SAMJ
CLINICAL NUTRITION

REVIEW ARTICLE

# Clinical nutrition — its critical future and new strategies

**Peter Fürst**

The evolution of nutrition science from clinical studies by Hippocrates in the 4th century BC to the current preoccupation with molecular biology and genetics is a unique Odyssey. Contributions by physicians to the recognition of the classic vitamin and amino acid deficiency diseases in the 18th and 19th centuries were followed by the development of models to explore the aetiology of these diseases in the 20th century. The development of enzymology and the identification of essential nutrients as co-enzyme precursors enabled investigators to study enzyme activity as a function of nutritional status. The discovery of the structure of DNA by Watson and Crick in 1953, the elucidation of the genetic code, the exploration of gene structure and the regulation of gene expression have led to the expansion of 'biochemical genetics'. These new concepts and methods in molecular biology have, in turn, led to the cloning of genes, the identification of genomic receptors for hormones and growth factors and the implementation of genetic engineering.

In the past two decades, scientific emphasis on defining energy and lipid metabolism has opened a new era of understanding of the nutritional basis of extremely common diseases such as obesity, hypertension, atherosclerosis, diabetes and others. We now recognise that many of these diseases, including those with highest mortality, are intrinsically related to diet. Other common nutritional disorders carry potentially life-threatening complications. This implies that in a very short time, nutritional disorders have moved from relative obscurity to the forefront of clinical medicine. In this context, a modern definition of clinical nutrition is pertinent.[1] Clinical nutrition might be defined as a scientific and clinical discipline concerned with diseases that are caused by abnormalities in the intake, intestinal absorption and metabolism of dietary constituents. Nutritional disorders might be receptive to treatment by changes in the amounts and composition of nutrients, by modifications of the responses to diseases and/or nutritional treatment and finally by utilisation of novel substrates and endogenous mediators.[1,2]

A comprehensive review of all of the mosaics involved in the fascinating development of clinical nutrition is beyond the scope of this article. Instead it evaluates novel therapeutic measures aimed to modify and/or reverse abnormalities caused by dietary factors. Three general aspects form the basis for this discussion: (*i*) the concept of molecular nutrition; (*ii*) alterations of stress response; and (*iii*) provision of tissue-specific substrates. These approaches, whether employed alone or in combination, may emphasise the potential benefits that new gains in technology will have on the practice of clinical nutrition.

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## Molecular biology — the concept of molecular nutrition

A logical start to this approach would be the demonstration of the utility of molecular biology in certain areas of clinical nutrition. Molecular biology can serve as a bridge between the fundamental science of biophysics and biochemistry and the knowledge of organs and tissues which is more familiar to a clinical audience, though seldom in terms of conventional nutrition. The applications of molecular biology actually relate to all levels of nutritional science like: (*i*) basic research used to understand the fundamental mechanisms of metabolic disease and how they may be favourably modified; (*ii*) 'therapeutics' whereby specific nutrients or regulatory factors are used to modify gene expression and whole body metabolism; and (*iii*) 'diagnostics' used for the definition of metabolic and nutrition disorders that might involve genetic basis.

For the clinical nutritionist, the latter two categories are the most inviting, probably because they minimise the need for a fundamental biochemical background.[3]

### *Complementary DNA*

The core of modern molecular biology employs novel methods based on the concept of 'complementary DNA' or cDNA cloning. The use of cDNA enables many powerful new experimental approaches for the study of metabolic regulation at the molecular level. Detailed discussion of the steps involved in the cDNA cloning is not feasible in the context of this article, and the reader is referred to many relevant textbooks.[4,5] In brief, mRNA is isolated and purified from a tissue of interest, and it is then used as templates on which strands of DNA complementary to the RNA sequences are formed, i.e. the designation cDNA-'complementary-DNA'. Once a specific cDNA is isolated, it is an easy process to insert it into a sc. plasmid and then generate millions of copies by replicating the plasmid in a bacterial host. Following sequencing of cDNA, numerous nutrition-relevant protein sequences have been derived, as shown in Table I.[6,7] This technique can readily be applied in animal or human nutritional studies with RNA preparations obtained from needle biopsy samples. Fig. 1 depicts changes in the expression of mRNA for IGF-1 in fetal liver tissue during maternal fasting. All IGF-1 mRNA transcripts are markedly decreased in quantity.[8] The hormone IGF-1 is an essential determinant of tissue growth rates and thus regulation of its transcription during various nutritional conditions is likely to be important. Changes in nutrient intake have been shown to alter mRNA levels for growth hormone receptors in the liver, IGF-1 in the liver and other tissues, IGF-1-binding proteins, and IGF-1 receptors.[6,7] The method offers great promise for defining the molecular basis for the co-ordinated relationships between nutrient status and growth regulatory pathways.

**Table I. Nutritionally relevant protein sequences derived from cDNA**

| Function | Example(s) |
|---|---|
| Carbohydrate metabolism | Phosphoenolpyruvate carboxykinase |
| | Pyruvate kinase |
| | Insulin receptor |
| | Glucose transporters |
| | Low-density lipoprotein receptor |
| Lipid metabolism | Apolipoproteins |
| | Adipsin |
| | Uncoupling protein of brown fat |
| | Growth hormone |
| Growth control | ICFs |
| | Growth factor receptors and binding proteins |
| Micronutrient action | Retinol binding protein |

Adapted from Smith.[7]

