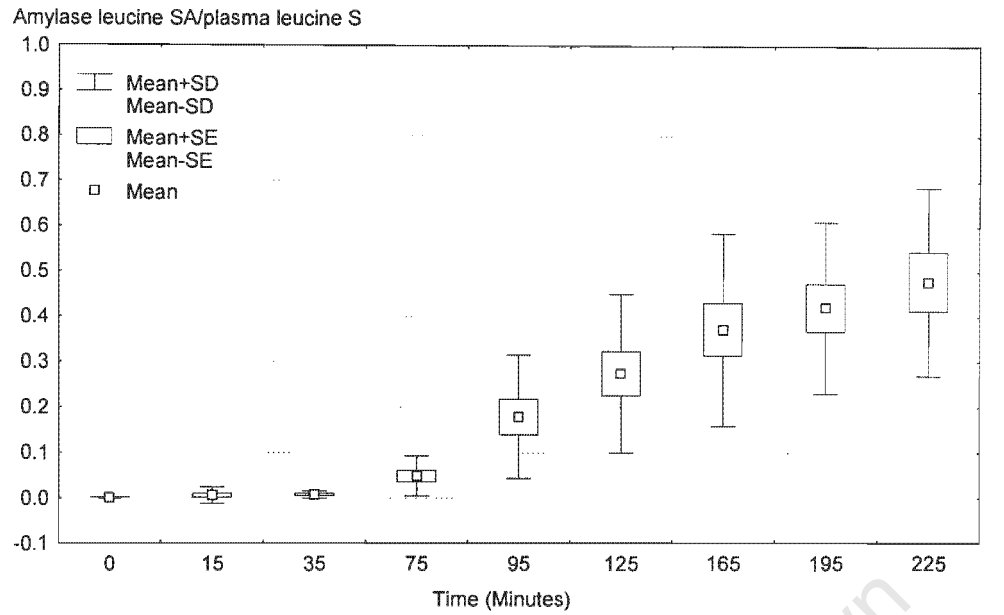


Figure 8.4: Box and whisker graph of pancreatic amylase leucine specific activity (enzyme leucine SA/plasma leucine SA) in aspirated jejunal juice during stimulation with CCK-8, plotted against time, in malnourished patients before nutritional support.

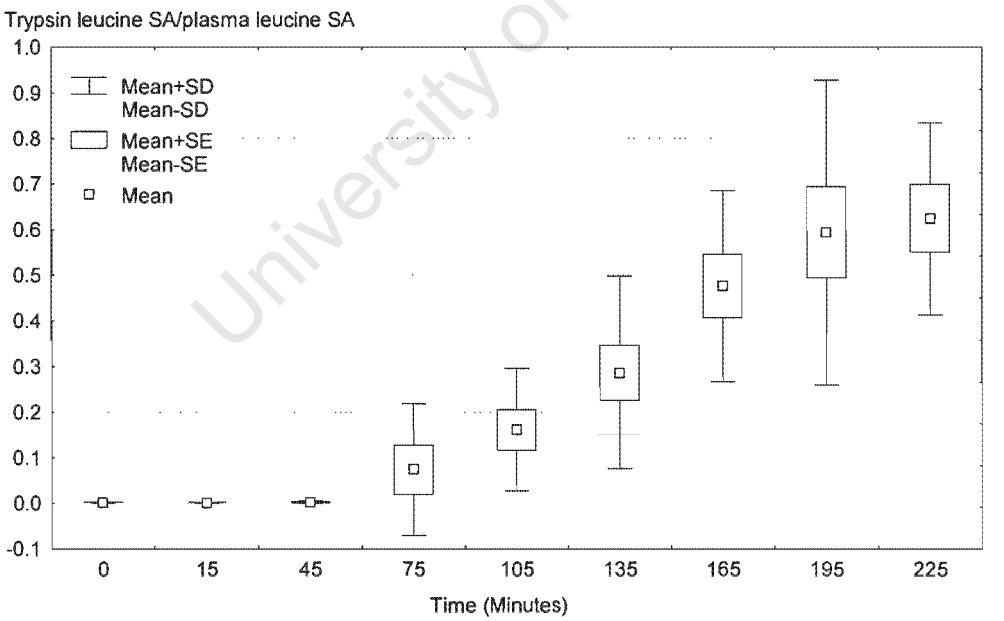


Figure 8.5: Box and whisker graph of pancreatic trypsin leucine specific activity enzyme leucine SA/plasma leucine SA) in aspirated jejunal juice during stimulation with CCK-8, plotted against time, in malnourished patients before nutritional support.

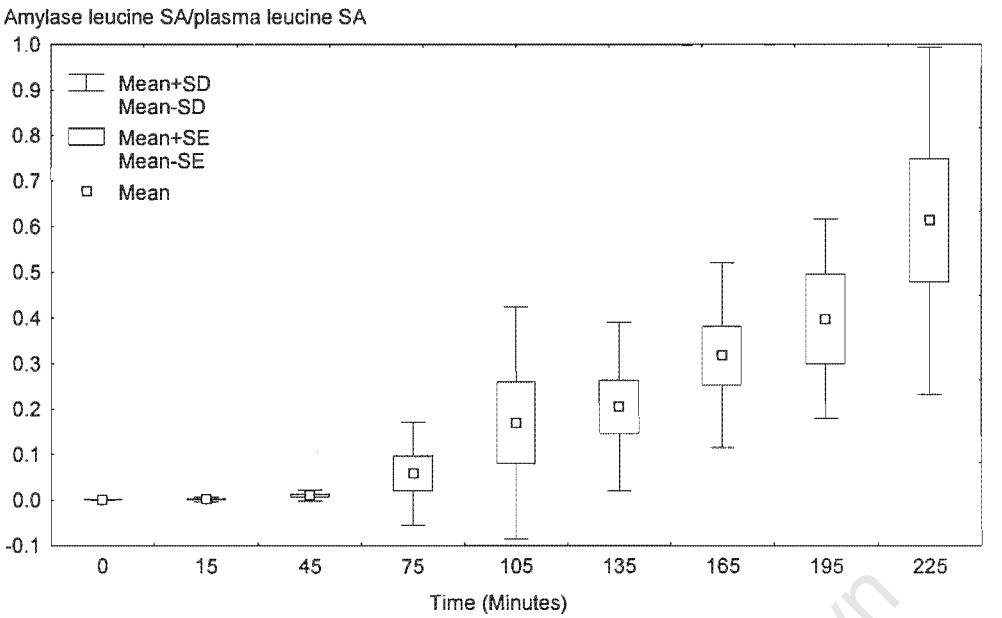


Figure 8.6: Box and whisker graph of pancreatic amylase leucine specific activity (enzyme leucine SA/plasma leucine SA) in aspirated jejunal juice during stimulation with CCK-8, plotted, against time, in malnourished patients after nutritional support.

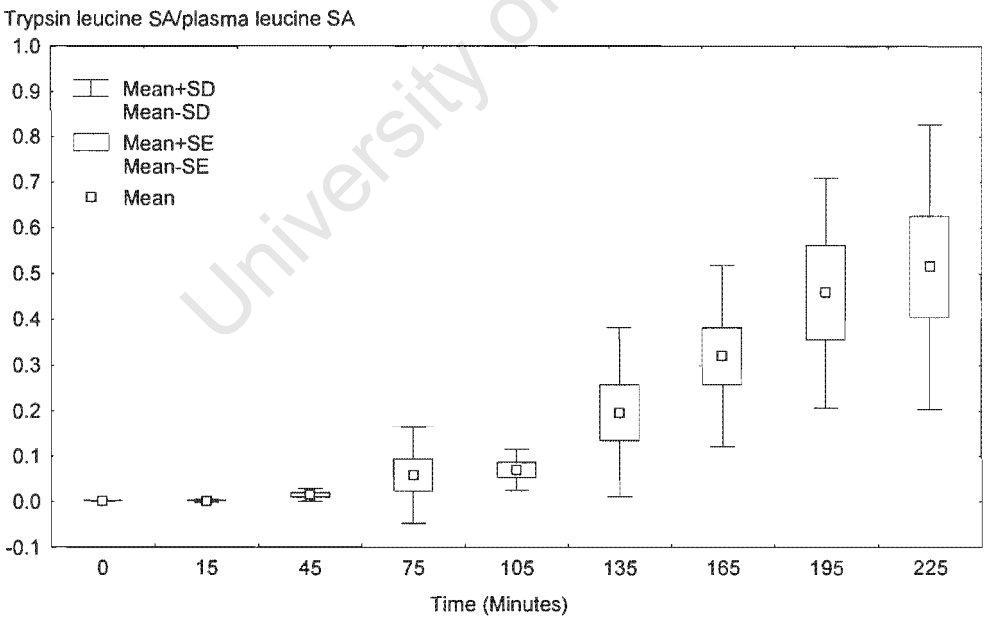


Figure 8.7: Box and whisker graph of pancreatic trypsin leucine specific activity (enzyme leucine SA/plasma leucine SA) in aspirated jejunal juice during stimulation with CCK-8, plotted against time, in malnourished patients after nutritional support.

From the individual graphs, and from the measurement of enzyme output during the period of isotope incorporation, enzyme production times, zymogen pool turnover rates, and zymogen pool sizes were calculated, and the results shown in Table 8.1,and Figure 8.8 and Figure 8.9.

Table 8.1:

Pancreatic Enzyme (Amylase and Trypsin) Secretion, Production Times, Zymogen Pool Turnover (TO), and Zymogen Pool Sizes in Controls and Malnourished Patients Before, and After Nutritional Support.

	Controls	Patients before	Patients after
Amylase Secretion (U/h)	2025 (337.9)	636.0 (110.5)*	1845 (267.0)#
Amylase Production (h)	1.16 (0.06)	1.03 (0.09)	0.96 (0.10)
Amylase TO (%/h)	30.99 (3.83)	26.30 (3.72)	23.53 (4.79)
Amylase Pool (U)	6838 (848.4)	2177 (417.2)*	10566 (2031)#
Trypsin Secretion (U/h)	312.7 (40.82)	115.7 (15.75)*	192.2 (16.92)**##
Trypsin Production (h)	0.96 (0.04)	1.25 (0.10)**	0.95 (0.08)##
Trypsin TO (%/h)	35.24 (3.13)	37.88 (5.70)	22.59 (4.29)**+
Trypsin Pool (U)	932.4 (142.3)	379.5 (84.60)*	1027 (176.0)#

Results are illustrated as means (SEM). * $P < 0.001$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.01$ vs. patients before nutritional support, ## $P < 0.05$ vs. patients before nutritional support, + $P = 0.05$ vs. patients before nutritional support.

The enzyme production time represents the minimum time taken for synthesis, and secretion of enzymes. The mean production time of amylase in the malnourished patients was similar to that of the controls (1.03 ± 0.09 vs. 1.16 ± 0.06 h), whereas that of trypsin was significantly delayed (1.25 ± 0.10 vs. 0.96 ± 0.04 h; $P < 0.05$). Zymogen pool turnover rates of both amylase and trypsin in the undernourished patients before feeding were similar to control values (amylase 26.30 ± 3.72 vs. 30.99 ± 3.83 %/h: trypsin 37.88 ± 5.70 vs. 35.24 ± 3.13 %/h). Zymogen pool sizes of both amylase and trypsin were significantly reduced in the undernourished patients (amylase 2177 ± 417.2 vs. 6838 ± 848.4 U; $P < 0.001$: trypsin 379.5 ± 84.60 vs. 932.4 ± 142.3 U; $P < 0.001$).

Following nutritional support, the mean production time of amylase remained unchanged (0.96 ± 0.10 vs. 1.03 ± 0.09 h), whereas there was a significant improvement in the production of trypsin (0.95 ± 0.08 vs. 1.25 ± 0.10 h; $P < 0.05$), to a level similar to the control value. Following re-feeding, the mean amylase zymogen pool turnover rate was unchanged (23.53 ± 4.79 %/h), whereas that of the trypsin zymogen pool was reduced, compared to levels prior to nutritional support (22.59 ± 4.29 vs. 37.88 ± 5.70 %/h; $P = 0.05$), and compared to control values (22.59 ± 4.29 vs. 35.24 ± 3.13 %/h; $P < 0.05$). After the period of nutritional support there was a significant increase in the zymogen pool sizes of both amylase (10566 ± 2031 vs. 2177 ± 417.2 U; $P < 0.001$) and trypsin (1027 ± 176.0 vs. 379.5 ± 84.60 U; $P < 0.001$), to levels not significantly different to control values.

