General Discussion at the 3rd Amino Acid Assessment Workshop (3rd AAAW)1

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Young: Now we will turn to the general discussion that we have included in the program. What we thought we would like to do is give all of you an opportunity to contribute to the discussion: to think about how your presentation possibly might lead us closer to identifying, or help us to identify, upper levels of amino acid intake. But before throwing this to the audience, we are going to ask three individuals to make five minutes worth of viewpoints, comments, or reactions. And these three individuals will be Dr. Kraisid Tontisirin from FAO, Dr. John Fernstrom from University of Pittsburgh, and Dr. Kyoichi Kishi from University of Tokushima. Would you like to start, Kraisid? Say whatever you want that is useful to the purpose of this workshop.

Tontisirin: Thank you for inviting me to participate in this very important workshop. I would like to present some of my thoughts, and share with you some of the related work that FAO has been doing during the last two to three years. Recommendations of energy and nutrient requirements are one of the most important areas in which FAO and WHO have been working together to provide science-based recommendations to member countries and other international bodies. From time to time we revisit and organize expert consultations to update information on nutrient requirements. For example, during the last two years we have organized several consultations. One was on human energy requirement, which was convened in October, 2002. I am pleased to inform you that the report will be published in the next few months.

Another expert consultation was on protein and amino acid requirements chaired by Peter Garlick; many participants in this room are expert members including Vernon Young, Joe Millward, Dennis Bier, Paul Pencharz, William Rand, and Peter Fürst. I expect that the report will be published next April or May. With regard to the upper level of nutrient intakes, it is in our work plan during the next two years to organize expert consultations to look into upper levels of vitamins and minerals.

For the upper level of amino acids, it appears from this workshop that there is need for more data and information in particular on the database for each individual amino acid in terms of intake, metabolism, and requirement. It seems appropriate to apply “risk analysis” to study the upper levels of amino acid at high intake levels because Codex has emphasized and elaborated this process for food safety. Risk analysis (RA) is composed of three components: risk assessment, risk management, and risk communication. RA is well established for chemical hazards, and FAO and WHO are now extending the experience and expertise developed from RA of chemical hazards to that of microbiological hazards. It will require extensive work and expertise to apply this process to nutrients including amino acids. As shown by Vernon Young in his slides during the opening session, risk assessment is composed of four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. Risk assessors will perform risk assessment and provide data and information to risk managers. At the global level, the Codex Alimentarius Commission sets up food standards, guidelines, and recommendations by taking into account risk assessments carried out at the international level by the Joint FAO/WHO Expert Committee on Food Additive and Contaminants (JECFA), the Joint FAO/WHO Meeting on Pesticide Residue (JMPR), and other expert bodies.

From my observations, I realize that risk assessments for chemical and microbiological hazards are at an advanced stage and are proceeding satisfactorily, but the work for scientific advice to Codex and member countries on nutrition, including safety aspects of high intake of nutrients, has been rather slow. Sometimes it has been quite frustrating. I would like to come back to this meeting. I really appreciate the intention and determination of the International Council of Amino Acid Science (ICAAS) and the scientific expertise of the group, chaired by Vernon Young, to further advance the research in assessment of amino acid intakes. This will stimulate the international community to do more research and will lead to the generation of more databases for individual amino acids; eventually the public will benefit from the information and from appropriate guidelines for consumers.

I am pleased to inform you that FAO has recently organized the Technical Workshop on Energy Conversion Factors and the report is now available at the FAO web site at the Food and Nutrition Division web page. There is also a plan to update the food composition tables including the table for amino acid composition. This needs your collaboration in some part, too.

1 Presented at the conference “The Third Workshop on the Assessment of Adequate Intake of Dietary Amino Acids” held October 23–24, 2003 in Nice, France. The conference was sponsored by the International Council on Amino Acid Science. The Workshop Organizing Committee included Vernon R. Young, Yuzo Hayashi, Luc Cynober, and Motoni Kadowaki. Conference proceedings were published as a supplement to The Journal of Nutrition. Guest editors for the supplement publication were Vernon R. Young, Dennis M. Bier, Luc Cynober, Yuzo Hayashi, and Motoni Kadowaki.

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3 The 3rd Amino Acid Assessment Workshop is dedicated to Vernon R. Young who recently passed away.