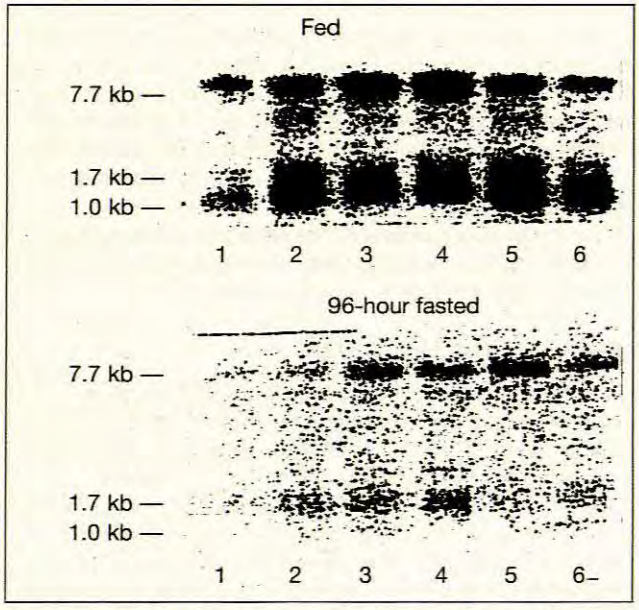


**Fig. 1. Effects of maternal fasting on fetal liver IGF-1 mRNA levels. Each lane in this Northern blot represents an individual fetus. (Reproduced from Strauss *et al.*[8] with permission.)**

## Recombinant DNA

One of the numerous approaches in the field of molecular biology relates to production of proteins with recombinant DNA. In this method the host bacteria become cDNA-producing biological factories. In Table II some nutritionally relevant proteins are listed produced by recombinant DNA technology. Recombinant human insulin has largely replaced beef- or pork-derived insulin. The potential implication of human growth hormone (HGH) includes improved utilisation of energy and enhanced rate of protein synthesis. HGH stimulates synthesis of collagen and bone matrix presumably mediated via the somatomedins like the previously mentioned IGF-1. Other growth factors like epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factors (TGF and TGF-β) promote healing in soft tissues and bone. The sc. colony-stimulating growth factors (GM-CSF, G-CSF) are interesting substances in haematology and they may serve as more therapeutic efforts in association with chemotherapy and total body irradiation.[7,9]

**Table II. Nutritionally relevant proteins produced by recombinant DNA technology**

| |
|---|
| Insulin |
| Insulin-like growth factors (IGFs) |
| Growth hormone |
| Epidermal growth factor (EGF) |
| Colony-stimulating factor (GM-CSF) |
| Tumor necrosis factor (TNF) |
| Interleukins |

From Smith.[7]

## Transfection

A powerful tool to study normal and abnormal protein is the process called transfection, whereby a foreign cDNA is introduced into mammalian cells. With this method, cDNA encoding proteins involved in nutritionally important pathways, like receptors, have been transfected into cultured cells. By studying the consequences of the expression of transfected proteins at high levels on the cell surface, one may gain insight into the normal effects of these proteins on cellular metabolism.[7,10]

Naturally occurring mutant cDNA sequences isolated from patients with inherited metabolic disorders can similarly be transfected into cultured cells and the consequences of the mutations for function of the protein can be investigated. Accordingly it has been possible to demonstrate the molecular basis for certain rare forms of diabetes mellitus.[11] The genetic basis for inherited propensities to many nutrition-related diseases like obesity, lipid and digestive disorders, and hypertension, undoubtedly will be defined in the next few years. As genetic abnormalities are identified, studies in transfected cultured cells should continue to provide a valuable approach for establishing the functional properties of the abnormal proteins that form the molecular basis for clinical disease.[6]

## Transgenic animals

An exciting recent application of transfection techniques is the introduction of cDNA into intact animals. An animal that has received foreign DNA in this manner is referred to as transgenic.[12] The implication of transgenic animals illustrates the use of a fusion gene composed of a nutrient-sensitive regulatory region and a region coding for a selected protein, like growth-promoting protein. The remarkable ability to transfer genes across species lines has been demonstrated, and the generation of human proteins in large quantities from animal 'bioreactors' is already a reality.[13] The use of gene transfer methods to ameliorate certain disease processes is currently undergoing experimental as well as clinical evaluation. The therapeutic prototype of gene therapy is illustrated in reports describing the construction of a transgenic animal model expressing the LDL-receptor. The isolated LDL-receptor gene[14] has been expressed in cells where it has been shown to be transcriptionally regulated by cholesterol.[15,16] In transgenic animals the rate of LDL-receptor production is not subject to the normal inhibitory influences of cholesterol. This over-expression of

the LDL-receptor gene results in a reduced plasma cholesterol which presumably could influence the course of atherogenesis in a positive manner.[17] Not only does this model yield valuable information regarding how gene expression is regulated, it yields practical benefits in the form of genetically engineered animals that may exhibit 'super properties'. Thus, transgenic methods also have potential implications in modifying the quality and composition of natural nutritional products obtained from plant and animal sources. It has become near-future science fiction to envision 'transgenic supercattle' producing low-fat milk and low-cholesterol beef.[18,19] Actually, since cDNAs for human proteins have been cloned recently,[20] the creation of transgenic cattle that produce human milk is feasible.[21]

Indeed, the application of molecular biology and especially genetic technology to the discipline of nutrition and medicine has made tremendous strides. It has evolved from a field that was burdened with a negative public image to one that currently enjoys an unprecedented level of activity and interest. A number of anticipated areas of progress in molecular biology and nutrition are listed in Table III. With the rapid development of new methods and knowledge, we are entering an exciting time for research in clinical nutrition designed as 'molecular nutrition'. Molecular nutrition will surely continue to increase its influence on the biomedical sciences, eventually yielding rich rewards for the area of clinical nutrition.