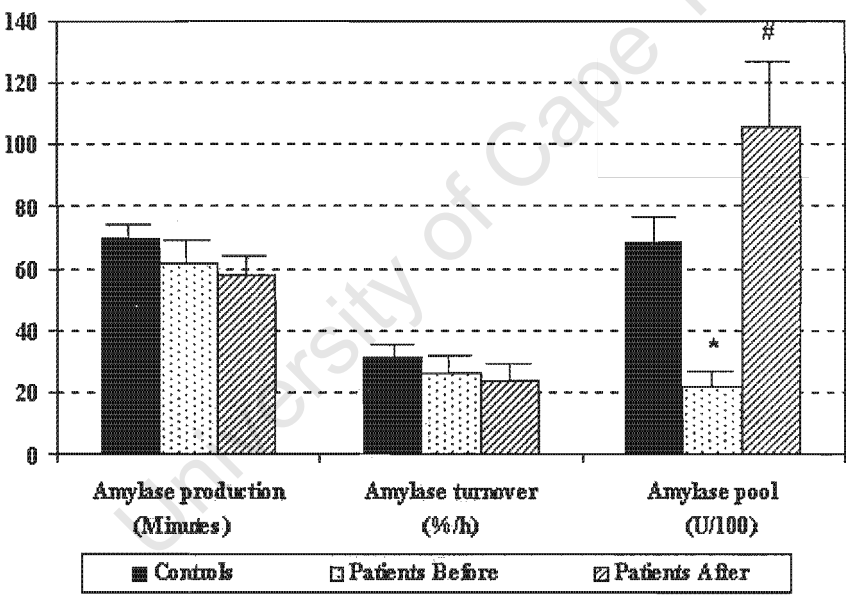


Figure 8.8: Amylase production time, zymogen pool turnover, and zymogen pool size in controls, and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.001$ vs. controls, # $P < 0.01$ vs. patients before nutritional support.

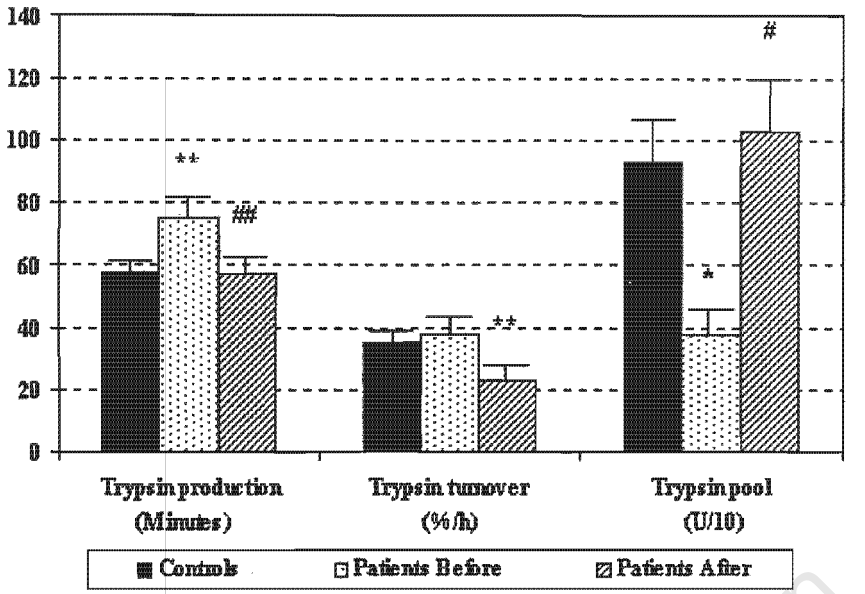


Figure 8.9: Trypsin production time, zymogen pool turnover, and zymogen pool size in controls, and malnourished patients before, and after nutritional support. Bars represent means (SEM). * $P < 0.001$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.01$ vs. patients before nutritional support, ## $P < 0.005$ vs. patients before nutritional support.

8.4.2 Pancreatic Enzyme Synthesis in Anorexia and Disease Patients

The results of the pancreatic enzyme protein synthesis studies performed in the *anorexia patient* group, and in the *disease patient* group prior to nutritional support are shown in Table 8.2.

Table 8.2:

Pancreatic Enzyme (Amylase and Trypsin) Secretion, Production Times, Zymogen Pool Turnover (TO), and Zymogen Pool Sizes in Controls, and in the Anorexia Patient and Disease Patient Groups Before Nutritional Support.

	Controls	Anorexia patients	Disease patients
Amylase Secretion (U/h)	2025 (337.9)	1000 (108.5)**	393.19 (110.8)*#
Amylase Production (h)	1.16 (0.06)	1.00 (0.07)	1.04 (0.13)
Amylase TO (%/h)	30.99 (3.83)	39.38 (5.16)	19.76 (3.48)**#
Amylase Pool (U)	6838 (848.4)	2733 (485.7)**	1868 (610.4)*
Trypsin Secretion (U/h)	312.7 (40.82)	134.2 (24.96)*	103.29 (20.40)*
Trypsin Production (h)	0.96 (0.04)	1.15 (0.13)	1.31 (0.14)**
Trypsin TO (%/h)	35.24 (3.13)	53.36 (10.11)**	29.28 (5.28)##
Trypsin Pool (U)	932.4 (142.3)	278.6 (85.53)*	435.5 (128.1)**

Results are illustrated as means (SEM). * $P < 0.01$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.01$ vs. *anorexia patient* group, ## $P < 0.05$ vs. *anorexia patient* group

In both groups of patients, mean amylase production time was similar to control values (*anorexia patient* group, 1.00 ± 0.07 h; *disease patient* group, 1.04 ± 0.13 h; controls 1.16 ± 0.06 h) (Figure 8.10). Amylase zymogen pool turnover was, however, significantly reduced in the *disease patient* group compared to controls (19.76 ± 3.48 vs. 30.99 ± 3.83 %/h; $P < 0.05$), whereas that in the *anorexia patient* group was similar to the control value (39.38 ± 5.16 vs. 30.99 ± 3.83 %/h). Amylase zymogen pool sizes were significantly reduced in both the *anorexia patient* group and in the *disease patient* group (*anorexia patient* group, 2733 ± 485.7 vs. 6838 ± 848.4 U; $P < 0.02$: *disease patient* group, 1868 ± 610.4 vs. 6838 ± 848.4 U; $P < 0.001$). There was no significant difference between the mean amylase zymogen pool sizes in the two groups

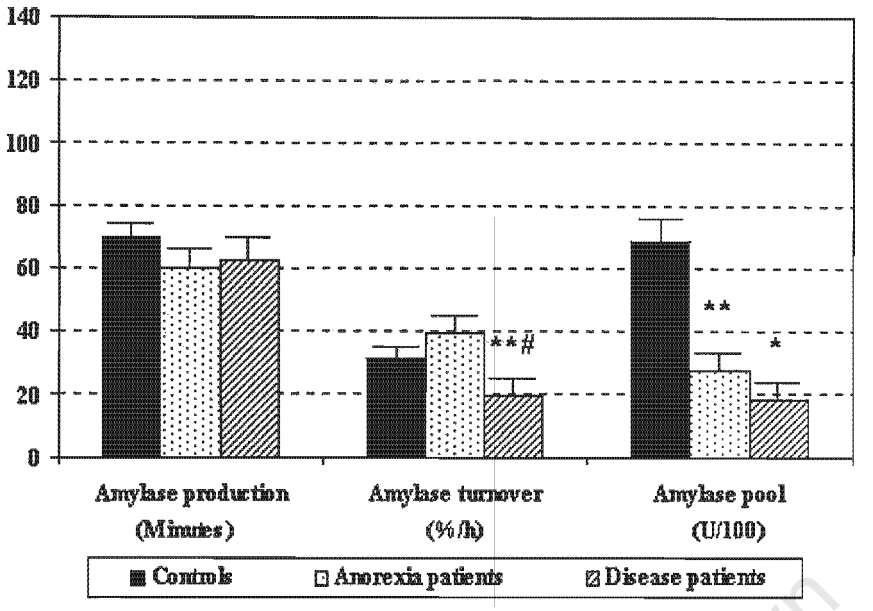


Figure 8.10: Amylase production time, zymogen pool turnover, and zymogen pool size in controls, and in the anorexia patient and disease patient groups before nutritional support. Bars represent means (SEM). * $P < 0.001$ vs. controls, ** $P < 0.05$ vs. controls, # $P < 0.01$ vs. *anorexia patient* group.

Mean trypsin production time in the *anorexia patient* group was not significantly different to control values (1.15 ± 0.13 vs. 0.96 ± 0.04 h), whereas that in the *disease patient* group was significantly delayed (1.31 ± 0.14 vs. 0.96 ± 0.04 h; $P < 0.05$) (Figure 8.11). Trypsin zymogen pool turnover was significantly reduced in the *disease patient* group compared to the *anorexic patient* group (29.28 ± 5.28 vs. 53.36 ± 10.11 %/h; $P < 0.05$), which was, significantly greater than the control value (53.36 ± 10.11 vs. 35.24 ± 3.13 %/h; $P < 0.05$). Trypsin zymogen pool sizes were similar in the *anorexia patient* group and in the *disease patient* group, and significantly reduced compared to controls (*anorexia patient* group, 278.6 ± 85.53 vs. 932.4 ± 142.3 U; $P < 0.01$: *disease patient* group, 435.5 ± 128.1 vs. 932.4 ± 142.3 U; $P < 0.02$).

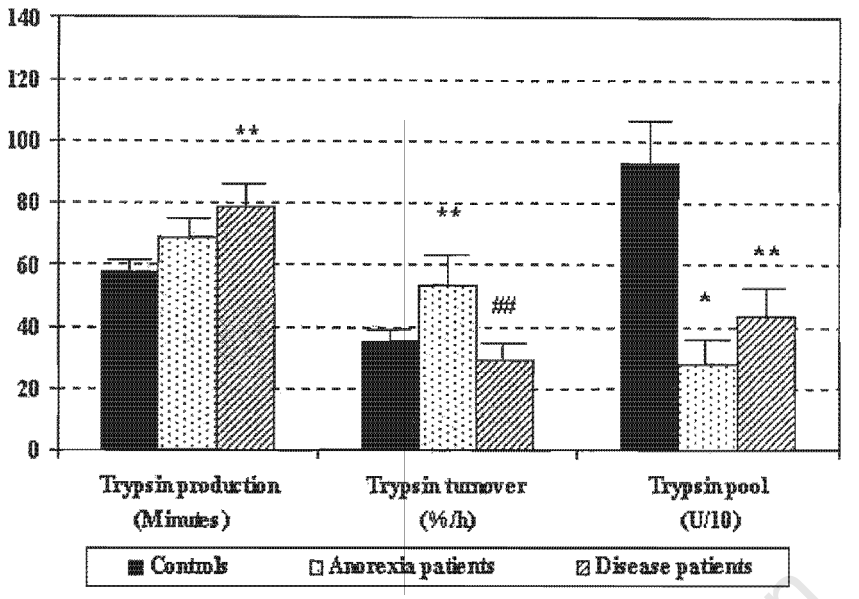


Figure 8.11: Trypsin production time, zymogen pool turnover, and zymogen pool size in controls, and in the anorexia patient and disease patient groups before nutritional support. Bars represent means (SEM). * $P < 0.001$ vs. controls, ** $P < 0.05$ vs. controls, ## $P < 0.05$ vs. *anorexia patient* group.

The results of the pancreatic enzyme protein synthesis studies performed following nutritional support are shown in Table 8.3.

Table 8.3:

Pancreatic Enzyme (Amylase and Trypsin) Secretion, Production Times, Zymogen Pool Turnover (TO), and Zymogen Pool Sizes in Controls, and in the Anorexia Patient and Disease Patient Groups After Nutritional Support.

	Controls	Anorexia patients	Disease patients
Amylase Secretion (U/h)	2025 (337.9)	1526 (380.7)	2140 (403.1)
Amylase Production (h)	1.16 (0.06)	0.91 (0.22)	0.99 (0.10)
Amylase TO (%/h)	30.99 (3.83)	25.52 (5.42)	22.39 (7.15)
Amylase Pool (U)	6838 (848.4)	6675 (1706)	12789 (2777)*
Trypsin Secretion (U/h)	312.7 (40.82)	174.7 (57.71)	201.9 (43.10)
Trypsin Production (h)	0.96 (0.04)	0.90 (0.20)	0.98 (0.06)
Trypsin TO (%/h)	35.24 (3.13)	23.87 (4.92)	21.85 (6.41)**
Trypsin Pool (U)	932.4 (142.3)	739.74 (185.2)	1192 (242.8)

Results are illustrated as means (SEM). * $P < 0.05$ vs. controls, ** $P = 0.06$ vs. controls.