FAO is also working with WHO on the guidelines of food fortification, and the report will be finalized soon. FAO and WHO will jointly organize the “Expert consultation on nutrient requirements for infants and young children under two years of age.” This consultation will also cover protein and amino acid requirements of these age groups.

Food consumption surveys in developing countries will be promoted with the main objective to generate a high-quality database of food and nutrient intakes to link with nutritional status, diet, and disease relationship and as a part of exposure assessment to hazards, with the hope that these data will be used for food and nutrition programs. Last year FAO also engaged with WHO to organize the expert consultation on diet and nutrition in prevention of chronic diseases, and the report of the experts was published in April of this year. This report received extensive attention from the press because it has population nutrient-based recommendations in particular for fat, carbohydrates, and added sugar. Can we think along this line by looking at certain amino acids with specific functions in nutrition, health, and disease?

Finally, I would like to share with you another activity in FAO relevant to nutrition. It is a modernization of FAO statistics, which will include food availability by country and region. This updated data certainly will be of interest to a statistician like Will Rand who may wish to spend a few months in Rome to analyze protein and amino acid availability at the regional and country levels in relation to requirements. With that, I think I have shared enough information with you. Thank you.

Young: Well, thank you very much for being so positive about our meeting. And we wish you luck at FAO. John, would you like to give us some thoughts that are useful?

Fernstrom: I looked last night at the proceedings of the second ICAAS meeting, and was drawn to the article and discussion offered by Rodricks [Rodricks, J. V. (2003) J. Nutr. 133: 2025S–2030S; Cynober, L. & Young, V. R. (2003) J. Nutr. 133: 2101S–2107S]. It led me to reflect on the goal of the ICAAS efforts. One of the statements in his manuscript was particularly cogent: “Unless the world’s best talent in amino acid science is brought to these questions, it is likely that regulators will eventually step into the breach and seek to force upon amino acids the approach to risk assessment designed to deal with foreign chemicals.” In the general discussion of the meeting, he attempted to lead the participants in part to design a set of animal experiments that regulators might find useful in arriving at a set of standards to be used to define safety and toxicity issues for amino acids. In reading the discussion of this interchange, it seemed either that there was a lack of understanding of his message, or that the issue of defining amino acid safety in a diverse human population is almost impossible, given the enormous number of interactions. In the pharmaceutical industry, testing of safety and efficacy begins with animal studies, then proceeds to limited human studies and clinical trials. If safety and efficacy are established, this leads to introduction into the population, after which study of the exposed population continues to make certain that with large-scale human exposure, safety issues do not arise that could not be seen in smaller controlled trials (because of power considerations). Conceivably, as a point of departure, such a process could be considered for amino acids. In any case, Dr. Rodricks challenged the group with issues of design, with animal studies as the starting point, and discussed study length and end-point measures. When he challenged the group with the question “Is there any reason I should not do that for an amino acid?”, the responses were rather mild, and I wonder if he walked away with very many good answers. So I raise this issue for discussion because I think we need to restate it and then examine the end point of these exercises. The end point should be the definition of safe intake levels for individual free amino acids in the food supply. We have to do this, because, as Dr. Rodricks pointed out, in the U.S. all amino acids except tryptophan are still sold freely to anybody, and ultimately if we do not carefully work through related safety issues, someone else will.

Young: Thank you very much.

Renwick: I read that discussion with interest. I agree with you, I think there were a lot of issues that were raised that were not resolved at that meeting. We do need positive hazard identification methods, and I think that in vivo studies should incorporate the use of biomarkers related to both exposure and response. Key questions are: what those biomarkers might be, how they will allow us to bridge from animal to man, and how we could incorporate them into human studies. I think that in this day and age, you would be crazy if you were doing a 90-d study, and not build in basic marker measurements that correlate with the change in the adverse effect.

