sarily a prerequisite for obtaining a large supply of that nutrient.

In public health and preventive medicine, emphasis is at present laid on the use of dietary modifications (and interventions) for controlling the chronic (non-communicable) and degenerative diseases that lead to high morbidity and mortality. Dietary guidelines for disease prevention have been set out by various international expert committees emphasizing which nutrients should be increased in the diet (for example complex carbohydrates, dietary fibres and some minerals) or reduced (total fats and saturated fats) as compared to current intakes. It should be realized that in spite of the large amount of work in progress in the nutrition field there are numerous important questions which still deserve further investigation. The various papers presented here should not only provide updated information but also stimulate further research on both the basic and the applied aspects of nutritional science.

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Effects of exaggerated amino acid and protein supply in man

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Summary. A general update review of the dynamic aspect of protein metabolism is presented. The effect of excess protein level on protein metabolism has been the object of a limited number of studies in man. From the information available, it appears that the primary regulatory pathway for body protein homeostasis is the process of amino acid (protein) oxidation.

Key words. Protein synthesis; protein breakdown; protein turnover; N balance; protein overfeeding; strength training; body composition.

Introduction

The present day understanding of protein metabolism stems from Rudolf Schoenheimer's pioneering work with stable isotopes during the 1930s. A summary of most of these investigations can be found in a commemorative book which was published in 1942, shortly after his death [12]. In a series of studies in rats, Schoenheimer and his colleagues demonstrated two very important aspects of protein metabolism. The first was that amino acids from the diet, once absorbed, were kinetically indistinguishable from those already present in the body. This implied, therefore, that all amino acids, irrespective of whether they originated from the diet or from endogenous protein, could be considered kinetically as a single homogeneous metabolic pool within the body – a finding that was in direct opposition to the accepted opinion held at the time. The second and equally important observation was that the proteins which make up the body of the adult animal are not metabolically inert but are in a continuous state of being broken down into free amino acids and subsequently reincorporated back into protein. Although the concept of 'protein turnover' was actually first considered by Borsook and Kieghley, in 1935 [1], it was Schoenheimer and his associates [12] who provided the firm experimental evidence to support its existence. This concept was also contrary to the generally accepted view held at that time which, except in the case of normal growth and tissue repair, regarded body protein as being metabolically inert. These two aspects, studied some fifty years ago in rats, form the basis of our present understanding of human protein metabolism – as outlined in figure 1 – and are important for appreciating the effects of changing dietary amino acid or protein supply.

Schoenheimer's studies [12] generated a great deal of research interest in protein metabolism, especially in the influence that the composition of the diet had on it. The greater part of the work done subsequently, however, concentrated either on developing methods for assessing rates of protein synthesis and breakdown (turnover) or on the effects of protein and energy deficiencies on these processes (for reviews see Reeds and Garlick [10], and Waterlow [15]). In contrast, very little work has been done on the consequences of an excessive dietary intake of amino acids and protein.

This account will cover the main features of whole-body amino acid and protein metabolism and will also describe a study in which the rate of protein turnover was measured in subjects consuming either normal or relatively high amounts of dietary protein.

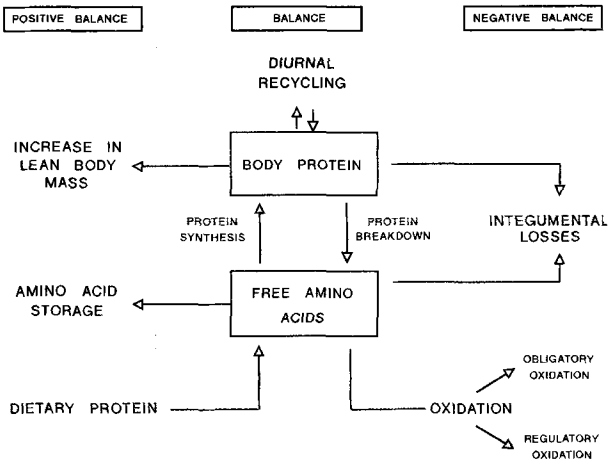

Figure 1. Schematic diagram of protein metabolism in man (adapted from refs 5, 13). When protein intake is exaggerated the disposal of excess amino acid takes place primarily by stimulation of the regulatory pathway of amino acid oxidation, despite the fact that both protein synthesis and breakdown (i.e. protein turnover) are also increased. As a result there is no substantial net body protein deposition.