Following nutritional support, mean amylase production times in both the *anorexia patient* group (0.91 ± 0.22 h) and the *disease patient* group (0.99 ± 0.10 h) remained similar to control values (1.16 ± 0.06 h). The amylase zymogen pool turnover rates in both groups were also similar to control values (*anorexia patient* group 25.52 ± 5.42 %/h: *disease patient* group 22.39 ± 7.15 %/h: controls 30.99 ± 3.83). There was a significant increase in the amylase zymogen pool sizes in both groups (*anorexia patient* group 6675 ± 1706 vs. 2733 ± 485.7 U; $P < 0.05$: *disease patient* group 12789 ± 2777 vs. 1868 ± 610.4 U; $P < 0.001$), with the pool size in the *disease patient* group now significantly greater than the control value (12789 ± 2777 vs. 6838 ± 848.34 U; $P < 0.05$).

Following re-feeding, mean trypsin production times in both groups were similar to controls (*anorexia patient* group 0.90 ± 0.20 h, *disease patient* group 0.98 ± 0.06 h, controls 0.96 ± 0.04 h). There remained a trend for reduced trypsin zymogen pool turnover rates in the *disease patient* group (21.85 ± 6.41 vs. 35.24 ± 3.13 %/h; $P = 0.06$). Trypsin zymogen pool sizes were significantly increased in both groups (*anorexia patient* group 739.74 ± 185.2 vs.

278.6 ± 85.53 U; P < 0.05: *disease patient* group 1192 ± 242.8 vs. 435.5 ± 128.1 U; P < 0.02), and similar to the control value (932.4 ± 142.3 U).

8.5 Discussion

From the analysis of the individual plots of isotope incorporation into the pancreatic enzymes amylase and trypsin, considerable information was obtained regarding the synthesis, storage, and release of these enzymes. The results demonstrated that the enzymes secreted during the first hour were unlabelled, and therefore entirely derived from preformed stores. The first appearance of labelled enzyme in the jejunal juice represents the minimum time taken for the production of new enzyme. The studies indicated that the time taken for production of amylase in the malnourished patients was similar to that in control subjects, whereas that of trypsin was significantly prolonged. This time reflects the composite of the time taken for uptake of amino acid in the precursor pool into the enzyme protein synthesised on the ribosomes of the cell, the subsequent processing in the golgi body, transport into the zymogen pool, and the eventual secretion from the cell. Since the pancreatic ductal volume is only 4 ml, and the mean rate of flow of pancreatic juice has been reported at 3.5 ml/min,[424] the major portion of this time will represent intracellular events associated with enzyme synthesis.[420] Using the present technology we are, unfortunately, unable to determine precisely where in the cascade of intracellular events, the delay is actually occurring.

The rate of increase of specific activity in the secreted enzyme thereafter represents the rate of turnover, or replacement, of the zymogen pool. This rate depends on both the rate of entry of newly synthesised enzyme into the pool, as well as the size of the zymogen pool. Measurement of both the rate of replacement of the pool (%/h), as well as the actual secretion of enzyme (U/h) during the time of isotope incorporation allowed calculation of the size of the zymogen pool. The results indicated a marked reduction in zymogen pool sizes in malnourished patients, with that of amylase reduced to 32% of the control value, and trypsin pool reduced to 41%. These findings are in keeping with the histological evidence of pancreatic atrophy documented in autopsy studies of malnourished patients.[183-188] Although the zymogen pool turnover rates of the malnourished patients were similar to the controls, in view of the significantly reduced pool sizes, this indicates impairment of the rates of synthesis of both amylase and trypsin.

These findings complement, and extend the findings of our studies of CCK-8 stimulated pancreatic enzyme secretion, where the profile of secretion in normal controls suggested initial release of enzyme from preformed stores, with a constant release of enzymes thereafter. The constant release of enzyme, in a steady state, is likely to parallel the synthesis of new enzyme. The rate of release of enzyme during that period therefore probably reflects the rate of synthesis of new enzyme. In the malnourished patients, the profile of the release of enzymes suggested decreased zymogen stores, and this was confirmed in the synthesis studies.

Following nutritional support, the production time of amylase remained unchanged, whereas that of trypsin improved significantly to a level similar to the control value. There was a significant improvement in the zymogen pool sizes of both amylase and trypsin, to levels similar, or in the case of amylase, slightly higher than controls. The zymogen pool turnover rate of amylase was unchanged from the pre-feeding level, and similar to the control value. However, in view of the marked increase in pool size, this indicates that the rate of amylase synthesis had increased, and was now normal. This was reflected by the normal rate of secretion of amylase documented in these patients after re-feeding. Trypsin pool turnover, on the other hand, was now significantly slower than the control value, indicating that despite restitution of the trypsin pool, synthesis rates remained impaired. The impaired secretion of trypsin in the malnourished patients demonstrated after re-feeding, although significantly improved compared to the pre-feeding values, is likely to reflect this continued reduced synthesis..

The studies of pancreatic enzyme secretion indicated that the secretion of the three enzymes measured were decreased proportionately in relation to nutritional status, although, in the anorexia patients, lipase appeared relatively less affected than amylase or trypsin. The period of nutritional support resulted in a non-parallel restitution of secretion, and this is confirmed in the synthesis studies. Synthesis and secretion of amylase appears to be positively induced, with rates recorded higher than anticipated from the improvement in nutritional status, whereas restitution of trypsin secretion appeared somewhat retarded. Studies in rats have indicated that dietary manipulation can result in non-parallel enzyme synthesis. High carbohydrate diets have been reported to result in increased synthesis of amylase,[425,426] whereas protein-rich diets induced protease.[427,428] Pancreatic secretogogues, such as cholecystokinin have also been reported to result in non-parallel pancreatic enzyme synthesis

in rats,[429-431] and in humans.[432] However, the rehabilitation diets administered to the patients were considered "balanced", and our malnourished patients received identical pancreatic stimulation to our control subjects. The reason for the non-parallel restitution of pancreatic enzymes in the malnourished patients therefore remains unclear. It is tempting to speculate that as trypsin is a proteolytic enzyme, excessive induction of synthesis may predispose to premature activation within the pancreatic parenchyma, with subsequent development of pancreatitis. It is possible that the relative tardiness of restitution of trypsin synthesis and secretion actually represents a protective mechanism. It is interesting that pancreatitis and pancreatic calcification have been documented in association with malnutrition in certain tropical regions (tropical pancreatitis).[433-435] It would therefore be of interest to study the production of pancreatic protective factors, such as trypsin inhibitor in relation to malnutrition and re-feeding.

The studies of the *anorexia patient* and *disease patient* groups demonstrated similar reduction in zymogen pool sizes of amylase and trypsin in the two groups. Zymogen pool turnover rates were, however, significantly impaired in the *disease patient* group, indicating that enzyme synthesis was more affected in the *disease patient* group than in the *anorexia patient* group. The significant reduction of amylase secretion in the *disease patient* group during the period of isotope incorporation, compared to the *anorexia patient* group, possibly reflects this impairment of synthesis. The increased trypsin pool turnover noted in the *anorexia patient* group, compared to controls, is likely to represent the marked reduction in pool size, resulting in a relatively faster turnover rate. Nutritional support resulted in significant increases in zymogen pool sizes of both amylase and trypsin in both groups, with the pool sizes returning to levels equivalent, or greater (in the case of amylase in the *disease patient* group) than the controls. In both groups, amylase pool turnover rate was similar to the control value indicating that amylase synthesis was now normal. Trypsin turnover, particularly in the *disease patient* group, was reduced indicating continued impaired synthesis of enzyme.

These results indicate that the reduced secretion of pancreatic enzymes in malnourished patients, documented under conditions of CCK-8 stimulation, is a consequence of impaired pancreatic enzyme protein synthesis and reduced zymogen stores. They also provide further evidence of the additional adverse effect of co-existent disease on digestive function. The non-parallel restitution of pancreatic enzyme synthesis and secretion following nutritional support is of interest, and requires further study.

CHAPTER 9

CONCLUDING SUMMARY AND CLINICAL APPLICATION

Nutrition and digestive function are intimately interrelated. Normal digestive function is dependent on an adequate supply of digestive enzymes, and an efficient absorptive mucosal surface. Production of these enzymes, and maintenance of the mucosal surface are, in turn, dependent on an adequate supply of nutrients. This thesis investigates this cycle of events, and considers the effects of severe malnutrition (undernutrition), and subsequent re-feeding on digestive function in human subjects.

Patients entered into the study were severely malnourished with a mean body mass index (BMI) of 13.39 kg/m^2. Results indicated that this degree of undernutrition was associated with significant impairment of digestive function. The patients had evidence of impaired gut absorption, as assessed by D-xylose absorption and in-vivo fat absorption, and villous atrophy was present in 36% of cases. Studies of gastro-duodenal mucosal protein fractional synthesis demonstrated the rapid mucosal turnover in the gut, with the mucosa replaced every 3-4 days. This is likely to result in substantial demand on the body's resources. Villous growth and absorptive cell function are dependent on nutrition, which may explain the observations of partial villous atrophy and decreased D-xylose and fat absorption documented in the undernourished patients. Although no significant difference was noted in mucosal protein fractional synthesis between undernourished patients and control subjects, there was evidence that the co-existence of overt disease had an adverse effect on mucosal protein synthesis in undernourished patients. The role of nutrition in the restoration and maintenance of gut mucosal function was illustrated by the marked trophic effect, particularly on the duodenal mucosa, following the period of intensive nutritional support, with improvement in villous atrophy, and gut absorptive function.

Malnutrition was associated with significant impairment of gastro-pancreatic secretion. Basal achlorhydria was evident in 70% of the undernourished patients, and stimulated gastric acid output (maximum acid output) was reduced to 26% of normal. Secretion of the pancreatic enzymes amylase, lipase and trypsin was reduced to 36% of normal. The

reduction of both basal, and stimulated gastric acid secretion in the malnourished patients is likely to affect the initiation of the digestive process, and predispose to bacterial overgrowth, with further interference of digestion and intestinal transit. Although the human digestive system has considerable reserve, 42% of the malnourished patients had lipase levels reduced more than 90%, a level that has been associated with fat malabsorption.[415] However, in patients with co-existent gut mucosal dysfunction, consequent to disease or to the malnourished state, more modest impairment of pancreatic enzyme secretion may be sufficient to result in malabsorption. The correlation between lipase output and the parameters of malabsorption (stool frequency and faecal fat excretion), indicated that pancreatic enzyme insufficiency was contributing significantly to the maldigestion and malabsorption documented in these patients.

Although there was evidence of gut mucosal dysfunction, which may affect enteral stimulation of gastric and pancreatic secretion, the studies indicated that the reduction of gastric acid and pancreatic enzyme output in undernourished patients was principally consequent to primary gastric parietal and pancreatic acinar cell dysfunction. The decreased pancreatic enzyme secretion was associated with impaired synthesis of enzyme protein, and reduction of zymogen stores to 32% and 41% of normal.

The group of patients with anorexia nervosa (*anorexia patient* group) are of special interest. Despite having similar severity of malnutrition to the group of patients with associated overt disease (*disease patient* group), as determined by the body mass index (BMI), and similar impairment of gut absorptive function as assessed by D-xylose absorption, they did not demonstrate clinical evidence of digestive dysfunction. The digestive system, therefore, has considerable reserve, and at least at the level of malnutrition studied, the clinical manifestation of the adverse effects of malnutrition on the digestive system appeared to relate to the presence of co-existent disease. Gastric acid output was reduced in both groups, although significantly more so in the *disease patient* group, and the secretion of the pancreatic enzymes amylase and trypsin were similarly impaired. Lipase output, although significantly reduced in the *disease patient* group, was relatively preserved in the *anorexia patient* group. Lipase output correlated with both faecal fat excretion and stool frequency, and the preservation of secretion in the anorexia patient group may explain the lack of clinical expression of digestive dysfunction in these patients.

The reason for the relative sparing of lipase secretion noted in the *anorexia patient* group, as opposed to the *disease patient* group, is unclear. Studies indicated impaired whole-body protein flux and synthesis, expressed in relation to body weight, in the *disease patient* group, but not in the *anorexia patient* group suggesting that the metabolic consequences of the added burden of co-existent disease were adversely affecting protein synthesis in these patients. This may explain the differences in the rates of gut mucosal protein turnover, and the production of pancreatic enzymes between these two groups.

It should be noted, however, that there might be other differences associated with undernutrition due to anorexia nervosa, and that associated with disease, which may contribute to the differences noted between the two groups. The negative energy balance in anorexia may be associated with a high quality diet, consumed in insufficient quantities, whereas that of the disease patients may have been deficient in both quantity and quality. The role of the nature of the antecedent diet, with the possibility of the development of specific micronutrient deficiencies resulting in the various physiological abnormalities noted in malnourished patients requires further investigation.