Fernstrom: To respond to your comments, you might wish to do studies in animals that actually mimic human use. For example, humans might not mix amino acid supplements into their normal foods, but might take them directly as pills in association with an intended activity (e.g., exercise). Hence, exposure would be intermittent and separated from meals, and thus quite different from the design of a standard animal toxicity study (agent is mixed in with the food). In animal studies, therefore, one can imagine that an amino acid of interest might be given as a gavage (in lieu of a pill) at times of the day when they are not eating. Such an approach would also fortuitously avoid the problem of palatability, which sometimes leads animals to reduce their food intake, when the agent included in the diet has an undesirable taste, giving the false impression of an adverse effect (weight being a standard measure of adverse effect). Denny Bier also made some points that struck me as highly relevant regarding clinical studies. For example, regarding the choice of end point of measure, how can you define sample size without knowing your end point, to make power calculations? And, considering the institutional review board (IRB) as a filter for study approval, you cannot gain approval for a study without considering risk-benefit analysis and including an analysis of the likely incidence of side effects (e.g., you have to list, detail by detail, what the likely incidence of side effects is at the 10% level, 1% level, 0.1% level, and so on). Hence, there are many experimental issues in need of discussion, both in animals and humans, to arrive at a well-constructed experimental plan for evaluating the safety of individual amino acids.

Renwick: I think you are right. It also depends on the nature of the beneficial effect and therefore the pattern and nature of human intake. If it is to be taken in capsules or tablets, then diet palatability in the animal studies is not an issue, because humans would not show a decrease in food intake because it tastes bad. So we would have to deal with that. You have to think about the intended human exposure scenario and that has to be taken into account in the design of the animal experiments.

Caldwell: To go a bit further, I think that John Fernstrom is going in the right direction here, because decreased weight gain is an extremely crude index. The only use you would make of it, in a pharmaceutical or environmental chemical toxicity test, would be to set those limits for long-term studies. You would simply work to what the FDA chooses to call the “maximum tolerated dose,” which is described as one that does not cause a more than 10% decrement in weight gain over a 3-mo dose-ranging study. The key thing, of course, is what these
compounds are actually doing inside the animal. The specific example that two or three of us have been talking about among ourselves surrounds phenylalanine. And, of course, the central nervous system (CNS) issue, because I perhaps asked a rather naive question, and that is what do phenylketonuria (PKU) patients actually suffer from? Is it a phenylalanine overdose or tyrosine deficiency? It seems to have elements of both. If we cannot answer a question like that, about what is almost certainly, based on everybody’s evidence, the best-studied amino acids among the 21, I can see that we have a great need for a framework. You do not want to get anywhere near the National Toxicology Program. Some of you might have had something to do with it, but if you have not, it really is demanding. You do not want to get anywhere near it. It is testing to destruction! But quite clearly, we have to find a way of steering through something that relates to the establishment of sensible upper limits. Sensible upper limits can be derived without going to the extent of all the issues involved in formal toxicity testing. Pencharz: I think your points are well taken, John (Caldwell), but just for your information and other people’s, that was one of the motivations for the series of studies we did in PKU. In fact, these children are given supplements of tyrosine, and some people have gone to much higher doses of tyrosine, with the theory that they would have beneficial CNS effects. My co-author, Joe Clarke, was involved in a study to determine whether there was a change in the learning and IQ performance of the children who were given high levels of tyrosine intake. They did not find any benefits.

Gibney: I would like to comment on the issue John Fernstrom raised. I suggested that you are going to have to be imaginative. And I think that the approach you suggested, John, is not going to help. First of all, because the simple fact is it is going to cost an awful lot of money. Even if you do it with a mixture of all the amino acids, when you give one amino acid at high dose, it affects other amino acids. It is an endless question. And the question you have to ask yourself is: “What is the social value of all that?” And that is why I think you are going to have to be imaginative. I do not know what the solution is, but I think testing them to death as drugs is not the answer because drugs are in a very managed environment. These are not. Even if you were to come up with safe upper levels, that would not stop people from buying a packet of pills and taking them. So you will not have necessarily solved the problem.

Garlick: I would just like to emphasize two points that I have in my conclusions that would be necessary for any testing. One is the fact that people really would take supplements in all sorts of different ways, which would have to be accounted for. Particularly, amino acids would be consumed without food, so you would get amino acids spiking to very high concentrations, presumably absorbed very quickly and probably smoothed out. And it would not be all that important. The second thing is that, obviously, there is no way, absolutely no way, that the testing can be done for all amino acids together, or even groups of amino acids in terms of their effects. There are quite a few amino acids that cause changes in neurological effects, but different mechanisms are involved. So it is not going to be something that you could do as a block thing. Similarly, the notable thing about the two most toxic amino acids, the two sulfur amino acids, is that their toxicity is characterized by completely different mechanisms. The amino acids all have to be treated as completely independent things.