**Table III. Future applications of molecular biology in nutrition**

| |
|---|
| cDNA cloning of nutritionally important molecules |
| Definition of molecular regulatory mechanisms |
| Development of new products |
| Definition of molecular basis for nutrition-related disorders |
| Obesity |
| Atherosclerosis |
| Neoplasia |
| Inborn errors of metabolism |
| Modification of genetic factors |
| Gene modification |
| Gene replacement |
| Directed nutrient regulation of specific gene expression |

From Smith.[7]

## Alteration of stress response — cytokines and nutrition

A fascinating approach in modifying the response to a disease and/or treatment relates to endogenous mediators. The most important mediators are the cytokines. They are large polypeptide hormone-like substances synthetised in response to specific signals by a variety of cell types that mediate normal host immune responses. Cytokines produced by mononuclear phagocytes are often referred to as monokines, whereas those produced by activated lymphocytes are called lymphokines (Fig. 2).[22]

Conditions ranging from bacterial infections and parasitic infestation to rheumatoid disease and severe trauma have many symptoms in common. Evidence of anorexia, changes in metabolic rate, tissue catabolism, alterations in tissue composition, changes in substrate flows between tissues and stimulation and proliferation of cells of the immune system can be observed. Practically each of these effects can be mimicked by administration of individual cytokines.[23,24] As the cytokines communicate between neural endocrine and immune systems they affect a number of tissues and participate in the regulation of various processes including tumor growth and suppression or activation of immune cells.[9]

When discussing the role of cytokines in clinical nutrition one should keep certain essential points in mind. Firstly, nutritional factors always affect cells of the immune system from which cytokines are produced. Secondly, nutritional factors generally affect tissues of the liver, muscle and connective tissues which are actually the targets of cytokine action. Consequently, in traumatised, infected or malnourished patients, nutrients directly and nutritional status indirectly influence the production of cytokines and thus may have an input on the subsequent cytokine action. This action includes metabolic changes resulting in the provision of nutrients for the activated immune system, and the synthesis of substances which will increase its effectiveness while protecting the host against the potentially damaging action of products derived from the system.[24]

Although the complex mechanisms which determine the

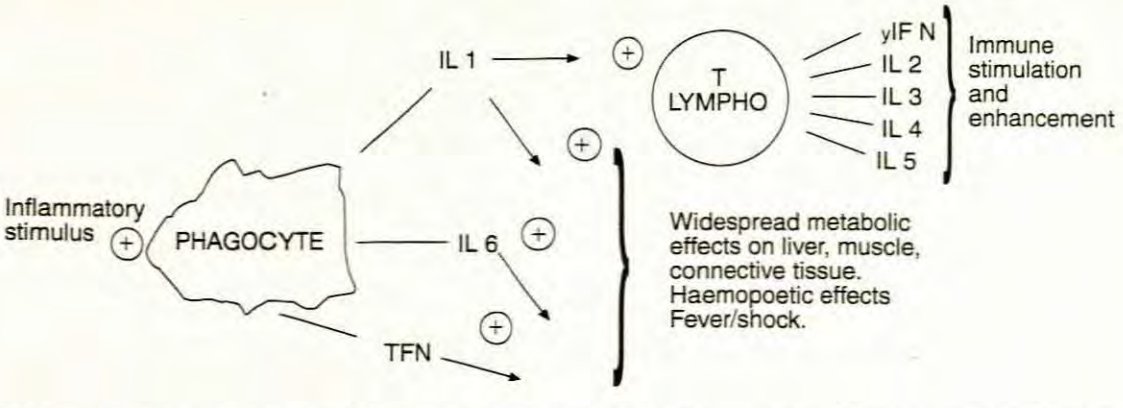


**Fig. 2. General scheme for cytokine-mediated changes in inflammatory states. γIFN = γinterferon; IL-1 = interleukin 1α and β; T: lympho = T: lymphocyte. (Reproduced from Grimble[23] with permission.)**

different metabolic responses of nutrients in a variety of diseases are not yet fully understood, it is reasonable to postulate that endogenous mediators produced by activated immune cells interact in an immune regulatory network to modulate the alterations in carbohydrate (glucose), protein and lipid metabolism.[25] In Fig. 3 a simplified diagram illustrates the confirmed and proposed effects of two selected cytokines, IL-1 and TNF, on substrate metabolism during episodes of stress or infection. As shown, both IL-1 and TNF inhibit lipoprotein lipase which ultimately results in elevated triglyceride concentration. Both TNF and IL-1 stimulate thermogenesis (fever) and acute phase protein synthesis. However, only IL-1 stimulates ACTH secretion with subsequent elevation in glucocorticoid levels. On the other hand, TNF causes a direct increase in catecholamines, which suppresses IL-1 production.[26] IL-1 stimulates the secretion of insulin and glucagon and thus appears to be responsible for an increase in glucose production. TNF causes the increased mobilisation of amino acids from the peripheral tissues, and there probably may be a synergistic effect with IL-1.