Following the initial studies, the patients received intensive nutritional support, which was initiated using a semi-elemental, hydrolysed formulation (Vital or Alitraq), and subsequently a polymeric formulation (Ensure). Four patients required a period of parenteral nutrition. Intensive nutritional support resulted in significant improvement in body mass index, gut absorption, gastric and pancreatic secretion, and protein synthesis. Alitraq contains additional glutamine, however, there was no significant difference in the results of the groups who initially received Vital, Alitraq, or parenteral nutrition.

The improvements in mucosal absorption, and in the secretion of gastric acid and pancreatic enzymes in the patients following intensive nutritional support, indicate that the dysfunction was nutritionally mediated. Although histological studies of the pancreas in patients who died of malnutrition have demonstrated fibrotic change,[184,187,188] implying irreversibility, our results indicate significant functional disturbance of both gastric acid and pancreatic secretion. The studies demonstrated that re-feeding resulted in improvement in enzyme synthesis, and restitution of zymogen stores. Amylase and lipase secretion was generally quickly restored with nutritional support, whereas trypsin and gastric acid output, although significantly improved, remained impaired compared to normal values. The reason for this

non-parallel restitution of synthesis and secretion remains unclear, and demands further study. It is interesting that the production time of trypsin, but not of amylase was impaired in undernourished patients and this feature may, in some way, relate to the subsequent non–parallel restitution of secretion following nutritional support. Full recovery of gastric acid secretion may depend on the time taken to regenerate the parietal cell mass.

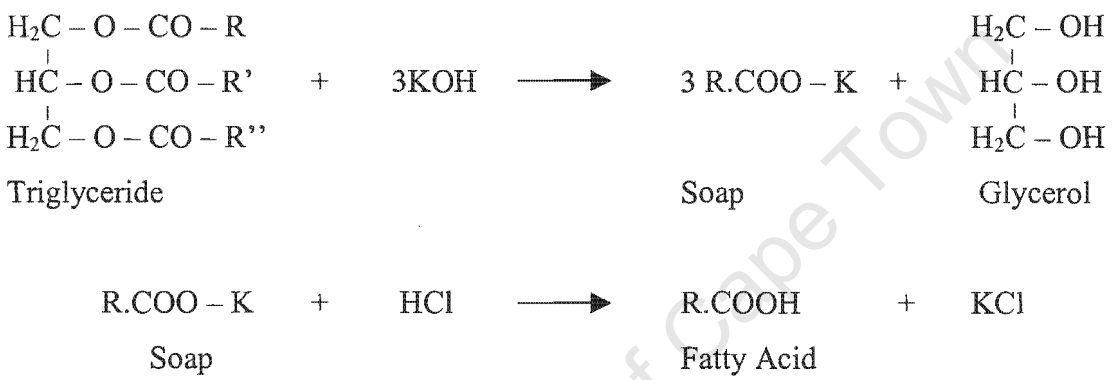
These findings of significant gut mucosal dysfunction, and impaired digestive enzyme activity associated with severe undernutrition have special relevance with regard to the management of such patients in hospital, and may help explain some of the problems, such as diarrhoea, occurring on attempting to feed such individuals. Because of the variability in digestive reserve, it is important to attempt an initial trial of oral feeding once critical fluid and electrolyte disturbances have been corrected. Intolerance, with exacerbation of diarrhoea predicts the possibility of a vicious cycle of malnutrition, malabsorption and diarrhoea. In many cases, the diarrhoea will gradually subside, but in those in whom it continues, specialised feeding techniques will need to be implemented in order to break the cycle, and prevent further deterioration. Digestive capacity could be maximised by converting the food supply to a continuous infusion of a liquid formula diet. In order to make allowances for defects in enzymic digestion, the use of a hydrolysed or semi-digested (semi-elemental) diet should be considered. Intravenous feeding may become necessary if diarrhoea persists despite the use of pre-digested formulas. Nevertheless, as demonstrated by three of the four patients in the study who required parenteral nutrition, the need for intravenous feeding is generally of short duration, as the recovery of digestive function appears to be relatively rapid.[198] Some form of oral feeding should be continued during intravenous feeding, despite continuing malabsorption and diarrhoea, because topical nutrients are the greatest stimulus to mucosal growth and recovery.[382]

The observations of this thesis illustrate the interplay of multiple factors in the normal functioning of the human digestive system, which are adversely affected by a poor nutritional state and the presence of associated disease. Malabsorption may be a consequence of combined defects in enzyme secretion, nutrient digestion, mucosal absorption and intestinal transit. Disruption of the intimate interrelationship between the various processes may result in a vicious cycle of malnutrition, malabsorption, diarrhoea, and further exacerbation of the malnourished state.

APPENDICES

Appendix 1. Determination of Fatty Acids

Determination of total fatty acids in a sample involves saponification in alcoholic potassium hydroxide under reflux. The fatty acids are liberated from the soaps by acidification and extracted into toluene. The extracted fatty acids are then titrated with standard alkali.



Free fatty acids in the samples are determined by direct extraction into toluene, and titration with standard alkali.

Reagents:

1. **Potassium hydroxide** (KOH) 66% $^{w}/_{v}$ aqueous
2. **Ethyl/amyl alcohol** 96% ethyl alcohol + 0.4% amyl alcohol
3. **Ethyl alcohol** 96% (neutral to thymol blue)
4. **Hydrochloric acid** (HCl) 77% $^{v}/_{v}$ (equivalent to 28% $^{w}/_{v}$)
5. **Toluene A.R.**
6. **Thymol blue** 0.2% $^{w}/_{v}$ in 50% ethanol
7. **Sodium hydroxide** (NaOH) 0.1 N
8. **Triolene control** (glycerol trioleate B.D.H. 1 ml = 3.118 mEq oleic acid)

Method:

A. Saponification and Extraction

1. Duplicate reagent blanks (10 ml water) and duplicate triolene controls are processed with each batch of determination.
2. The CONTROLS are set up as follows. 0.10 ml glycerol trioleate is washed into a flask containing 10 ml alcohol by means of a 0.1 ml TC pipette, 10 ml water and 5 ml KOH are then added.
3. To the BLANKS and the TESTS (1 ml infusate/aspirate) add 5 ml 66% KOH and 40 ml ethanol containing 0.4% amyl alcohol.
4. All the flasks are brought to the boil, and boiled for 20 minutes under reflux. They are then cooled under the tap, and then, while still cooling in water, 15 ml 77% HCL are slowly added.
5. To each flask, add 50 ml toluene from a 100 ml burette. Stopper the flask and shake vigorously by hand for 1 minute, and then allow to stand for 10 minutes for the phases to separate.

B. Titration of Fatty Acids

1. 25 ml of the upper toluene layer is transferred to a clean flask using a bulb pipette and propipette. The toluene is evaporated to dryness on a boiling water bath under reduced pressure and the residue is dissolved in 10 ml ethyl alcohol.
2. The standard 0.1 NaOH is titrated at least twice against the standard oxalic acid with thymol blue as indicator.
3. All the flasks are then titrated against the standard alkali using 2 drops thymol blue as indicator.

C. Calculation:

1. The titration value for the reagent blank is subtracted from those obtained for the controls and tests.
2. The concentration of fatty acids is calculated as follows:

$$\text{Fatty acids (mmol/l)} = N \times V \times \frac{50}{25} \times 1.19 \times 10^3$$

where:

N = Normality of NaOH

V = Volume of titration (- blank)

The factor 1.19 is an empirically determined correction factor which compensates for the fact that the volume of toluene is increased due to solution of ethanol in it, and for the fact that the extraction of fatty acid from alcohol-water by toluene in one step is incomplete.

Appendix 2. Assay of Polyethylene Glycol

Polyethylene glycol, molecular weight 4000, (PEG 4000) is a non-absorbable marker, used to determine recovery of duodenal juice aspirated from the upper gastro-intestinal tract. The principal of the assay is based on the creation of an oil-in-water emulsion of the water soluble PEG, when trichloroacetic acid (TCA) is added in the presence of the divalent barium ion. The turbidity of the emulsion is then read spectrophotometrically at 650 nm. Substances, such as proteins and sulphates may interfere with the estimation, and it is therefore necessary to precipitate these materials with barium chloride, barium hydroxide and zinc sulphate, and remove them by filtration before the TCA-barium chloride mixture is added.

Reagents:

1. **Barium Chloride** ($BaCl_2.2H_2O$)
   0.41 M (97.7 g/l)
   Store at room temperature.
2. **Barium Hydroxide ($Ba(OH)_2.8H_2O$)**
   0.18 M (56.7 g/l)
   Store at room temperature.
3. **Zinc Sulphate ($ZnSO_4.7H_2O$)**
   0.17 M (48.8 g/l)
4. **TCA/Barium Chloride mixture**
   TCA 1.84 M (300 g/l)
   BaCL2 0.21 M (50 g/l)
   To make up 500 ml: Dissolve 25 g $BaCl_2$ in 200 ml H_2O and add 300 ml 50% TCA.

5. **Standards**

Standard solution: 0.25 g PEG 4000/100 ml distilled water

Standard curve:

	Vol Std. (ml)	Vol H_2O	g/l
Blank	0.0	1.0	0.00
S1	0.1	0.9	0.25
S2	0.2	0.8	0.50
S3	0.3	0.7	0.75
S4	0.4	0.6	1.00
S5	0.5	0.5	1.25

6. **Samples**

0.2 ml sample + 0.8 ml H_2O

Method:

1. Into a test tube pipette:

   10 ml distilled H_2O

   sample/standard + H_2O

   1 ml $BaCl_2$

   2 ml $Ba(OH)_2$

   2 ml $ZnSO_4$

2 Vortex the mixture, and maintain at room temperature for 15 minutes to allow complete precipitation.

3. Filter through Whatman No. 1 filter paper.
4. Mix equal volumes of the filtrate and a solution of TCA/$BaCL_2$.
5. After 5 minutes exactly, read turbidity at 650 nm.
6. Standards, infusates and samples are read against the blank.
7. A standard curve is plotted, and sample and infusate concentrations are read off, and multiplied by 5 to get g/l.

Appendix 3. Determination of Amylase Activity

A known quantity of starch is digested by amylase in the sample of duodenal juice. The amount of starch remaining, as determined by its reaction with iodine, is used to calculate the amount digested, which is proportional to the amount of amylase in the sample. One Pimstone unit (U) of amylase activity = mg starch digested by 1 ml duodenal juice in 30 min at 37° C.

Reagents:

1. **Stock Starch** (Analar 2%)
   a. Dissolve 2 g soluble starch in 60 ml distilled H_2O.
   b. Boil, constantly stirring.
   c. Add 2 g NaCl.
   d. Pour into 100 ml volumetric flask, rinsing containerAdd 5 drops toluene, and make up to 100 ml with distilled H_2O.
   e. Store at 4°C.
2. **Starch (Phosphate) Buffer** (0.1 M, pH 7)
   a. Make up 0.1 M $NaH_2PO_4 \cdot 2H_2O$ solution (15.6 g/l).
   b. Make up 0.1 M NaH_2PO_4 solution (14.2 g/l).
   c. Mix ± 450 ml (a) with ± 1000 ml (b) until pH + 7.0.
3. **Buffered Starch Solution**
   a. Boil 15 ml stock starch + 35 ml starch buffer until clear.
   b. Store at 4°C for up to 4 days.
4. **5% H_2SO_4**
   Add 25 ml conc. H_2SO_4 to 475 ml H_2O.
5. **1.5% NaCl**
   Dissolve 30 g NaCl in 2000 ml distilled H_2O.
6. **Iodine** (1:100)
   a. 1:10 stock solution. Mix 12.7 g I_2 + 40.0 g KI and make up to 1000ml.
   b. 1:100 working solution. Using 10 ml of 1:10, make up to 100 ml with distilled H_2O.

c. Store in dark bottle at 4°C.

Method:

1. Set the waterbath to 37°C and the spectrophoyometer to 660 nm, UV source off.
2. Boil starch buffer solution (reagent 3).
3. Label 4 x 50 ml glass test tubes per sample (2 per test and 2 per blank).
4. Label one 200 ml volumetric flask per sample.
5. Dilute duodenal juice sample 1 in 200 with 1.5% NaCl.
6. Pipette 1 ml of buffered starch in all tubes (blanks and samples).
7. Leave in waterbath to equilibrate at 37°C.
8. Add 2 ml of 5% H_2SO_4 to both blanks of each sample (to prevent activity).
9. At exactly timed intervals (30 secs) add 0.1 ml from each sample of diluted duodenal juice to all 4 tubes (2 samples and 2 blanks) for that sample.
10. Incubate for exactly 30 mins (10 mins for high activity).
11. At exactly timed intervals add:
    a. To Sample Tubes:

       2 ml of 5% H_2SO_4 (to stop enzyme activity), a dash of distilled H_2O, 1 ml iodine working solution, then make up to 50 ml mark with distilled H_2O and invert the tube.

    b. To Blanks:

       Add a dash of distilled H_2O, 1 ml iodine working solution, and then make up to 50 ml mark with distilled H_2O and invert the tube.
12. At the spectrophotometer:
    a. Place H_2O in the reference cuvette.
    b. Place H_2O in the sample curvette and set A_{660} to zero.
13. 30 mins after addition of iodine, record readings of blanks (B), and samples (S).