Renwick: Just to comment about this idea of testing mixtures. That would tell you what you want to know about the mixture itself, because we have heard that amino acids, such as the sulfur amino acids, are closely interrelated. So you could have one amino acid masking the toxicity of another one. We really have to adopt a reductive approach, and look at each one separately. Of course we would also have to prioritize. But personally, I do not see any way around the reductive approach of looking at them individually, and then using knowledge and understanding of metabolic pathways to predict circumstances where there may be concern about possible interactions, based on the data of individual amino acids. To test a mixture could potentially mask a problem.

Caldwell: Just to add to that, if you start to go down this road, inevitably it requires you to demonstrate toxicity. You have to have frank effects, hopefully with very high doses. I was looking at some data last week where people were feeding animals a new drug at 14% (w/w) of the diet, which they regarded as the maximum feasible dose. The animals were excreting white feces, because they could not absorb the compound, which had very low solubility. And they regarded that, not as maximum tolerated dose but the maximum feasible dose. In fact, they have gone way over what was really practical and, even so, the FDA told them to try harder. And so what I am saying is, you want to stay as far away as you can from that sort of regulatory approach. It would inherently be more relevant to pay more attention to the essential amino acids, rather than the ones that are synthesizable, just in terms of looking at the issues about intakes and all the rest of it. Because if you’re supplementing a natural background, that’s one thing, but if you are supplementing something you need to take into the diet, then that may represent a possibly hazardous intervention.

Baker: Could I just respond to Dr. Renwick very quickly? You referred to “palatability.” I would like to suggest that those of us who work in the area from a nutritional standpoint do not like to use the term “palatability” because we really do not know why food intake is reduced in animals when amino acids are consumed in excess. The animals cannot tell us. We use the term “acceptability.” I agree with what Vernon Young was, I think, inferring: we assume that food intake is a physiological response, rather than a taste response. Although with some of the high levels I was reporting, it could very well be taste. Among the 22 or so amino acids I have worked with over the years, some are essentially tasteless, some are bitter, some are salty, and some are sweet. One nice thing about the avian model is that chickens have very little taste perception. In choice studies in both chickens and pigs, we have found that these animals will self-select a diet with 4% excess dietary leucine over one containing a normal level (about 1%) leucine. Moreover, Quinton Rogers at the University of California, Davis, has observed that cats actually prefer a diet containing as much as 10% leucine. It should be noted, however, that in both our studies and those of Dr. Rogers, both isoleucine and valine were surfet in the diet to which excess leucine was added.

Fernstrom: Well, there is literature on this. Every amino acid has a taste; Dr. Torri can elaborate on this issue. So it is a given that taste is going to affect the palatability of food. And that has to be considered in the experimental design of animal studies that include amino acids in the diet. I understand what you are saying, but the bottom line is that absent any positive move on this group’s part in developing an experimental design for doing this sort of study, somebody else is going to impose that experimental design. For example, the next time the DRI exercise occurs, the committee may decide: “Well, the last DRI committee did not assign upper levels. And it’s 10 years since, which should have been sufficient time to collect the needed data to make an informed assessment. So, whatever data are available now should be used to assign upper levels.” And numbers will be picked based on available data at that time.
course, this is a worst-case scenario. But, absent new data, numbers may be assigned anyway. The reasons not to do something do not help. We need reasons to do something, and then do it. And hopefully, if this is the decision, Peter Garlick will have a better database/literature the next time from which to extract valid numbers for making calculations of safe intakes.

And finally, tyrosine and phenylalanine are co substrates for tyrosine hydroxylase, suggesting that one does not need to give tyrosine to a PKU patient to produce catecholamines. The phenylalanine should be adequate as the catecholamine precusor. Seymoun Kaufman demonstrated this more than 20 years ago using a purified enzyme preparation [Katz, I., Lloyd, T. & Kaufman, S. (1976) Biochim. Biophys. Acta 445: 567–578], and we showed it to be true a few years ago in PC12 cells [DePietro, F. R. & Fernstrom, J. D. (1999) Brain Res. 831: 72–84.]