Lipid nutrients also exert important modulation on cytokine biology, as illustrated in Fig. 4. These include producing changes in the substrate availability for eicosanoid synthesis, altering membrane fluidity and altering the production of non-eicosanoid secondary messengers.[24] Indeed, inflammatory symptoms of rheumatoid arthritis, psoriasis, Crohn's disease, and ulcerative colitis are all relieved by fish-oil preparations whether or not directly related to cytokine production. Consumption of 15 g EPA per day, for 6 weeks, reduced the production of IL-1 $\alpha$ and $\beta$ as well as TNF $\alpha$ and $\beta$ in response to an endotoxin stimulus by more than 30% for 10 weeks. The anti-inflammatory effects of fish oil may also include decreased production of inflammatory substances, like leukotrien-B4 and PAF, released by the action of cytokines as well as a large reduction of cytokine-induced synthesis of $PGE_2$ and thromboxane $B_2$ in the colonic mucosa.[27-29]

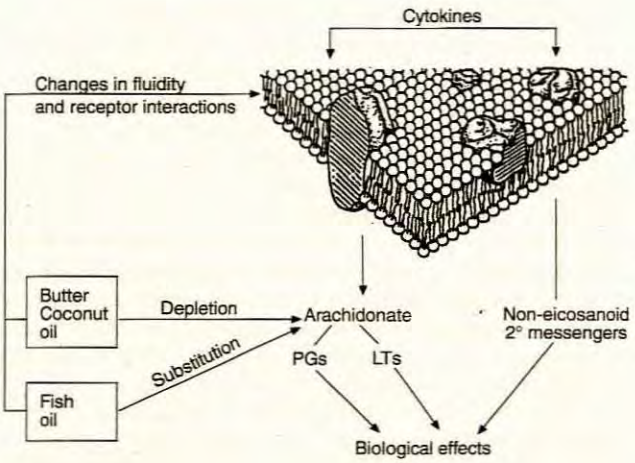


**Fig. 4. Influences of dietary fat on cytokine actions. (Reproduced from Grimble[24] with permission.)**

It is obvious that modulation of cytokine biology by nutrients provides, in theory, an intervention strategy for manipulating events so that cytokines can express their beneficial effects rather than their potentially damaging properties in patients. Certain patients who are acutely ill may benefit from high cytokine activity. In contrast, prolonged effects of TNF and of IL-1 during ongoing chronic disease may cause severe symptoms and increased mortality.[2,9,25,30] Consequently, some critically ill patients may benefit from the administration of specific cytokines in the acute phase response. In the majority of patients, however, harmful prolonged production of cytokines should be suppressed. If cytokine and lipid-mediator signals are central to the initiation of responses to inflammation, their production can be blocked or at least their activity diminished by preventing any of the following 4 events: (*i*) the initial stimulus; (*ii*) gene transcription and translation of cytokines; (*iii*) stimulation of the effector cells; and (*iv*) transduction of the cell signal.[31]

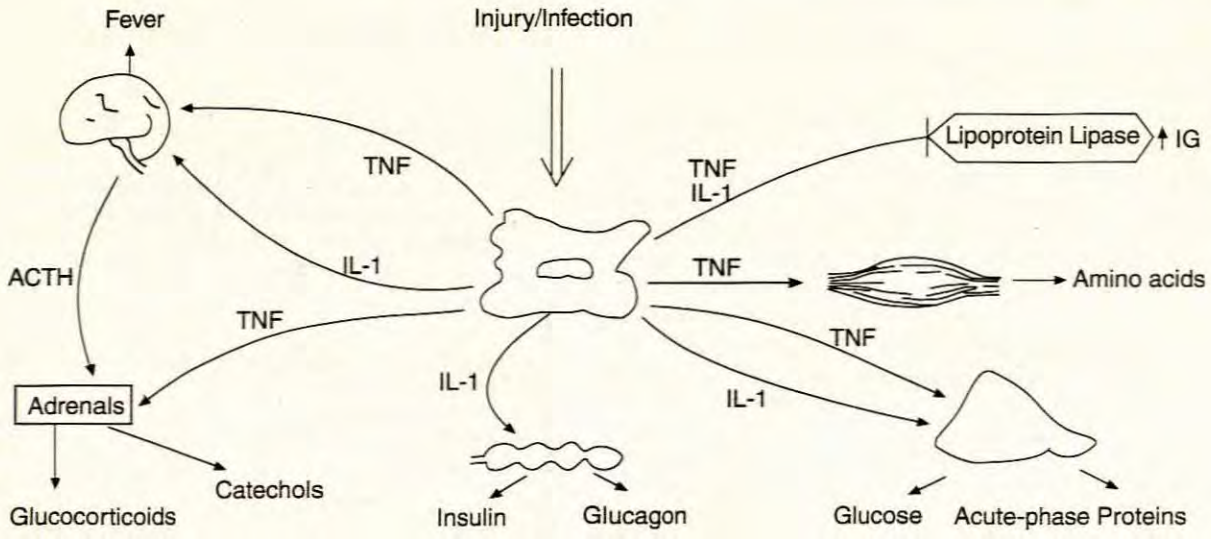


**Fig. 3. Effects of IL-1 and TNF on host substrate metabolism. This simplified diagram depicts the confirmed and proposed effects of IL-1 and TNF on substrate metabolism. For simplification, the effects of other monokines which also participate in this complex network have been omitted. Only the end products of stimulation or inhibition are shown. (Modified from Pomposelli *et al.*[25] with permission.)**

Gene transcription of cytokines can be interrupted by treatment with glucocorticoids during or before signal initiation.[32] This treatment is, however, ineffective once cytokines have been released. This might be the reason that glucocorticoids do not benefit patients with sepsis.[33] A clinically more useful technique is pre-treatment with blocking monoclonal antibody to TNF or PAF. The antibody will interfere with the coupling between the cytokine and receptor situated on the cell membrane and thus attenuate host responses which might be deleterious.[34,35] A fascinating novel way to modify responses to stress is to block signal transduction within the effector cell. Wilmore and co-workers administered a non-steroidal anti-inflammatory drug, ibuprofen, to inhibit the effects of arachidonic acid cyclo-oxygenase in normal subjects receiving endotoxin.[36] Blood TNF concentrations and white blood cells remained elevated, but many of the symptoms associated with the endotoxin, like fever, malaise, myalgia, tachycardia and diaphoresis, were less severe or absent. The results indicate that the cyclo-oxygenase pathway is necessary for the manifestation of the major responses to the cytokine TNF.