Calculation:

$$\text{Amylase activity (Pimstone units)} = \frac{B - S}{B} \times 6 \times \frac{30}{T} \times \frac{FV}{1} \times \frac{1}{D}$$

Where

B	=	A_{660} of Blank (reading for 6 mg starch in I ml)
S	=	A_{660} of Sample
6	=	mg starch in control sample
30	=	minutes
T	=	incubation time (minutes)
FV	=	dilution factor of sample
D	=	volume of diluted juice

Appendix 4. Determination of Lipase Activity

$$\text{Triglycerides} \xrightarrow{\text{lipase}} \text{Di- and Monoglycerides} + \text{Fatty Acids}$$

Triolein (olive oil) with a bile acid (to act as an emulsifier) are incubated with the sample containing lipase. After removal of the samples from the 37°C waterbath, the reaction is stopped by the addition of ethanol containing phenolphthalein and thymolphthalene indicators. The liberated fatty acids are detected by titration against NaOH. Lipase activity is expressed as the amount of fatty acid (expressed as µmol) liberated in one minute at 37°C (International Units IU).

Reagents:

1. **Buffer 0.05 MTris pH 8.0**
   a. Weigh 6.057 g Tris (hydroxymethyl) methylamine and make up to 700 ml with distilled H_2O.
   b. Add ± 2 ml conc. HCl to make pH 8.0 Make up to 1000 ml with distilled H_2O.
2. **NaOH**
   a. Make 1000 ml of 1.0 M NaOH using Titrosol reagent in ampule.
   b. Dilute on day of use to 0.05 M (10 ml to 200 ml).
3. **pH Indicator in ethanol**
   a. Conc. 2% thymolphthalein
      2 g thymolphthalein in 100 ml 96% ethanol.
   b. Working solution
      Take 20 ml of (a) and make up to 250 ml with 96% ethanol.
      Add 0.25 g phenolphthalein.
4. **20% Sodium Taurocholate (NaT)**
   Weigh 20 g and make up to 100 ml with distilled H_2O.

5. **Substrate** (Olive Oil)
   a. Weigh 37 g gum acacia (emulsifies allowing mixing of the substrate solution) and 1 g sodium benzoate (preservative).
   b. Add these to 500 ml distilled H_2O in a wide necked reagent bottle.
   c. Shake well and hold under hot water.
   d. Take care not to allow solids to adhere to the bottom.
   e. Mix for 10 minutes with ultra turrax, then allow to stand for 5 minutes.
   f. Add 500 ml olive oil in three separate portions, mixing for 10 minutes every time and cooling between.
   g. Store in dark bottle in fridge.
6. **1.5% NaCl**

   Weigh 30 g of NaCl and make up to 2000 ml with distilled H_2O.

Method:

1. Mix substrate and buffer 1:1 and ultraturrax for 3 minutes.
2. Prepare sample dilutions on ice.
   a. Make 1:10 dilution in 1.5% NaCl (0.5 ml sample + 4.5 ml 1.5% NaCl). Vortex.
   b. Take 0.5 ml from each 1:10 dilution and add to 4.5 ml 1.5% NaCl to give 1:100 dilution. Vortex.
3. Prepare tubes for test
   a. Using a 1 ml graduated pipette, place 0.1 ml NaT into all tubes.
   b. Add 1 ml pH indicator to 2 blanks for each sample.
   c. Pipette 0.2 ml of 1:100 sample dilution into tubes.
   d. Add 3 ml of substrate-buffer mix to all tubes.
   e. Vortex and incubate for 30 minutes at 37°C.
   f. Make up to 0.05 M NaOH and flush titrator (whilst incubating samples).
   g. Remove rack from waterbath and place in ice.
   h. Add 1 ml pH indicator to all samples (not to blanks).
   i. Titrate all tubes with 0.05 M NaOH until colour changes from white to pink, matching the colour or each pair of samples to blanks (N.B. titrate blanks before the samples).

Calculations:

$$\text{Lipase activity (IU/l)} = T - B \times \frac{100}{1} \times \frac{1000}{0.2} \times \frac{1}{30} \times 50$$

where:

$\frac{100}{1}$	=	Sample dilution
$\frac{1000}{0.2}$	=	Sample volume (l)
$\frac{1}{30}$	=	To express results per minute
50	=	50 µmol per ml NaOH

Appendix 5. Determination of Trypsin Activity

The protease trypsin splits N-benzoyl –L-arginine ethyl hydrocbloride (BAEE) to produce N-benzoyl –L-arginine (BA) and ethyl hydrochloride (EE).

$$\text{BAEE} \xrightarrow{\text{trypsin}} \text{BA} + \text{EE}$$

At 253 nm, BA has a higher absorbance than BAEE. As the enzymic action continues, and the increase in absorbance may be plotted on a graph, from which the enzyme activity may be determined.

Reagents:

1. **Substrate: BAEE (N-benzoyl-L-arginine ethyl hydrochloride)**
   0.25 mM BAEE in 0.05 M phosphate buffer, pH 8.0
2. **0.001 N HCl**
3. **Phosphate buffer**
   0.05 M sodium phosphate buffer, pH 8.0
4. **Standard:**
   0.2 mg/ml 0.001 M HCl Serazac trypsin (3174 BAEE U/mg material)

Method:

1. Dilute sample appropriately (1:5 – 1:40) with 0.001 N HCl.
2. Place the following in curvettes at 25°C and mix:

	Control	Reference
Substrate	3.0 ml	3.0 ml
0.001 N HCl	0.2 ml	

3. Set the wavelength of the spectrophotometer at 253 nm. Place the reference curvette in the reference position, and the control curvette in the sample position. Adjust the absorbance to read 0.05. Set the recorder correspondingly.

4. Place 3.0 ml substrate and 0.2 ml standard in a curvette, mix and place in the sample position. Start recorder, let it run for 3 minutes after a straight line is achieved.
5. The samples are assayed in the same way as the standard, with each assay repeated.
6. The machine is reset at 0.05 with each assay and the graph reset.

Calculation:

$$\text{Trypsin activity (BAEE U/ml)} = \frac{\Delta A\text{sample}}{0.2} \times \text{dilution factor} \times 0.003$$

where:

ΔAsample	=	change in absorbance of sample per minute at 253 nm
0.2	=	volume of sample (ml)

Appendix 6. Determination of Plasma Leucine Specific Activity

Plasma leucine activity is determined by first deproteinising the plasma sample, then measuring the specific activity of the deproteinised plasma by liquid scintigraphy, and finally determining the concentration of leucine in the sample.

1. Collect 10 ml heparinised blood.
2. Spin at 3000 rpm for 10 minutes.
3. Aspirate plasma into clean mailing vials.
4. Store plasma frozen.
5. Defrost plasma and spin at 300 rpm for 10 minutes.
6. Pipette 2 ml plasma into a clean 5 ml tube.
7. Add 40 µl 10 mM homocysteic acid (internal standard). Mix by vortexing.
8. Add 50 mg 5-sulphosalicylic acid. Mix by vortexing. Allow to stand for 10 minutes.
9. Freeze samples (-20°C) overnight.
10. Thaw samples. Mix well and spin at 2500 rpm for 15 minutes.
11. Decant supernatant and filter through a 0.45 µ filter into a 1.5 ml Eppendorf tube.
12. Pipette 0.5 ml of filtrate into a counting vial. Add 0.33 ml hydrogen peroxide and incubate at 37°C for 1 hour.
13. Add 7 ml Instagel, and count ^{14}C DPM for 10 minutes.
14. Determine leucine concentration of filtrate by HPLC (Appendix 10).
15. Leucine specific activiy = DPM/nmol leucine.

Appendix 7. Determination of Mucosal Protein Specific Activity

The method involves hydrolysis of the mucosal protein to constituent amino acids. The specific activity (^{14}C) is then determined by liquid scintigraphy. Leucine concentration of the sample is then determined by HPLC, and the specific activity of the incorporated leucine calculated (DPM/nmol leucine).

1. Wash biopsy in 5 ml 0.9% NaCl.
2. Homogenise biopsy in 2 ml 0.2% Triton X100 in 0.95 NaCl.
3. Add 2 ml 105 TCA to homogenate.
4. Freeze at –20°C.
5. Thaw specimen.
6. Spin at 3000 rpm for 10 minutes. Discard supernatant.
7. Using a glass rod, re-suspend precipitate in 4 ml 10% TCA. Spin at 3000 rpm for 10 minutes. Discard supernatant.
8. Repeat step 7 twice more.
9. Add 2 ml 6 N HCl and 50 µl 10 mM L-homocysteic acid (internal standard) to sample.
10. Transfer sample to a 4 ml pyrex vial.
11. Seal vial tightly and heat for 24 hours at 100°C.
12. Allow hydrolysate to cool to room temperature and transfer to 50 ml glass tube.
13. Add 20 ml absolute alcohol to each sample and evaporate the hydrolysate to dryness under nitrogen. Add 20 ml absolute ethanol to the sample and re-dry.
14. Dissolve the sample in 1 ml distilled H_2O and filter through a 0.45 µm filter.
15. Add 0.5 ml of the sample to 7 ml Instagel and count ^{14}C DPM for 10 minutes.
16. Determine leucine concentration by HPLC (Appendix 10).
17. Mucosal protein specific activity = DPM/nMol leucine.

Appendix 8. Purification of Amylase and Trypsin from Duodenal Juice

The pancreatic enzymes amylase and trypsin are extracted, and purified, from duodenal juice using agarose affinity columns, with the ligands acarbose (amylase) and anti-trypsin antibody (trypsin).

Reagents:

1. **Amylase buffer #1:** 0.02 M NaH_2PO_4/Na_2HPO_4 (pH 7.0), 0.5 M NaCl 0.2% aprotinin (Midran or Trasylol)
   a. Dissolve 145 g NaCl in ± 4 litre H_2O.
   b. Add 6.7 ml 85% H_3PO_4.
   c. Adjust pH to 7,0 with 10 N NaOH.
   d. Adjust volume to 5 litres.
   e. Add 6 ml aprotonin (Midran or Trasylol).

2. **Trypsin buffer #1:** 0.02 M NaH_2PO_4/Na_2HPO_4 (pH 6.0), 0.5 M NaCl
   a. Dissolve 145 g NaCl in ± 4 litre H_2O.
   b. Add 6.7 ml 85% H_3PO_4.
   c. Adjust pH to 6,0 with 10 N NaOH.
   d. Adjust volume to 5 litres.

3. **Amylase buffer #2:** 0.02 M NaH_2PO_4/Na_2HPO_4 (pH 7.0), 0.15 M NaCl
   a. Dissolve 8.7 g NaCL in ± 900 ml H_2O.
   b. Add 1.33 ml 85% H_3PO_4.
   c. Adjust pH to 7.0 with 10 N NaOH.
   d. Adjust volume to 1 litre with H_2O.

4. **Trypsin buffer #2:** 0.025 M sodium citrate (pH 2.5), 0.025 M $CaCL_2$
   a. Dissolve 5.3 g citric acid and 3.7 g $CaCl_2$ in ± 900 ml H_2O.
   b. Adjust pH to 2.5 with 10 NaOH.
   c. Adjust volume to 1 litre with H_2O.

5. **0.1 NaOH**
   a. Dissolve 4 g NaOH in ± 900 ml H_2O.
   b. Adjust volume to 1 litre with H_2O.