Gibney: Is there any evidence available that taking amino acids does good? In which case that has to be weighed against the toxicity levels, and that have to be documented. I am not too sure that you have substantial evidence to say that taking megadoses of amino acids is good. You do not have any evidence to say that taking them is not good either. It is not as though we are giving people things that, you know, might not have any good. They might.

Young: Could I ask Dr. Kishi to make a quick presentation?

Kishi: Firstly, I would just like to thank the organizers, Dr. Young, Dr. Cynober, Dr. Kadawaki, and also ICAAS for inviting me to this meeting. I will focus on a fundamental issue in amino acid nutrition. That is the amino acid balance. Unbalanced amino acid intake has adverse effects. Harper et al. classified amino acid imbalance into four categories, that is: defeciency, excess, imbalance, and antagonism. The balance of dietary nitrogenous constituents influences the amino acid requirement; one of them is total nitrogen intake or protein intake, the second is the indispensable amino acid (IAA) pattern, and the third is the indispensable to dispensable amino acid ratio (I/D ratio). Our data [Ikemoto, S., Miyashita, M., Yamanaka, C., Shizuka, E., Kido, Y., Kishi, K., Sugawara, Y., Kori, H., & Yamamoto, S. (1989) Nutr. Rep. Int. 39: 477–485] showed that maximum weight gain was obtained at lower I/D ratio when the amino acid mixture diets simulating an egg pattern rather than a casein pattern were fed. Also the IAA requirement is influenced by I/D ratio and total N intake [Hosoi, A., Kishi, K., & Inoue, G. (1988) J. Nutr. Sci. Vitaminol. 34: 245–259]. Thus, the IAA requirement depends on the amino acid composition of the basal diet. It means that amino acid requirement values are valid only under the conditions under which they are determined. Here, I want to emphasize the relativity of the amino acid requirement, although the amino acid requirement is usually determined as an absolute amount (mg/kg body weight/d). We had better express each amino acid requirement as a relative amount, relative to total IAA or total AA (or protein).

Usually, the amino acid scoring pattern (mg/g protein) is obtained by dividing the estimate of each IAA requirement (mg/kg body weight) by the safe level of protein intake (g/kg body weight) determined independently in separate studies. The pattern would change with the change in the estimate of the protein requirement even with the same IAA requirement figures. That happened in the adult amino acid requirement pattern between the 1973 FAO/WHO report and the 1985 FAO/WHO/UNU report. IAA requirement estimates did not change but the safe level of protein intake increased from 0.55 in the 1973 report to 0.75 g/kg/d in the 1985 report, resulting in the lower estimate of adult amino acid pattern.

Also toxic effects of amino acids depend on protein levels. Animal experiments showed that when given with a low-protein diet, excess amino acids have great adverse effects. But at higher protein intakes, the same level of amino acid may have no toxic effect. In conclusion, when considering amino acid nutrition, we should always keep amino acid pattern in mind.

Young: Thank you very much. Would anybody like to comment?

Cynober: I think that Dr. Kishi raises a very important issue: the way we are expressing the amount of amino acids given as a supplement. In my opinion, we need to express the supplementation in mg/kg/d versus percentage of total N intake and the essential amino acid to nonessential amino acid ratio (EAA/NEAA). This mode of expression allows one to know what a rat or a pig actually receives. Reading the literature I often have some difficulty in determining what the animal actually received because the data are expressed as a percentage of the total diet. If you do not know the total food intake, you are not able to determine what the dosage of supplementation was.

Gibney: I actually thought that was a brilliant suggestion of using the impact of high levels on total amino acid composition, because it gives you an opening to begin to look at the impact of an amino acid on other amino acids. There is, I think, some knowledge about balances of amino acids and their impact. And I would certainly see it as an opportunity to be exploited in terms of intake, because the g/kg/d is going to be done in dozens and dozens of studies. You could perhaps have some sort of conceptual approach, and maybe linkage to amino acid requirements. I think it is a very good idea.