The quoted clinical studies are scarce. The bulk of the available investigations have been conducted on experimental animals. The challenge for the future will be to put the complex knowledge gained from all available studies into the clinical context. In that way, nutritional therapy can improve the chances of survival and healing in conditions in which cytokines have been elicited.[24] The ability to produce at will and control the degree of the metabolic response to stress and infection by exogenous means would be a powerful tool for physicians.

## New nutrition substrates

As both common and specific mechanisms for alterations in substrate metabolism are being discovered, there arise unique opportunities to intervene in the disease process. Undoubtedly, the efficacy of providing substrate to the injured, immunocompromised and/or malnourished host has caused a renaissance in the clinical application of dietary intervention in the treatment and prevention of disease.[37-40]

Many investigators proceeded on the assumption that 'tailor-made preparations' will increase the benefits of nutritional efforts for specific patient groups. Thus, specific amino acid mixtures have been developed for the treatment of renal and liver disease or to optimise the growth of young infants. Other investigators still hope for benefits from products enriched in branched-chain amino acids.[9,40,41] Although many of these nutrition formulae are now available, they were designed to improve tolerance of nitrogen load in the presence of illness, malnutrition or organ dysfunction rather than to provide specific nutrients for individual organs or tissues.[42]

Current research directions consider individual substrates as tissue- or organ-specific single nutrients as an alternative approach. Certain diseases accompanied by deficiencies, antagonism or imbalances in a particular compartment or in various organ tissues might selectively require one or more nutrients to support the attenuated organ and/or tissue.[41] Administration of required substrates might thus greatly facilitate an anabolic response to a life-threatening disease.[31] The major questions are: which nutrients should be considered, and in what amounts should they be administered?

Great interest is devoted to new substrates. As a therapeutic alternative to the long-chain triglycerides (LCT), the potential enteral and parenteral use of medium-chain triglycerides (MCT) has been discussed. The development of so-called 'structured lipids' is a further alternative to the MCT-LCT-mixtures. A novel approach is the possible therapeutic implication of $n^3$ fatty acids. 'Magic bullets' as integrated emulsion particles should be specifically tailored to be taken up exclusively by defined receptors, cells or tissues. Supplementation with the glutamine analogue alpha-ketoglutarate or its ornithine salt is seen as a physiological way of providing glutamine during nutritional therapy. The use of stable and highly soluble dipeptides shows great promise as an avenue for the provision of amino acids that may otherwise be difficult to deliver.[40]

### *New substrates in clinical nutrition — amino acids/dipeptides*

The general approach to the nutritional care of the critically ill, malnourished or stressed patient involves delivery of a balanced diet including an adequate amount of protein or suitable amino acid preparation that reflects a high biological value, like egg protein.[2,43] This approach however is not feasible in clinical practice since poor solubility and/or instability prevent inclusion of glutamine, tyrosine and cystine into the currently available amino acid preparations.

Cystine and tyrosine are known to be indispensable for newborn infants,[44,45] tyrosine in chronic renal failure,[46] and both amino acids are claimed to be essential in severe liver failure.[47,48] With the recognition of stress-induced intracellular glutamine depletion,[49,50] various approaches of glutamine nutrition received revitalised interest.[51-53] Glutamine is considered today as a conditionally essential amino acid in critically ill, stressed and malnourished patients.

Glutamine is the most prevalent free amino acid in the human body. In skeletal muscle, glutamine constitutes more than 60% (> 19.5 mM/l ICW) of the total free amino acid pool. Glutamine not only acts as a precursor for protein synthesis, but is also an important intermediate in a large number of metabolic pathways. It is a precursor that donates nitrogen for the synthesis of purines, pyrimidines, nucleotides and amino sugars. Glutamine is the most important substrate for renal ammoniagenesis, and thus takes part in the regulation of the acid-base balance. As the most abundant amino acid in the bloodstream, glutamine serves as nitrogen transporter between various tissues. Owing to its diverse participation in transamination reactions, glutamine can be classified as a true regulator of amino acid homeostasis.[40]

It is well known that glutamine represents an important metabolic fuel for the cells of the gastro-intestinal tract (enterocytes, colonocytes),[54-56] a trait it shares with many rapidly proliferating cells, including those of the immune system.[57,58] In addition, there is much evidence that hypercatabolic and hypermetabolic situations are accompanied by marked depressions of the intramuscular glutamine concentration. This has been shown to occur after elective operations, major injury, burns, infections and pancreatitis, irrespective of nutritional attempts at repletion

(Fig. 5). Reduction of the muscle-free glutamine pool thus appears to be the hallmark of the response to injury, and its extent and duration is proportional to the severity of the illness.[50,59,60]

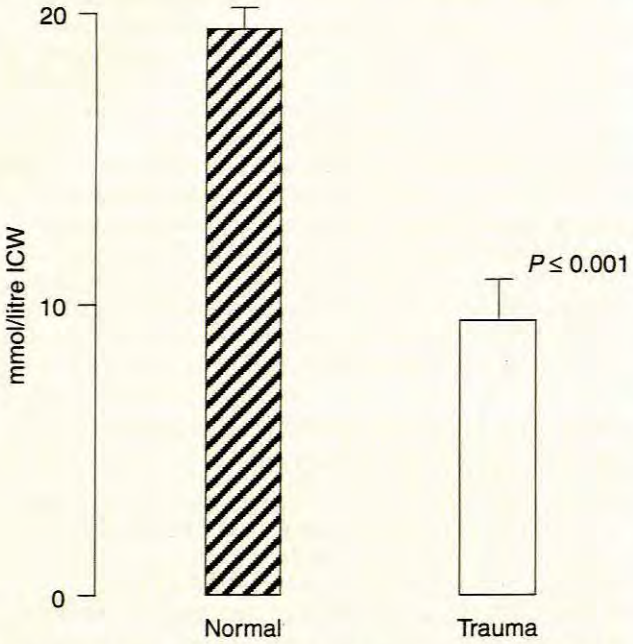


**Fig. 5. Trauma-induced intracellular glutamine depletion in human muscle tissue (mean ± SEM).[41, 50, 80] (ICW = intracellular water.)**