Whole-body nitrogen regulation

As mentioned by Waterlow[14] whole-body nitrogen metabolism is perhaps the crudest way of looking at the metabolic events that involve nitrogen in the body. It is simply an overall view of events (a weighted average) which is based on the biological mass of individual organs or tissues and their respective rates of nitrogen metabolism. The concept is analogous to that of whole-body energy metabolism in that the overall rate of oxygen consumption or carbon dioxide production by the body is the sum of the rates of individual tissues or organs. Although both may appear to be unrefined models, they are nevertheless very useful in appreciating any clinical and nutritional changes that occur.

Figure 1, adapted from Waterlow et al.[13] and Millward and Rivers[5], illustrates a simplified model for the homeostasis of body protein (nitrogen). The model includes two distinct pools of amino acids within the body: 1) amino acids bound in body protein and 2) amino acids existing as 'free' metabolically active metabolites. The free pool provides amino acids for both protein synthesis and oxidation (either directly or indirectly) and is itself replenished by amino acids generated by the breakdown of body protein. It is also supplied with amino acids from the diet. The sizes of these two pools are totally different, however. In a 70-kg-heavy man, for example, the bound pool (body protein) contains about 10 kg of amino acids whereas the free pool consists of no more than ~ 100 g of amino acids[7]. Thus the size of the free amino acid pool of the body is only one percent of that of the protein-bound pool. It is, in fact, similar in quantity to the amount of amino acids consumed each day in western diets.

General reactions that occur in protein metabolism can be classified into three broad types: 1) those that serve to increase the size of the nitrogen pools in the body (positive-balance reactions); 2) those that, although they may have short-term effects, have no net effect on a daily basis (balance reactions); and 3) those which operate to decrease the amount of nitrogen in the body (negative-balance reactions).

With respect to the positive-balance reactions there are two possibilities: the first is by storage of amino acids in the free form, the second is by increasing the net amount of body protein itself (i.e. increase in lean body mass). Storage of free amino acids, although it does occur occasionally in certain situations, is quantitatively not important[4]. Increases in lean body mass can also occur but again, generally speaking, in adults this is quantitatively not a significant pathway for disposing of excess amino acids. For instance, in the study of Oddoye and Margen[8], where surfeit levels of protein were fed to healthy subjects, less than 4% of dietary nitrogen intake could be accounted for by an increase in lean body mass (positive nitrogen balance). Appreciable increases in lean body mass do occur, of course, in growth phases in the young and during pregnancy in women but in adults increases in the amount of body protein, apart from diurnal cycling (see below), are limited.

Diurnal recycling is the transient postprandial deposition of dietary amino acids into body protein followed by the subsequent remobilization of this protein during the postabsorptive state to release free amino acids. Although undoubtedly important in the short term, diurnal recycling has no direct significance on a longer term basis since there is theoretically no net change in the amount of body protein (it is a balanced reaction). Diurnal recycling appears to operate as a 'buffer' mechanism to prevent large increases in circulating free amino acids after the ingestion of a meal. This is suggested by the fact that the magnitude of the cycling appears to increase as the protein content of the diet increases[6].

There are two possible routes by which amino nitrogen can be lost from the body (negative-balance reactions): by physiological non-oxidative means i.e., through feces, urine and integumenta (skin, sweat and hair), and by metabolic mechanisms of oxidation. Integumental losses have been shown to increase when dietary protein is increased but on a fractional basis they collectively account for less than 8% of the intake of dietary nitrogen when subjects are maintained on a very high protein diet[8].

The principal way in which homeostasis of protein metabolism is achieved both in the short and long term is clearly through oxidation of amino acids. Again from the data of Oddoye and Margen[8], the amount of urinary nitrogen excreted (primarily as urea and ammonia) represented 88% of the nitrogen intake on both a normal and high protein diet. Oxidation itself can be differentiated into two forms – 'obligatory' and 'regulatory'. These

correspond, respectively, to the basal rate in the postabsorptive state and to the net increase in the rate in the postprandial state. In both cases the absolute value for the rate is affected by the level of protein in the antecedent diet.