Fernstrom: I like the idea of having both pieces of information available. Looking back at some of Harper’s work examining the chronic level of protein intake and its effect on the induction of amino acid catabolic enzymes, one can see that the background level of protein intake will affect the rate of metabolism of any amino acid that might be taken separately as a supplement. This notion was amply demonstrated by another group [Tanaka, H., Nakatomi, Y., Takahashi, K. & Ogura, M. (1991) Agric. Biol. Chem. 55: 531–537] that looked carefully at the rate of metabolism of many amino acids in rats as a function of the chronic level of dietary protein intake. Below ~10% protein intake (as a percent of daily energy intake), the catabolism of individual amino acids falls off rapidly. Above 10%, the catabolic rate is maximal for each. Hence, if one is thinking about the body’s exposure to an amino acid supplement, it’s useful to know what the background protein intake is, not just the dose of the amino acid itself.

Millward: Can I pick up on the comment that Peter Garlick made about just expressing intakes in relation to only total protein or amino acid intakes? If we did this, rather than expressing intakes as total absolute intake, it would assume a potential mechanism of action that will only be realized when it is consumed at the same time as protein. In fact what we need to know is what the responses are when it is just taken on its own. In my view we need to know both values and I think Luc Cynober is right, that a single way of expressing intake, such as percentage of the diet on its own, isn’t good enough. It’s a useful value, but we need total intakes as well and we need to be able to do all the permanutations according to the paradigm that we are actually exploring.

Cynober: The absolute value by kg body weight gives us an idea about the burden of the supplementation. And with the ratio of total amino acids to total protein, we look at the character of imbalance. Of course, the significance of the supplementation is not the same. For example, if you make a supplementation with branched-chain amino acid to replace the 90% of the total amino acids, it is not the same thing as if
the same amount represents with only 10% of the total amino acids.

Young: Would you like to answer a few questions?

Cynober: Of course, we could discuss a number of issues. I would like to focus on some specific pathological states. One aspect, which has not been discussed, is the context of long-term parenteral nutrition. In this condition the intake of amino acids is not a burden. But we have to consider that there is a bypass of the splanchnic area for months or even for years, and it has been demonstrated that long-term parenteral nutrition has some relative side effects: for example, long-term TPN leads to calcireua, leading to bone resorption, and also steatosis. And it is clear that some amino acids may be involved in these long-term effects. Hence in my opinion, in the context of examining some nonspecific marker of exposure to long-term administration of amino acids we should give a look at the bone, kidney, and the liver functions.

Pencharz: I would actually say the liver is the main concern, rather than the kidney. Certainly for pediatric, long-term parenteral nutrition, cholestasis is a problem. It starts from very early on. If you use bile acids as a marker, you could find cholestasis quite early. A very real problem is that it is not known how much of the cholestasis is due to defunctioning of the gut or whether it's specifically related to intravenous (IV) feeding. There are various hypotheses from work done in Edmonton by a former colleague and trainee of mine, Johnny Van Aerde, who showed that the IV fat is at least a part of the problem. There are recent publications suggesting that sulfur amino acids, especially methionine, may be hepatotoxic. It is an area of very major concern that needs to be resolved, and we do have patients proceed toward liver transplantation because of end-stage liver disease associated with long-term parenteral nutrition.

Cynober: I think it's a problem also in the adult patient, if Peter Soeters could comment on that.

Soeters: Well, the pathogenesis of liver steatosis and ultimately cirrhosis is multifactorial. It has to do with the nutritional regimen. It probably has to do with the composition of fat, but it also has to do, for instance, with particle size of the fat emulsion. In addition, it has to do with bile-acid metabolism. I allude to the decrease in the bile acid pool size due to short bowel interfering with the intrahepatic cycling. This makes the liver more vulnerable to the toxic effects of, for instance, endotoxin in these short bowel patients, who very often, despite their shortness, have bacterial overgrowth. This is a very complicated issue, but patients die of it. Another thing is, they very often suffer from osteopenia or even osteoporosis. This is not exclusively due to the parenteral nutrition itself, but rather due to the fact that they have short bowel and develop steatorrhea and therefore lose much calcium. In the early period of the presence of short bowel and long-term parenteral nutrition, you tend to forget about it but if you do not start treating them early for osteoporosis prophylactically, as it were, you will ultimately end up with completely crippled individuals whose bones fall apart.

Finally, there is the kidney problem with oxoluria in these conditions. This causes kidney stone formation ultimately leading to repeated sessions of urology treatments, including shockwave treatments. This may lead to end-stage renal insufficiency. This is a very complicated issue, but I think it is very relevant. It is a small group of patients, but it is a model by which we can study metabolic abnormalities in their extreme forms. This may be very relevant to much larger populations with less severe forms of disease of the digestive tract and liver.