Two recent observations suggest that glutamine is involved in the regulation of muscle protein balance: firstly, the striking direct correlation between muscle glutamine and the rate of protein synthesis, and secondly, the positive effect of maintaining intracellular glutamine content on protein anabolic processes *in vitro*.[61,62] If maintenance of an intracellular glutamine pool promotes conservation of muscle protein, there is a theoretical case for glutamine supplements in the clinical nutrition of stressed and malnourished patients.[63] However, two specific chemical/physical properties hinder the inclusion of free glutamine in commercially available amino acid preparations: the quantitative decomposition of aqueous glutamine to cyclic pyroglutamic acid associated with ammonia liberation, and the limited solubility of L-glutamine in water.[41,64]

Recent knowledge concerning the efficient utilisation of intravenously supplied di- and tripeptides opens up the possibility of substituting available amino acid solutions with glutamine, cystine and tyrosine containing stable and highly soluble short-chain peptides. Undoubtedly this new approach has introduced a new dimension, although the explosion of new information about peptide assimilation is only a prelude, prior to intravenous use of peptides in common clinical practice.[40,59,65,66]

Basic studies in human and animal experiments with various highly soluble and stable synthetic glutamine-, tyrosine- and cystine-containing short-chain peptides provide convincing evidence that these new substrates are rapidly cleared from plasma after parenteral administration without being accumulated in tissues and with inconsequential losses in urine.[40,41,66-70] Considerable hydrolase activity ensures a quantitative peptide hydrolysis, the liberated amino acids being available for protein synthesis and/or generation of energy.[63,71,72] Following a bolus injection or under conditions of continuous TPN, these peptides provide tyrosine, cystine and glutamine, respectively, for maintenance of their intra- and/or extracellular pools.[65] Parenteral dipeptide nutrition promotes growth and nitrogen retention. Interestingly, intravenous provision of L-alanyl-L-glutamine (Ala-Gln) reduces muscle loss of glutamine[73] during stress and prevents intestinal mucosal atrophy in parenterally fed rats.[74-76] Current results indicate that Ala-Gln-supplemented parenteral nutrition may avoid TPN-related intestinal dysfunction[77] and atrophic response[78] in patients in the intensive care unit.

In patients undergoing major elective surgery, infusion of alanyl-glutamine supplemented TPN over 5 days resulted in an improvement of nitrogen balance and muscle intracellular glutamine on each postoperative day compared with controls receiving isonitrogenous and isoenergetic TPN without the peptides. The improved net nitrogen balance was associated with maintenance of the intracellular glutamine pool, whereas in patients receiving the control solution glutamine levels were markedly decreased compared with pre-operative values (Fig. 6). The peptides

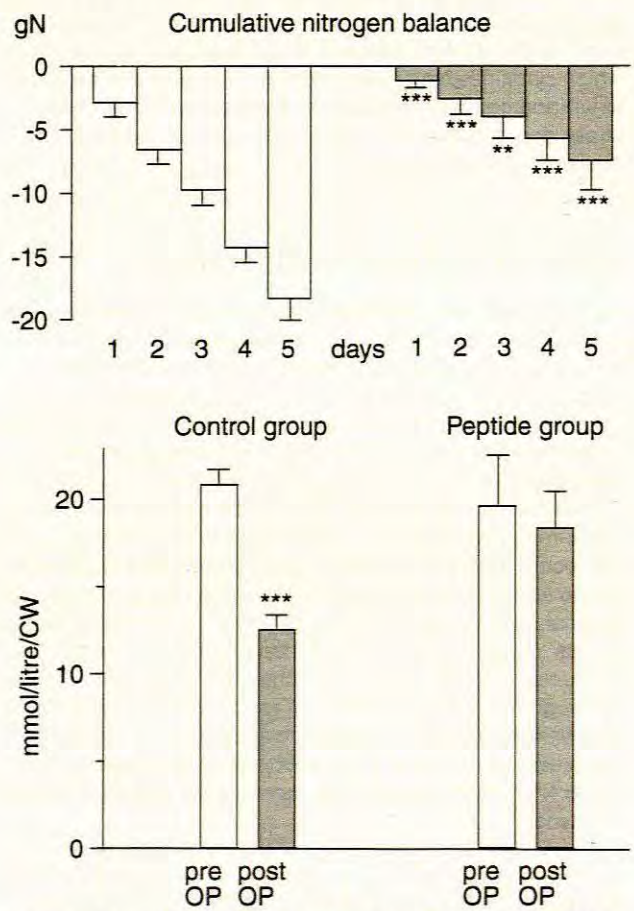


**Fig. 6.** Top: **Cumulative nitrogen balance (mean ± SEM, *N* = 6) in patients after major elective surgery receiving peptide-supplemented (dotted columns) or conventional (open columns) TPN. (****P* < 0.001; ***P* < 0.01.)** Bottom: **Intracellular muscle glutamine concentration (mean ± SEM, *N* = 6) in control patients and in patients receiving Ala-Gln-supplemented TPN (peptide group). (ICW = intracellular water; **P* < 0.05; ****P* < 0.001.)[50, 83, 84]**

were not detectable in plasma and muscle, and the plasma concentrations of the constituent amino acids did not differ between the treatment groups. Infusion of the solutions was free of any side-effects, and postoperative recovery was normal in each patient. In good agreement with these results, intravenous supply of alanyl-glutamine after cholecystectomy preserved the intracellular glutamine pool (91% pre-operative value) and the characteristic postoperative change in muscle ribosome profile was abolished.[79] Beneficial effects of short-term infusion of alanyl-glutamine on muscle protein synthesis assessed by ($^{13}$C) leucine incorporation were reported in post-surgical patients.[80]