Methods:

1. Thaw two 50 ml samples of duodenal juice which have been preserved with aprotinin (Midrand or Trasylol).
2. Dialyse each sample against litre 0.02 M NaH_2PO_4/Na_2HPO_4 (pH 7.0) containing 0.5 M NaCl and 0.2% aprotinin (amylase buffer #1).
3. Pack two columns (1.0 x 12 cm) each with 10 ml amylase affinity gel. (Amylase affinity gel consists of -amino hexyl agarose to which a spacer, N-(p-aminobenzoyl)-6-caproic acid, has been bound, and then the ligand acarbose).
4. Pack another two columns (1.0 x 12 cm) each with 10 ml trypsin affinity gel. (Trypsin affinity gel consists of 4% beaded agarose to which soybean trypsin inhibitor (STI) has been bound).
5. Equilibrate the amylase affinity columns with amylase buffer #1 and the trypsin affinity columns with trypsin buffer #1. Flow rate = 40 ml/hr. After 1 hr check that pH of eluants is 7.0 for amylase affinity columns and 6.0 for trypsin affinity columns. If not, equilibrate until pH's are correct.
6. Ultracentrifuge dialysate at 40 000 rpm (100 000 x g) for 1 hr at 10^0C. Decant supernatant and filter through 0.45 μm filter.
7. Connect bottoms of amylase affinity columns to tops of trypsin affinity columns. Load filtrates onto amylase and trypsin affinity columns at a flow rate of 10-15 ml/hr.
8. With columns still connected wash with amylase buffer #1 at flow rate of 10-15 ml/hr for 1 hr.
9. Disconnect columns. Wash each amylase affinity column with a minimum of 250 ml amylase buffer #1; flow rate = 17 ml/hr for 15 hr (overnight). Wash each trypsin affinity column with a minimum of 150 ml trypsin buffer #1: flow rate = 10 ml/hr for 15 hr (overnight).

10. Wash amylase affinity columns with amylase buffer #2 at a flow rate of 40 ml/hr for 1 hr.
11. At the same time, wash first trypsin affinity column with trypsin buffer #2 at a flow rate of 40 ml/hr and collect 5 ml fractions of eluant. While eluting the first trypsin affinity column, continue washing the second trypsin affinity column with trypsin buffer #1.
12. Read the absorbance of the fractions against trypsin buffer #2 at 280 nm. Combine fractions which have absorbance above baseline (generally 3-5 fractions after and including the fraction in which absorbance increases and pH decreases) in a 50 ml glass tube.
13. Repeat steps 11 and 12 on second trypsin affinity column.
14. Wash the first amylase affinity column with 0.1 N NaOH at a flow rate of 40 ml/hr and collect 5 ml fractions of eluant. While eluting the first amylase affinity column, continue washing the second amylase affinity column with amylase buffer #2.
15. Read the absorbance of the fractions against 0.1 N NaOH at 80 nm. Combine fractions where absorbance peak occurs (generally the first five fractions after the fraction where absorbance and pH first increase) in a 50 ml glass tube.
16. Repeat steps 14 and 15 on second amylase affinity column.
17. Evaporate all combined fractions under nitrogen at $50^{O}C$ to ± 1 ml. Transfer evaporated fractions to 4 ml glass vials, washing out 50 ml tube with 1 ml H_2O, and evaporate to dryness.
18. Samples can be frozen at this stage.

Appendix 9. Determination of Amylase and Trypsin Specific Activity

1. If purified amylase or trypsin samples were frozen after dryings, thaw.
2. Add 2 ml 6 N HCl and 50 µl 10 mM L-homocysteic acid (internal standard) to each sample.
3. Seal vials tightly and heat for 24 h at 100^0C.
4. Allow hydrolysates to cool to room temperature and transfer to 50 ml glass tubes.
5. Add 20 ml absolute ethanol to each sample and evaporate the hydrolysate to dryness under nitrogen. Add 20 ml absolute ethanol to the sample and re-dry.
6. Dissolve each sample in 2 ml distilled H_2O and filter through a 0.45 µm filter.
7. Count 1 ml of each filtrate in 10 ml Instagel.
8. Determine leucine concentrations by HPLC.
9. Enzyme SA = DPM/nmol leucine.

Appendix 10. HPLC Amino Acid Analysis

Equipment:

1. Spectra-Physics ternary solvent delivery system (SP880/8810)
2. Spectra-Physics UV detector
3. Spectra-Physics integrator

Solvent System:

Chromatography of amino acids is carried out using a five-stage gradient of acetonitrile in sodium phosphate buffer.

Distilled water, further purified by a Millipore-Q Water system is used for preparing the two mobile phase solvents:

Stock 300 mM phosphate buffer

Add 20 ml 85% phosphoric acid to ± 800 ml H_2O. Adjust the pH to 7.2 with 10 N NaOH. Make up to 1 litre.

Solvent A.

15 mM phosphate buffer pH 7.2.

Dilute 50 ml of the stock solution to 1 litre. Mix well.

Solvent B.

50 % acetonitrile in 15 mM phosphate buffer.

Dilute 25 ml of the stock solution to 500 ml.

Add 500 ml of HPLC grade acetonitrile (210 nm cut off). Mix well.

Gradient:

Time	%A	%B	Flow (ml/min
0.00	100	0	2.00
0.50	85	15	2.00
12.00	50	50	2.00
15.00	50	50	2.00
19.00	40	60	2.00
22.00	38	62	2.00
25.00	0	100	2.00
30.00	0	100	2.00
33.00	100	0	2.00
36.00	100	0	2.00

Standard:

Make up the following solutions in new glass counting vials:

10 mM homocysteic acid	=	18.32 mg/10 ml
10 mM asparagine	=	13.21 mg/10 ml
10 mM glutamine	=	14.61 mg/10 ml
10 mM tryptophan	=	20.42 mg/10 ml

The standard comprises 100µl Sigma L-amino acid standard solution and 25 µl each of 10 mM homocysteic acid (internal standard), asparagines, glutamine and tryptophan, diluted with 2.3 ml H_2O.

Derivatization:

Amino acids are chromatographed as 0-phthaldialdehyde derivatives, which are formed "pre-column".

This achieves three purposes:

1. Improves the chromatography by decreasing the polarity of the amino acid, and therefore increases retention by the C18 stationary phase.
2. Increases solute selectivity, as only those compounds with a free amino group will act with the derivatizing reagent.
3. Allows detection of the amino acids by UV absorbance (340 nm max) or by fluorescence.

Reagents:

1. **Potassium borate** 0.5 M, pH 10.5

   Dissolve 31 g boric acid (H3BO3) in 1 litre H_2O.
   Adjust to pH 10.5 with potassium hydroxide pellets.
2. **O-phthaldialdehyde** (Sigma P1378)

   10 mg/ml in methanol. Protect from light.
3. **Ethanethiol** (Merck)

   10 µl/ml in methanol. Protect from light.

   Perform derivitization under fume hood.

Method:

1. Pipette 50 µl of sample (standard, deproteinised plasma, or hydrolysate).
2. Add 300 µl borate buffer.
3. Add 100µl ethanethiol reagent.
4. Add 100 µl 0-phthdialdehyde reagent.
5. Mix and inject 200µl of mixture into HPLC.

Calculation:

$$[AA] = \frac{\text{AA area}_{sample}}{\text{I.S. area}_{sample}} \times \frac{\text{I.S. area}_{standard}}{\text{AA area}_{std}} \times \frac{[AA]_{std}}{[I.S.]_{std}} \times [I.S.]_{sample}$$

where:

$[AA]$ = amino acid concentration (nmol/ml)

AA area_{sample} = area of amino acid peak in the sample

$\text{I.S. area}_{sample}$ = area of the internal standard peak in the sample

AA area_{std} = area of the amino acid peak in the standard

I.S. area_{std} = area of the internal standard peak in the standard

$[AA]_{std}$ = concentration of the amino acid in the standard (=100 nmol/ml)

$[I.S.]_{std}$ = concentration of the internal standard in the standard (=100 nmol/ml)

$[I.S.]_{sample}$ = concentration of the internal standard in the sample

= 200 nmol/ml for deproteinized plasma

= 500 nmol/ml for mucosal protein hydrolysates

= 250 nmol/ml for trypsin and amylase hydrolysates

$$SA_{protein}\ (\text{DPM/nmol}) = \frac{\text{Specific Activity (DPM/ml)}}{[\text{leucine}]\ (\text{nmol/ml})}$$

Appendix 11. Data Sheets

Whole Body Metabolism

Control Subjects:

Diagnosis	RQ	REE (kcal/d)	REE (kcal/kg/d)	WBP Flux (g/d)	WBP Flux (g/kg/d)	WBP Oxid (g/d)	WBP Oxid (g/kg/d)	WBP Syn (g/d)	WBP (g/kg/
Control	0.90	1641	26.05	192.2	3.05	23.67	0.38	168.5	2.68
Control	0.77	1581	24.32	230.4	3.54	24.83	0.38	205.5	3.16
Control	1.42	2641	30.01	325.9	3.70	71.79	0.82	254.1	2.89
Control	0.93	1480	25.09	330.7	5.61	19.00	0.32	311.7	5.28
Control	0.70	1905	21.17	320.7	3.56	30.26	0.34	290.5	3.23
Control	0.84	1835	24.15	423.6	5.57	44.31	0.58	379.2	4.99
Control	0.84	1673	22.61	502.8	6.80	48.53	0.66	454.3	6.14
Control	0.85	1479	24.65	403.2	6.72	31.83	0.53	371.4	6.19
Control	0.86	2286	35.17	272.3	4.19	37.12	0.57	235.1	3.62
Control	0.77	1550	20.40	466.4	6.14	29.39	0.39	437.0	5.75
Control	NA	NA	NA	489.6	6.48	53.40	0.71	436.2	5.77
Control	NA	NA	NA	380.8	6.35	74.76	1.25	306.1	5.10
Control	NA	NA	NA	417.4	5.88	42.05	0.59	375.4	5.29
Control	NA	NA	NA	281.6	3.26	37.88	0.44	243.7	2.82
Control	NA	NA	NA	336.5	4.10	64.19	0.78	272.3	3.32
Control	NA	NA	NA	256.0	3.88	52.47	0.80	203.6	3.08
Control	NA	NA	NA	304.2	4.06	64.11	0.86	240.1	3.20
Mean	0.89	1807	25.36	349.1	4.88	44.09	0.61	305.0	4.27
Std Dev	0.20	381.9	4.36	91.15	1.35	17.24	0.24	89.22	1.31

RQ = Respiratory Quotient
REE = Resting Energy Expenditure
WBP Flux = Whole Body Protein Flux
WBP Oxid = Whole Body Protein Oxidation
WBP Syn = Whole Body Protein Synthesis
NA = Not Available

Whole Body Metabolism

Malnourished Patients Before Nutritional Support:

Diagnosis	RQ	REE (kcal/d)	REE (kcal/kg/d)	WBP Flux (g/d)	WBP Flux (g/kg/d)	WBP Oxid (g/d)	WBP Oxid (g/kg/d)	WBP Syn (g/d)	WBP Syn (g/kg/d)
Anorexia	0.72	753	20.92	147.9	4.109	10.86	0.302	137.1	3.808
Anorexia	0.7	1060	42.4	208.8	8.35	15.18	0.61	193.7	7.74
Anorexia	0.94	1691	52.84	212.4	6.64	40.73	1.27	171.6	5.36
Anorexia	0.71	702	21.27	167.0	5.06	15.64	0.47	151.3	4.59
Anorexia	0.98	1087	33.97	130.4	4.08	23.11	0.72	107.3	3.35
Anorexia	1.06	1546	38.65	157.0	3.93	32.39	0.81	124.6	3.12
Anorexia	0.77	929	28.41	NA	NA	NA	NA	NA	NA
Anorexia	0.92	698	21.15	NA	NA	NA	NA	NA	NA
Crohn's	NA	NA	NA	73.6	1.94	6.22	0.16	67.39	1.77
Crohn's	0.83	1100	27.5	120.5	3.01	13.29	0.33	107.2	2.68
Crohn's	0.67	2692	59.82	156.3	3.47	41.83	0.93	114.5	2.54
Crohn's	0.9	1174	40.48	119.5	4.12	13.95	0.48	105.6	3.64
Crohn's	0.74	1132	28.3	147.1	3.68	14.59	0.37	132.5	3.31
Crohn's	0.78	1802	45.05	132.6	3.32	18.40	0.46	114.2	2.85
Crohn's	0.69	936	28.36	101.4	3.07	5.38	0.16	96.05	2.91
Crohn's	NA	990	27.5	72.5	2.01	16.84	0.38	58.67	1.63
Tuberculosis	0.84	781	22.31	169.7	4.85	8.74	0.24	161.2	4.61
Tuberculosis	0.68	NA	NA	NA	NA	NA	NA	NA	NA
Tuberculosis	NA	NA	NA	185.0	5.97	29.55	0.96	155.5	5.02
IPSID	NA	NA	NA	172.6	4.01	21.77	0.51	150.8	3.51
IPSID	NA	1792	35.86	108.6	2.17	20.59	0.41	87.98	1.76
Short Bowel	NA	1340	27.91	179.8	3.75	14.80	0.31	164.9	3.44
Amyloid	NA	NA	NA	225.2	5.00	52.37	1.64	172.8	3.84
Melanoma	0.68	898	24.6	131.3	3.60	9.45	0.26	121.9	3.34
Ca Lung	NA	NA	NA	135.8	4.38	29.80	0.96	105.8	3.41
Mean	0.80	1216	33.02	148.0	4.11	20.70	0.58	127.4	3.56
Std Dev	0.12	500.9	11.06	40.98	1.50	12.37	0.38	35.18	1.35