Millward: May I just make a comment relating to bone, because I think there are parallels with situations in people who are being orally fed with high-protein intakes? The issue of net endogenous acid production needs to be considered. We know that there is a very high susceptibility of bone, at the level of bone demineralization, to changes in pH, as in renal calcium reabsorption. Certainly in long-term TPN, we do not normally give appropriate consideration to balancing sulfate production from amino acid oxidation with appropriate base. In the normal diet, we know that it is very important to balance protein with potassium salts of weak organic acids, which mainly come from fruits and vegetables, to avoid bone demineralization. With diets that are very high in protein, and very low in carbohydrates, such as the Atkins diet, where intakes of fruits and vegetables are low, one of the potential deleterious effects is that net endogenous acid production is much higher, because of a lack of base, which comes from fruits and vegetables. This could be a key issue.

There may well be another issue of amino acid toxicity that relates to overall dietary balance that we have not started to think about here. Do the TPN experts actually pay attention to long-term acid-base balance?

Pencharz: Actually Bill Heird and Ralph Dell first started to worry about acid base balance in neonates. They made us aware of amino acids, which were net hydrogen ion acceptors, which are glutamate and aspartate. Conversely, other amino acids are net hydrogen ion donors like lysine and arginine. By balancing these four amino acids they showed that it is possible to create an amino acid mixture for parenteral nutrition that is neutral from the acid-base point of view.

Cynober: There is also probably a direct interaction at the kidney level, especially concerning the problem of tubular reabsorption of calcium. We have to remember that there is an inherited disease that associates general hyperaminoaciduria and hypercalcuria. It has been demonstrated that perfusion of amino acids with only minimal carbohydrates leads to calcium loss in urine in healthy subjects.

If there are no further comments, I have a question for Dr. Fukagawa. You remarkably demonstrated yesterday that elderly people have some specific requirements. Now my question is: Do elderly people have specific susceptibility to high levels of amino acid supplementation? Are there some data indicating that certain supplementation could be more deleterious than in the young adults?

Fukagawa: I do not really know the specific data, with respect to that, but I would say that the associated diseases that they have will increase their vulnerability. However, if I were to look at normal, healthy animals and modulate amino acid intake along those lines, we could not find differential responses as a consequence of age, with respect to specific metabolism. From that standpoint, one could say that there may be areas that are riskier (or safer) than others.

Cynober: We studied old rats a few years ago using glycine as a placebo. Of course we studied in parallel old rats and young rats. It was clear that in the old rat, we have a huge accumulation of glycine and serine not found in the young rat. I think, with the fact that the population is aging and there is a relationship between health, nutrition, longevity, and so on, that the market of products designed specifically for elderly people will dramatically increase in the forthcoming years. And we have to take care to look at the specifics, not only requirements, but also possible side effects of high amino acid intake in this population.

Fukagawa: I would think that it would seem logical that it would be important to consider the vulnerability of the older population, with respect to supplementation, just as it is with respect to other supplements that also clearly differ with advanced age. With respect to your study on the accumulation
of glycine and serine, as I recall, it was published and there were plasma changes, correct?

Cynober: Plasma and tissues, such as muscle.

Fukagawa: I will not comment about that actually. But on the other side of things, I believe that we should begin to think about animal models we might exploit to help us understand what the toxic consequences of the imbalanced intake are. I would suggest the use of transgenic animals. Natural experiments have occurred in live humans with metabolic diseases and they have provided some insight, with respect to phenylalanine intakes, for example. And it is quite possible that we could design a specific animal that could be used for other amino acids, at least in terms of areas that we should begin to look at. Then we will be able to apply the insights in the human. This would be another direction of work, which is sidestepping the age question, but I think it is a very important and feasible approach we should all consider.

Young: The area of aging and response to high amino acid intake is really quite intriguing. You’ve been working, Naomi, on the consequences of aging, its characteristics, and so on. A number of amino acids are oxidized within the mitochondria and are involved in the transport across mitochondrial membranes. That association would suggest to me that there is some interesting research in the context of aging and the response to getting rid of high loads of specific amino acids.