In a fascinating current hypothesis the essential importance of cellular hydration state as determinant of protein catabolism in health and disease is emphasised.[81] It is postulated that an increase in cellular hydration (swelling) acts as an anabolic proliferative signal, whereas cell shrinkage is catabolic and antiproliferative. The authors put forward the hypothesis that changes in cellular hydration state might be the variable linking muscle glutamine content with protein turnover and, because of the large muscle mass, the whole body nitrogen balance. Data from previous studies of the relation between intracellular glutamine content and catabolism in patients with various underlying disorders enabled the evaluation of the relation between muscle-cell water content and whole body nitrogen balance, showing an inverse relation (Fig. 7). The concentrative uptake of glutamine into muscle and liver cells would be expected to increase cellular hydration, thereby triggering a protein anabolic signal. Indeed, preparations containing glutamine dipeptides may facilitate aggressive therapeutic interventions in order to improve cellular hydration state and subsequently modify or reverse catabolic changes.

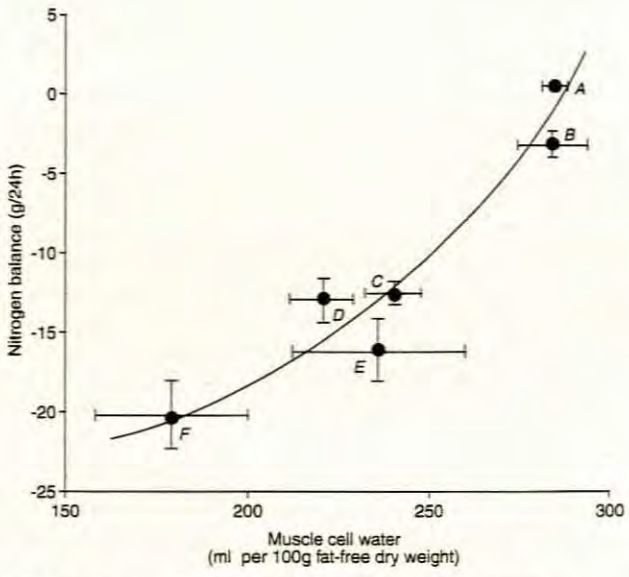


**Fig. 7. Whole-body nitrogen balance and cellular hydration of skeletal muscle. A = healthy subjects (*N* = 17); other subjects are patients suffering from liver tumours = B (*N* = 5); polytrauma day 2 = C and day 9 = D after trauma (*N* = 4). Skeletal muscle water was assessed in biopsy specimens from m. quadriceps femoris and the extra-/intracellular distribution was calculated by the chloride method, assuming normal membrane potential of –87.2 mV.[12,13] (From Häussinger *et al.*[81] with permission.)**

## *New substrate in clinical nutrition — lipids*

Over the past years a consensus has been reached concerning the use of lipid emulsions as regular component of parenteral nutrition. Several new emulsions are undergoing experimental evaluation. A number of novel lipid moieties, like MCTs, and structured lipids, n$^3$-FA and short-chain fatty acids are receiving increased attention as potential substrates in clinical nutrition.[82]

**Medium-chain triglycerides.** MCTs were introduced more than 30 years ago as a constituent of the first 'medical food', valued because of their rapid hydrolysis and absorption in the gastro-intestinal tract as well as their direct transport to the blood and liver. Intravenous MCT emulsions are now well established in Europe and presumably may soon be available in the USA. In early animal studies, infusion of pure MCT emulsions was associated with various undesirable symptoms, like hyperketonaemia, narcosis, hyper-lactacidaemia[83] and central nervous system toxicity.[84,85] These effects are due to the rapid hydrolysis of parenteral MCT, and the ability of the released FA to cross the blood-brain barrier (in contrast to LCT).

The threshold level of competition favouring LCT over MCT has been suggested to yield the optimum proportion of 66 - 50% LCT.[86] When this concentration of 33 - 50% MCT was attained, LCT outcompeted MCT for hydrolysis. The suggested physical mixtures of MCT and LCT are well tolerated when infused at relatively low rates.[87,88]

In clinical studies, administration of an MCT/LCT mixture revealed in many cases distinct advantages over an LCT emulsion. In surgical patients the mixed emulsion produced fewer circulating triglycerides and non-esterified FA than LCT, suggesting favourable utilisation.[89] The rapid plasma clearance[83] is associated with improved reticulo-endothelial system function[90] and thus results in less pulmonary sequestration of bacteria.[91] However, it should be said that the high lipid loads necessary to demonstrate this in experimental animal models may not apply to man.[40]

**Structured lipids.** A special new type of fat is constituted by the so-called 'structured lipids'. These man-made lipids were originally synthesised by varying combinations of LCT and coconut oils, resulting in a random assortment of triglycerides containing different medium-chain/long-chain FAs,[92,93] but they can also be created by interesterification of MCT and fish or safflower oils.[94] There are studies suggesting that structural lipids are superior to MCT/LCT mixtures. In animal experiments, higher albumin concentration, nitrogen retention and growth were observed with structural lipids than with physical mixtures of MCT and LCT, and lower rate of infection and improved survival are claimed following parenteral administration of structural lipids.[92,95] These effects are assumed to be due to a lower production of inflammatory and immunosuppressive eicosanoids. We can say tentatively that tailor-made structured lipids containing linoleic and/or alpha-linolenic acids may offer essential fatty acid components on the same glycerol molecule.