Whole Body Metabolism

Malnourished Patients After Nutritional Support:

Diagnosis	RQ	REE kcal/d)	REE (kcal/kg/d)	WBP Flux (g/d)	WBP Flux (g/kg/d)	WBP Oxid (g/d)	WBP Oxid (g/kg/d)	WBP Syn (g/d)	WBP Syn (g/kg/d)
Anorexia	1.05	1199	39.97	147.0	4.90	22.75	0.76	124.2	4.14
Anorexia	0.8	1170	25.44	225.2	4.90	49.58	1.08	175.7	3.82
Anorexia	0.99	972	22.61	229.4	5.34	24.82	0.58	204.6	4.76
Anorexia	1.25	1531	32.23	205.5	4.33	45.53	0.96	160.0	3.37
Anorexia	0.8	858	18.94	NA	NA	NA	NA	NA	NA
Anorexia	1.14	1069	26.73	NA	NA	NA	NA	NA	NA
Crohn's	NA	NA	NA	180.4	3.61	14.17	0.28	166.3	3.33
Crohn's	0.92	884	19.22	129.8	2.82	11.19	0.24	118.6	2.58
Crohn's	NA	NA	NA	169.8	3.65	14.81	0.32	155.0	3.33
Crohn's	0.88	1634	38.91	175.3	4.17	23.90	0.57	151.4	3.60
Crohn's	0.72	1016	25.40	191.1	4.78	30.49	0.76	160.6	4.02
Crohn's	NA	1171	28.56	212.8	5.19	29.18	0.71	183.7	4.48
Tuberculosis	0.95	1732	36.08	181.5	3.78	19.09	0.40	162.4	3.38
IPSID	NA	NA	NA	391.8	7.53	51.53	0.99	340.3	6.54
IPSID	NA	1763	31.77	283.9	5.12	63.22	1.14	220.7	3.98
Short Bowel	0.94	1458	25.14	109.0	1.88	11.60	0.20	97.35	1.68
Amyloid	NA	NA	NA	215.0	4.67	19.16	0.42	195.8	4.26
Melanoma	0.76	1049	27.61	156.8	4.13	17.48	0.46	139.3	3.67
Ca Lung	NA	NA	NA	205.2	5.70	39.16	1.09	166.0	4.62
Mean	0.93	1250	28.47	200.6	4.50	28.69	0.64	171.9	3.86
Std Dev	0.16	313.0	6.61	64.23	1.24	15.67	0.32	53.27	1.02

Gut Mucosal Fractional Synthesis

Control Subjects:

Diagnosis	Gastric Body (%/d)	Gastric Antrum (%/d)	Duodenum (%/d)
Control	29.9	21.2	27.0
Control	30.6	16.6	22.4
Control	22	54.9	24.9
Control	58.2	17.6	21.0
Control	51.9	17	24.9
Control	18.8	30.1	53.7
Control	45.6	41.5	39.3
Control	50.7	22.6	10.3
Control	35.9	27.1	23.7
Control	33.9	38.6	16.9
Control	43.2	27.6	39.4
Control	42.9	24.4	24.8
Control	35.3	14.5	11.7
Control	20.2	20.4	49.9
Control	21.4	7.1	4.5
Control	26.2	24.4	20.8
Control	12.9	28.3	15.6
Mean	34.09	25.52	25.34
Std Dev	13.10	11.34	13.39

Gut Mucosal Fractional Synthesis

Malnourished Patients Before Nutritional Support:

Diagnosis	Gastric Body (%/d)	Gastric Antrum (%/d)	Duodenum (%/d)
Anorexia	41.9	52.6	49.5
Anorexia	32.3	33.9	26.1
Anorexia	41.4	12.2	21.4
Anorexia	28.6	37.1	43.1
Anorexia	38.4	36.4	30.7
Anorexia	41.5	35.5	35.6
Crohn's	22.6	17.7	17.3
Crohn's	27.8	23.7	30.2
Crohn's	33.7	22.7	25.8
Crohn's	57.8	35.1	31.4
Crohn's	46.3	18.1	NA
Crohn's	47.6	26.9	31.7
Crohn's	35.2	12.7	21.6
Short Bowel	28.4	20.3	24.2
Tuberculosis	NA	18.9	19.3
IPSID	15.5	8.7	21.7
IPSID	11.9	17.3	14.5
Amyloid	14.2	38.6	15.5
TB	67.4	25.4	25.3
Ca Lung	9.7	11.5	21.2
Mean	33.80	25.27	26.64
Std Dev	15.38	11.5	9.07

Gut Mucosal Fractional Synthesis

Malnourished Patients After Nutritional Support:

Diagnosis	Gastric Body (%/d)	Gastric Antrum (%/d)	Duodenum (%/d)
Anorexia	53	24.3	35.60
Anorexia	42.1	33.4	34.00
Anorexia	63.8	54.9	36.90
Anorexia	57.3	31.5	40.10
Crohn's	44.5	22.7	33.60
Crohn's	NA	34.5	18.8
Crohn's	33.9	32.4	29.80
Crohn's	103.7	36.0	119.6
Melanoma	53.3	40.4	39.20
Short Bowel	44.3	51.9	123.0
Tuberculosis	24.4	15.6	23.20
IPSID	18.1	17.6	14.6
IPSID	25.2	38.1	36.10
Amyloid	1.8	4.1	30.8
Ca Lung	99.7	38.9	30.5
Mean	47.51	31.75	43.05
Std Dev	28.46	13.36	32.58

Gastric Acid and Pancreatic Enzyme Secretion

Control Subjects :
(Pentagastrin/CCK-8-Stimulation)

DIAGNOSIS:	Gastric Acid Output		Pancreatic Enzyme Secretion		
	BAO (mEq/h)	MAO (mEq/h)	Amylase (U/h)	Lipase (U/h)	Trypsin (U/h)
Control	NA	NA	1930	52.8	322.7
Control	NA	NA	546	33.0	203.3
Control	3.3	27.2	2700	170.6	616.7
Control	4.0	13.8	4614	103.4	306.7
Control	3.0	35.0	2504	199.6	400.7
Control	2.0	8.5	1586	85.6	209.3
Control	10	50.1	2770	212	443.3
Control	1.4	18.6	2158	134.1	397.3
Control	3.8	25.0	1340	95.0	208.7
Control	3.3	26.0	2900	100	305.3
Mean	3.85	25.53	2305	118.6	341.4
Std Dev	2.63	12.94	1096	59.73	129.0

Control Subjects:
(Enteral Meal-Stimulation)

DIAGNOSIS:	Gastric Acid Output		Pancreatic Enzyme Secretion		
	BAO (mEq/h)	MAO (mEq/h)	Amylase (U/h)	Lipase (U/h)	Trypsin (U/h)
Control	2.6	12.14	2670	98.22	560.0
Control	2.8	8.38	2822	80.3	563.3
Control	0	8.20	3298	88.56	595.3
Control	0	8.68	2028	83.34	754.7
Control	4.2	7.92	1590	44.06	196.7
Control	9.4	16.6	920	80.8	365.3
Control	0.14	14.4	2160	119.24	456.7
Mean	2.73	10.90	2213	84.93	498.9
Std Dev	3.37	3.51	801.5	22.65	179.6

BAO = Basal Acid Output MAO = Maximal (Stimulated) Acid Output

Gastric Acid and Pancreatic Enzyme Secretion

Malnourished Patients Before Nutritional Support: (Pentagastrin/CCK-8-Stimulation)

DIAGNOSIS:	Gastric Acid Output		Pancreatic Enzyme Secretion		
	BAO (mEq/h)	MAO (mEq/h)	Amylase (U/h)	Lipase (U/h)	Trypsin (U/h)
Anorexia	0	7.1	1052	42.8	162.9
Anorexia	0	10	1278	12.6	43.0
Anorexia	NA	NA	1058	121	203.3
Anorexia	0	13.4	770	80.06	118.0
Anorexia	0	15.2	934	76.48	131.3
Anorexia	1	5.5	1570	101.8	238.0
Crohn's	0	8.5	35.4	23.44	29.01
Crohn's	0	4.2	3024	103.4	122.0
Crohn's	2.2	14	122.5	2.92	15.25
Crohn's	1.45	9.9	324	8.24	99.14
Crohn's	0.6	5.6	16.4	1.82	30.67
Crohn's	NA	NA	840.4	32.76	100.0
Crohn's	0	4.8	1022	42.8	214/0
Crohn's	0.2	4.5	692	48.66	169.3
Melanoma	0	1.1	712	29.08	226
Short Bowel	0	0	480	3.12	72.0
Tuberculosis	0	2.8	780	1.00	135.3
Tuberculosis	0	0	189.2	14.28	99.33
Mean	0.34	6.66	827.8	41.46	122.7
Std Dev	0.66	4.82	700.1	39.08	69.52

Malnourished Patients Before Nutritional Suport: (Enteral Meal-Stimulation)

DIAGNOSIS:	Gastric Acid Output		Pancreatic Enzyme Secretion		
	BAO (mEq/h)	MAO (mEq/h)	Amylase (U/h)	Lipase (U/h)	Trypsin (U/h)
Amyloid	0.4	4.2	814	6.86	55.34
Ca Lung	0	4.22	189.1	4.06	91.33
Crohn's	2.4	7.9	1790	131.8	558
IPSID	1.6	1.7	338	11.96	181.3
IPSID	0	3.28	2662	41.48	248.7
Tuberculosis	0	4.11	137.2	4.72	37.22
Tuberculosis	NA	5.35	160.4	13.86	57.5
Mean	0.73	4.39	870.1	30.68	175.6
Std Dev	1.03	1.91	987.3	46.41	185.4

Gastric Acid and Pancreatic Enzyme Secretion

Malnourished Patients After Nutritional Support: (Pentagastrin/CCK-8-Stimulation)

DIAGNOSIS:	Gastric Acid Output		Pancreatic Enzyme Secretion		
	BAO (mEq/h)	MAO (mEq/h)	Amylase (U/h)	Lipase (U/h)	Trypsin (U/h)
Anorexia	1.2	7.8	1478	152	172.7
Anorexia	0	12.5	1698	69.8	154.0
Anorexia	0	14.2	1222	80.0	121.3
Anorexia	0	8.6	2602	270	342.0
Crohn's	14	12.9	834	20.42	52.0
Crohn's	0.8	22.4	4112	240	394.7
Crohn's	2.4	8.8	3358	126.3	315.3
Crohn's	5.4	9.9	3100	69.96	256.0
Crohn's	NA	NA	2810	113.6	253.3
Crohns	0.6	9.8	2336	56.2	266.0
Melanoma	0	2.9	1942	33.62	181.3
Short Bowel	0	14.3	1156	49.42	136.7
Tuberculosis	0	13.8	1858	62.0	149.3
Tuberculosis	NA	NA	774	77.42	126.6
Mean	0.98	11.49	2091	101.5	208.7
Std Dev	1.58	4.75	1004	74.13	97.64

Malnourished Patients After Nutritional Support: (Enteral Meal-Stimulation)

DIAGNOSIS:	Gastric Acid Output		Pancreatic Enzyme Secretion		
	BAO (mEq/h)	MAO (mEq/h)	Amylase (U/h)	Lipase (U/h)	Trypsin (U/h)
Amyloid	0.4	4.46	3440	6.8	136.0
Ca Lung	NA	NA	1124	7.66	153.3
Crohn's	0.8	9.17	4420	114	584.0
IPSID	1	2.7	1782	85.46	206.7
IPSID	0	1.27	2518	70.52	216.7
Tuberculosis	0	0	820	68.56	61.7
Mean	0.44	3.52	2351	58.83	226.4
Std Dev	0.45	3.50	1391	43.15	183.8

Isotope (^{14}C-Leucine) Incorporation into Pancreatic Enzymes (Enzyme Specific Activity/Plasma Leucine Specific Activity)

Control Subjects:

Amylase:

	0 mins	15 mins	45 mins	75 mins	105 mins	135 mins	165 mins	195 mins	225 mins
Control	0.000	0.000	0.018	0.062	0.154	0.255	0.471	0.540	0.584
Control	0.000	0.000	0.011	0.089	0.234	0.275	0.484	0.599	0.711
Control	0.000	0.000	0.000	0.031	0.151	0.227	0.393	0.409	0.512
Control	0.000	0.000	0.000	NA	0.161	0.232	0.475	0.579	0.550
Control	0.000	0.000	0.000	0.008	0.045	0.124	0.178	0.242	0.343
Control	0.000	0.000	0.000	0.009	0.031	0.065	NA	0.225	0.281
Control	0.000	0.000	0.037	0.050	0.147	NA	0.454	0.651	0.945
Control	0.000	0.000	0.000	0.061	0.184	0.320	0.355	0.482	0.539
Control	0.000	0.000	0.000	0.027	0.095	0.163	0.296	0.331	0.413
Control	0.000	0.000	0.000	0.052	0.207	0.398	NA	NA	1.006
Mean	0.000	0.000	0.007	0.043	0.141	0.229	0.388	0.451	0.588
Std Dev	0.000	0.000	0.012	0.027	0.066	0.101	0.108	0.157	0.239