Fukagawa: I agree, which is why I was embarking in research in that area. The underlying difficulty with the systems we have studied is that we do not know what denominator we should use when we try to compare age-related responses. Age is a continuous variable that influences responses over time. Other age-related changes, such as body protein mass and energy intake/utilization/production, influence whatever outcome measures we use. How to adjust for these multiple interactions is a challenge. “What is it we use to ‘normalize’ the responses to changes in specific amino acid intake?” I agree with your comments with respect to the role of mitochondria in amino acid metabolism and especially overall nutrient metabolism. Much of it, I think, relates back to issues of redox-sensitivity and balance. All of the pathways that exist within mitochondria end in final common pathways for the generation of energy. This also is a huge site for the production of free radicals, which can injure proteins that are in the mitochondria, which may then not function well, and thereby contribute to the senescence of the cells. But I agree, we need to do much more research in these integrated areas.

Young: Fascinating. Did you not have more questions?

Cynober: We need to have a placebo to determine whether the adverse effects of high intake of amino acids are specific or nonspecific. The question, first, is do we really need a placebo? And if yes, should this placebo be nitrogenous or non-nitrogenous? And if it’s nitrogenous, which type of nitrogen?

Bier: Well, I do not know whether we need a placebo because virtually no one has shown that we have a problem. So if we get to the point where we have enough studies that point to a direction, okay. In other words, if we have upper limits that we have to study in some way, then I think we may need the placebo—largely because the nonspecific symptoms that are associated with some of this are ones that you cannot discriminate from the background nonspecific symptoms that human beings exhibit when you do anything to them. If we get to the point where we know we need to do this, then I think we need a placebo. And what it is going to be? Who knows? In fact, I would think glycine is one of the things it would not be because it is another nitrogen-containing amino acid. For me, it would have to be a non-amino acid, probably not a nitrogen-containing compound, and something relatively neutral. And I do not know what that is.

Cynober: The problem with glycine is that several groups, especially Thurman’s group in North Carolina, have demonstrated that glycine at high concentration could have some pharmacological effects (e.g., in ischemic-reperfusion injury).

Bier: The problem with glycine is that you cannot test a single amino acid toxicity with the placebo that is another amino acid! I find that inconceivable!

Cynober: What do you think about the use of the combination of six, seven, or even eight nonessential amino acids?

Bier: Give me something nonspecific, in urine…

Cynober: Urea?

Bier: Not urea! That is another nitrogen-containing compound. It has to be something completely independent of the amino acid system that, by in large, is going to be innocuous.

Renwick: Assuming we are not testing toxicity in humans, we are going to be testing tolerability and developing a database that shows us what could be tolerated. We are not going to test toxicity in humans, but there will be testing of something at doses that you believe to be safe. So in that context, for me, it really does not matter what the placebo is, as long as it does not interfere with the biomarker that you are trying to use to relate to your animal studies. I do not think you have to match the amino acid and placebo for nitrogen or for anything. It is just a tolerability study.

Endo: I ask about the institutions of the United States or Europe. Are there any amino acids approved as drugs, by the FDA or the government? Are there no official studies of the activity of amino acids, even arginine or citrulline?

Cynober: In most European countries, all free amino acids are considered as drugs and are not allowed as an alimentary complement.

Endo: Is there no official record of testing the administration of an amino acid? So how about aspartame? What are the methods used to determine the toxicity of aspartame?

Young: Basically, phenylalanine concentrations—how they relate to concentrations and implications of phenylalanine levels in PKU patients.

Endo: So only PKU patients had been tested. What are the limits of aspartame ingestion for normal subjects?

Young: I was going to say 38 mg—very heavily supported by very extensive human studies.

Renwick: I agree. If you go down the aspartame route you will have to develop an extensive database for all amino acids.

Young: I agree.

I think it is time to call it quits! I do not intend to make any summary, except to say that I think we have been further guided as to the appropriate approach that we might think about taking in establishing upper levels of prioritized amino acids. It seems to me from what I have heard over the last day and a half that sulfur amino acids and arginine are what my priorities are. That may not be what you might agree with, but I don’t particularly care. We are drawing this to a close now. I would like to thank all of you for your excellent presentations and participation in the discussion, and for your efforts to prepare manuscripts. Thank you all for your efforts in making this workshop yet another success, as we walk down the road of amino acid assessment.