The value of physical combinations of emulsions or structural lipids as energy sources remains to be determined. Currently there is no commercial source of structural lipid emulsion, and so both preclinical and clinical experience is limited.

**$n^3$ fatty acids.** The current interest using fish oils has its origin in the epidemiological observation of lower incidences of atherosclerosis and age-adjusted mortality in Greenland Eskimos compared with the whole Danish population.[96] The Eskimos' diet contained total fat and cholesterol similar to the Danish diet but also a substantial amount of $n^3$-PUFA, also called $n^3$-FA. As already mentioned, there is the claim that $n^3$-FAs exert a protective effect in the development of cardiovascular[97] and inflammatory diseases, [98,99] and the beneficial effects of fish oil supplementation in many other chronic diseases have been advocated. Some preliminary observations suggest a potential role for fish oils in the treatment of atopic dermatitis[100] and psoriasis.[101] Dietary $n^3$-FA treatment offers exciting novel possibilities in malignant diseases.[102,103] There are also indications that premature infants have limited dietary support of the $n^3$-FA required for the normal composition of brain and retinal lipids.[104,105]

The major advantages of EPA- and DHA-acquired metabolites are summarised as follows:

1. EPA-derived thromboxane $A_3$ is less active in platelet aggregation than $A_2$, whereas the anticoagulant properties and relaxation of vascular smooth muscle are preserved.
2. Leukotriene $B_4$ enhances chemotaxis, while others like $C_4$, $D_4$ and $E_4$ augment vascular permeability and contractility.[106] EPA is converted into $B_5$, which has only a fraction of the activity of $B_4$ and PAF, resulting in decreased chemotactic migration and endothelial cell adherence. This would mean that $n^3$-FAs exert major effects on the synthesis of leukotrienes that promote inflammation.
3. Feeding with fish oils is associated with profound changes in immunoregulatory processes, including, as mentioned, the production and release of various cytokines. It is currently postulated that, partly as a result of these changes, the natural history and progression of diseases with an inflammatory or immunological component may be altered.
4. Consumption of EPA and DHA reduces serum cholesterol, LDL and triglyceride concentrations.

There are numerous reports regarding experimental and clinical trials following enteral (oral) application of various fish oil preparations. However, little is known about the effects of intravenously administered $n^3$-FA . In our laboratory, parenteral administration of a fish-oil preparation (10% Omegavenös; Fresenius AG, Germany) had no apparent influence on growth and nitrogen metabolism in catabolic rats.[107] This might tentatively be explained by the assumption that the catabolic response is virtually obligatory during the initial phase and might not be influenced by nutritional efforts.[108] TPN with fish oil resulted, however, in a decrease of plasma-free FA concentration and liver content of triglycerides, suggesting an increase in FA oxidation and reduced liver triglyceride synthesis. In our study,[107] fish oil feeding revealed a dose-dependent incorporation of $n^3$-FA into tissue total lipids and phospholipids at the expense of $n^6$-FA, as early as 3 - 4 days after starting infusion.

A word of caution is in order against over-interpretation of currently available information. Extrapolations from *in vitro* observations in the *in vivo* situation, and from animals to humans, may not be valid. There are several questions which may, however, be raised. In relation to the duration of treatment and the supplementary dose used, it is conceivable that the treatment and washout periods used in many studies were too short. In this respect one may consider delayed effects of $n^3$-FA on monocytes and IL-1 formation that are sustained several months after termination of the supplement. Definition of the optimum dose of fish oil that can produce beneficial action without showing undesirable side-effects (e.g. increased bleeding tendency, decreased insulin responsiveness) is warranted. Moreover, there is a need to identify which component of fish oil has the most potency in achieving the desired metabolic effect. There is clearly much to be learned before clinically useful fish-oil based emulsions become a reality.

Just over a century ago the relationship between nutrition and illness might best have been summed up by the old English proverb 'stuff a cold and starve a fever'. Today our understanding of that relationship has evolved to the point where scientific investigations are able to explore the role of diet in the regulation of gene expression. This article illustrates how far we have advanced in our understanding of the critical role of clinical nutrition in modifying treatment and/or the metabolic response to an acute or chronic illness. The challenge of integrating an imperfect background of widely diverse but pertinent knowledge was emphasised in trying to explore the role of nutrition during episodes of illness and malnutrition. Nutrition may interact with the metabolic response to stress in several ways. The catabolic response can be modified and recovery might be accelerated by various nutritional means, resulting in shortened length of hospitalisation and reduced convalescence.

Even as clinical nutrition is becoming established as an essential medical discipline, basic advances in nutritional biochemistry continue to push back the frontiers of knowledge of the relationship between basic mechanism of a disease and nutritional measures. The new frontiers of molecular biology also have immediate applications to nutrition science and clinical nutrition. Indeed, the combined knowledge has already been used by clinical investigators for novel therapeutic measures.

Modern clinical nutrition is a rapidly evolving and ever-changing discipline. In order to facilitate the general progress in the field, clinicians should be up to date with all fascinating facets of this development. It is my hope that this article will be of some value in promoting interest and understanding among the practitioners, clinical and basic scientists towards an advanced clinical nutrition. Continued rigorous critical evaluation of assumptions and hypotheses about relationships between diet, nutrition, health and disease will eventually provide reliable knowledge of what can and what cannot be achieved through nutrition.

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