Trypsin:

	0 mins	15 mins	45 mins	75 mins	105 mins	135 mins	165 mins	195 mins	225 mins
Control	0.000	0.000	0.025	0.113	0.186	NA	0.706	0.752	NA
Control	0.000	0.000	0.007	0.069	0.253	0.364	0.500	0.581	0.638
Control	0.000	0.000	0.000	0.052	0.283	0.324	0.434	NA	0.566
Control	0.000	0.000	0.000	NA	0.240	NA	0.643	0.623	0.701
Control	0.000	0.000	0.000	0.021	NA	NA	0.341	0.379	0.497
Control	0.000	0.002	0.007	0.011	0.036	0.114	0.149	0.339	0.330
Control	0.000	0.000	0.023	0.050	0.312	NA	NA	0.812	1.110
Control	0.000	0.000	0.000	0.057	0.236	0.473	0.473	0.587	0.801
Control	0.000	0.000	0.005	0.036	0.165	0.246	0.557	0.692	0.578
Control	0.000	0.000	0.011	0.090	0.370	0.500	NA	NA	0.873
Mean	0.000	0.000	0.008	0.055	0.231	0.337	0.475	0.595	0.677
Std Dev	0.000	0.001	0.009	0.032	0.096	0.144	0.175	0.167	0.229

Isotope (^{14}C-Leucine) Incorporation into Pancreatic Enzymes (Enzyme Specific Activity/Plasma Leucine Specific Activity)

Malnourished Patients Before Nutritional Support:

Amylase:

	0 mins	15 mins	45 mins	75 mins	105 mins	135 mins	165 mins	195 mins	225 mins
Anorexia	0.000	0.000	0.024	0.049	0.337	0.443	0.553	0.572	NA
Anorexia	0.000	0.000	0.000	0.151	0.430	0.595	0.635	0.411	NA
Anorexia	0.000	0.065	0.008	0.051	0.369	0.573	0.814	0.819	NA
Anorexia	0.000	0.017	0.009	NA	0.114	0.337	0.439	0.399	0.558
Anorexia	0.000	0.006	0.019	0.058	0.257	0.312	0.472	0.589	0.671
Crohn's	0.000	0.000	0.013	0.014	0.094	0.109	NA	NA	NA
Crohn's	0.000	0.000	0.007	0.012	0.069	0.096	0.136	0.162	NA
Crohn's	0.000	0.000	0.000	0.024	0.078	0.121	0.137	NA	0.266
Crohn's	0.000	0.000	0.009	NA	NA	0.313	0.367	0.358	0.447
Crohn's	0.000	0.000	0.000	0.102	0.133	0.145	0.153	0.251	0.307
Crohn's	0.000	0.000	0.000	0.000	0.018	0.096	0.119	0.133	0.254
Crohn's	0.000	0.000	0.000	NA	NA	NA	0.390	0.403	0.640
Melanoma	0.000	0.000	0.000	0.024	0.076	0.181	0.284	0.308	0.257
Short Bowel	0.000	0.000	NA	NA	NA	NA	0.339	0.483	0.524
Tuberculosis	0.000	0.000	0.011	0.049	0.172	0.256	NA	0.578	0.860
Mean	0.000	0.006	0.007	0.049	0.179	0.275	0.372	0.420	0.478
Std Dev	0.000	0.017	0.008	0.044	0.136	0.175	0.213	0.190	0.209

Trypsin:

	0 mins	15 mins	45 mins	75 mins	105 mins	135 mins	165 mins	195 mins	225 mins
Anorexia	0.000	0.000	0.000	NA	NA	0.568	0.617	1.144	NA
Anorexia	0.000	0.000	0.000	NA	0.395	0.625	NA	NA	NA
Anorexia	0.000	0.000	NA	0.069	0.304	0.512	0.700	0.918	NA
Anorexia	0.000	0.000	0.005	NA	0.180	0.355	0.388	0.468	0.667
Anorexia	0.000	0.000	0.009	0.399	0.275	0.107	0.596	0.631	0.764
Crohn's	0.000	0.000	0.003	0.013	0.034	0.086	NA	NA	NA
Crohn's	0.000	0.000	0.006	0.012	0.054	0.118	NA	0.185	NA
Crohn's	0.000	0.000	0.000	NA	0.038	0.103	NA	0.175	0.248
Crohn's	0.000	0.000	0.003	NA	NA	NA	0.720	1.049	0.813
Crohn's	0.000	0.000	0.000	NA	NA	0.018	0.080	0.327	0.354
Crohn's	0.000	0.000	0.000	NA	NA	0.507	0.723	NA	0.748
Melanoma	0.000	0.000	0.000	0.016	0.054	0.183	0.306	0.370	NA
Short Bowel	0.000	0.000	0.000	NA	NA	NA	0.373	0.544	0.621
Tuberculosis	0.000	0.000	0.000	0.005	0.119	0.419	NA	NA	0.774
Mean	0.000	0.000	0.002	0.085	0.161	0.300	0.500	0.581	0.624
Std Dev	0.000	0.000	0.003	0.155	0.134	0.220	0.225	0.349	0.210

Isotope (^{14}C-Leucine) Incorporation into Pancreatic Enzymes

(Enzyme Specific Activity/Plasma Leucine Specific Activity)

Malnourished Patients After Nutritional Support:

Amylase:

	0 mins	15 mins	45 mins	75 mins	105 mins	135 mins	165 mins	195 mins	225 mins
Anorexia	0.000	0.001	0.041	0.361	0.645	0.650	0.663	0.710	0.782
Anorexia	0.000	0.000	0.003	0.017	0.068	0.138	0.536	NA	0.556
Anorexia	0.000	0.018	0.020	0.021	0.044	0.117	0.127	0.169	0.264
Anorexia	0.000	0.000	0.020	0.011	0.074	0.141	0.292	0.423	0.513
Crohn's	0.000	0.000	0.000	0.056	NA	0.140	NA	0.216	0.224
Crohn's	0.000	0.000	0.006	NA	NA	0.429	0.392	0.475	0.981
Crohn's	0.000	0.000	0.006	0.019	0.054	0.113	0.202	NA	NA
Crohn's	0.000	0.000	0.000	0.016	0.078	0.138	0.172	NA	NA
Melanoma	0.000	0.000	0.011	NA	0.236	NA	0.547	NA	1.293
Short Bowel	0.000	0.000	0.005	0.012	0.029	0.058	0.106	NA	NA
Tuberculosis	0.000	0.000	0.000	0.016	NA	0.133	0.150	NA	0.299
Mean	0.000	0.002	0.010	0.059	0.154	0.206	0.319	0.399	0.614
Std Dev	0.000	0.005	0.012	0.114	0.209	0.185	0.203	0.218	0.380

Trypsin:

	0 mins	15 mins	45 mins	75 mins	105 mins	135 mins	165 mins	195 mins	225 mins
Anorexia	0.000	0.003	0.047	0.338	NA	NA	0.667	0.707	0.735
Anorexia	0.000	0.000	0.001	0.021	0.038	0.170	0.249	0.398	0.402
Anorexia	0.000	0.000	0.014	0.017	0.044	0.089	0.127	0.221	0.278
Anorexia	0.000	0.000	0.020	0.007	0.088	0.253	0.382	0.453	0.587
Crohn's	0.000	0.000	NA	0.053	NA	0.125	NA	0.179	0.257
Crohn's	0.000	0.000	0.006	NA	NA	0.670	0.444	0.798	0.541
Crohn's	0.000	0.000	0.021	0.013	0.053	0.137	0.218	NA	NA
Crohn's	0.000	0.000	NA	0.020	0.098	0.186	0.294	NA	NA
Melanoma	0.000	0.000	0.017	0.048	0.158	NA	0.603	NA	1.137
Short Bowel	0.000	0.000	0.005	0.010	0.023	0.067	0.079	NA	NA
Tuberculosis	0.000	0.000	NA	NA	NA	0.098	0.180	NA	0.195
Mean	0.000	0.000	0.016	0.058	0.072	0.200	0.324	0.459	0.516
Std Dev	0.000	0.001	0.014	0.106	0.047	0.185	0.197	0.251	0.311

Pancreatic Enzyme Synthesis

Control Subjects:

Diagnosis	Amylase			Trypsin		
	Production (h)	Turnover (%/h)	Pool (U)	Production (h)	Turnover (%/h)	Pool (U)
Control	0.94	29.39	6120	0.91	42.67	678.6
Control	0.90	32.97	1485	0.80	30.71	642.3
Control	1.12	27.00	9370	0.85	29.02	2032
Control	1.28	35.49	11560	0.94	37.16	751.4
Control	1.41	18.86	11719	1.19	26.23	1354
Control	1.48	16.82	7073	1.03	16.57	955.2
Control	1.11	44.01	6460	0.96	51.48	830.4
Control	0.92	26.51	6333	1.07	39.93	867.3
Control	1.2	22.14	3699	1.08	36.11	491.1
Control	1.25	56.69	4565	0.80	42.51	721.8
Mean	1.16	30.99	6838	0.96	35.24	932.4
Std Dev	0.20	12.11	3286	0.13	9.93	450.0

Production = Enzyme Production Time (h) Turnover = Zymogen Pool Turnover Rate (%/h)
Pool = Zymogen Pool Size (U)

Pancreatic Enzyme Synthesis

Malnourished Patients Before Nutritional Support:

Diagnosis	Amylase			Trypsin		
	Production (h)	Turnover (%/h)	Pool (U)	Production (h)	Turnover (%/h)	Pool (U)
Anorexia	0.78	33.69	3124	1.65	88.55	184.0
Anorexia	1.15	50.49	2532	0.89	57.50	74.78
Anorexia	1.02	53.17	961.2	1.06	52.42	189.9
Anorexia	1.15	28.82	3260	0.99	29.15	434.2
Anorexia	0.9	30.71	3786	1.15	39.18	510.2
Crohn's	0.83	10.52	178.5	0.94	7.60	318.4
Crohn's	0.83	8.60	NA	0.95	10.86	1282
Crohn's	1.03	11.86	1020	1.34	17.02	85.53
Crohn's	0.59	19.05	1341	1.34	57.14	151.5
Crohn's	0.49	11.32	60.08	NA	NA	NA
Crohn's	1.67	14.26	5775	2.32	35.42	335.4
Crohn's	1.74	39.61	720.0	1.08	37.90	328.6
Melanoma	1.16	20.04	3593	1.31	24.63	849.4
Short Bowel	1.05	25.51	1878	1.28	32.48	221.6
TB Bowel	1.08	36.83	2250	1.23	40.50	346.8
Mean	1.03	26.30	2177	1.25	37.88	379.5
Std Dev	0.34	14.41	1616	0.37	21.33	327.6

Production = Enzyme Production Time (h) Turnover = Zymogen Pool Turnover Rate (%/h)
Pool = Zymogen Pool Size (U)

Pancreatic Enzyme Synthesis

Malnourished Patients After Nutritional Support:

Diagnosis	Amylase			Trypsin		
	Production (h)	Turnover (%/h)	Pool (U)	Production (h)	Turnover (%/h)	Pool (U)
Anorexia	0.26	34.56	4321	0.30	35.65	442.8
Anorexia	1.24	32.67	3474	1.14	20.63	551.5
Anorexia	0.99	10.78	8096	1.11	12.36	690.3
Anorexia	1.16	24.07	10810	1.06	26.84	1274
Crohn's	0.40	8.94	10116	0.69	9.89	613.4
Crohn's	1.17	41.67	7584	1.03	38.90	963.1
Crohn's	1.01	13.21	25678	1.00	14.71	2144
Crohn's	1.08	15.48	19057	1.18	22.87	1044
Melanoma	1.09	56.40	3749	1.08	50.80	353.7
Short Bowel	0.99	6.89	11545	0.94	5.64	1841
TB Bowel	1.18	14.12	11795	0.97	10.15	1382
Mean	0.96	23.53	10566	0.95	22.59	1027
Std Dev	0.32	15.90	6738	0.25	14.23	583.7

Production = Enzyme Production Time (h) Turnover = Zymogen Pool Turnover Rate (%/h)
Pool = Zymogen Pool Size (U)

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