In healthy adult subjects who are eating and exercising normally, the whole system is practically balanced on a day-to-day basis; that is, there is no significant change in lean body mass or in the size of the free amino acid pool. However, when protein intake is either less than or greater than total nitrogen losses (oxidative and integumental) metabolic adjustments must occur, since the concentrations of amino acids in the free amino acid pool remain remarkably constant [13]. In the case of protein deficiency it is known that there is a general down-regulation of metabolic rates, especially that of protein turnover and of total oxidation of amino acids [15]. In contrast, very little is known of the changes that occur after excess protein intakes either in man or in animals. Today a controversy still exists between physiologists, nutritionists and athletic coaches as to whether or not a supra-physiological ingestion of protein may be beneficial in man under particular training conditions. It is common knowledge that body-builders consume very high levels of proteins [9], primarily in the form of protein powders in combination with megadoses of vitamins and mineral supplements. This excess protein intake is not only recommended by coaches for enhancing muscle mass but it is generally claimed that it also improves muscle strength in athletes, such as weight-lifters and shot-putters. Since in industrialized countries the habitual protein intake is already substantially greater than the recommended dietary allowances [16], it could be argued that the 'customary' protein intake in the West is largely sufficient to cover the extra protein needs during the anabolic phase of strength training.

In a recent study carried out at the Institute of Physiology, University of Lausanne, we have investigated the effect of dietary protein supplementation – in conjunction with strength training – on body weight, body composition and the rate of whole-body protein metabolism. The basic idea was to use independent methods to assess more accurately whether changes in lean body mass result from differences in the level of protein intake during a period of physical training.

Twelve young men (initial body weight 70 ± 7 kg, mean $\pm$ SD, age 24 ± 1 years, initial percentage body fat between 10 and 15%) were randomly allocated to 2 groups of 6 subjects:1) a protein supplementation group, who consumed 2 g protein/kg/day (protein powder) above their usual dietary intake of 1.3 g/kg/day, and 2) a control group who received a placebo composed of wheat bran (essentially acaloric). Following a 1-week baseline period, during which no strenuous exercise was allowed, a 4-week strength training was initiated in both groups (3 times per week, with each session lasting for one hour). The progressive resistance training consisted of a series of exercises directed towards 7 major muscle groups: arm flexors, arm extensors, pectoral muscles, dorsal muscles, deltoid muscles, knee extensors and ankle plantar flexors. In each session the loads were adjusted for each subject in order to match their individual maximal strength capacity. Body weight was measured daily and body composition weekly using skinfold thicknesses and underwater weighing. In addition, each week repeated 24-h urinary collections were made in a free-living situation to assess total nitrogen excretion and to calculate the apparent nitrogen balance. Finally, on the last day of the baseline period and at the completion of the training period, the subjects spent 24 h in a ventilated chamber (whole body indirect calorimeter) in which the temperature and humidity were controlled [3]. This made it possible not only to prescribe a standardized dietary intake but also to assess the rate of whole body protein turnover (i.e. protein synthesis and breakdown) using N15 glycine as a non-radioactive tracer. The single-dose method of Fern et al.[2] was used; after a single bolus of N15 glycine, administered intravenously, the enrichment of N15 in urinary urea and ammonia was measured over a 12-h period and the nitrogen flux was calculated from the average of both estimates.

In both groups, the results clearly showed different quantitative changes in all variables. The weight gain observed during the 4-week training period averaged 1.5 ± 0.6 kg in the placebo group of which nearly all could be explained by a gain in lean body mass. In the protein supplemented group, the body weight gain was significantly greater (2.8 ± 0.9 kg, $p < 0.01$). Estimates of body composition indicated a greater gain of lean tissue in the protein supplemented group relative to the placebo group ($p < 0.005$). The patterns of body weight changes are shown in figure 2.

It is interesting to interpret the above results with respect to the changes observed in nitrogen metabolism. These also appeared to be markedly influenced by the combination of muscle strength training and excess protein intake. A dramatic increase in total urinary nitrogen excretion – by a factor of 2.5 – was observed in the protein supplemented group whereas it remained practically unchanged in the control group (difference < 1%). Crude 24-h nitrogen balance, calculated between the pre-training period (baseline measurement) and the end of the study, was virtually unchanged in the placebo group ($+ 0.1 \pm 1.4$ (SD) g N/day) but increased appreciably in the protein supplemented group ($+ 3.4 \pm 3.3$ (SD) g N/day). This increased nitrogen retention, which is an indirect index of lean tissue storage, supported the observations, based on body weight and body composition changes, that there was greater protein deposition in the group consuming the extra dietary protein.

Concerning the dynamic aspects of whole body protein metabolism, there was a mean increase in protein synthesis and breakdown in the placebo group of 20% and 22%, respectively. The changes, however, were not

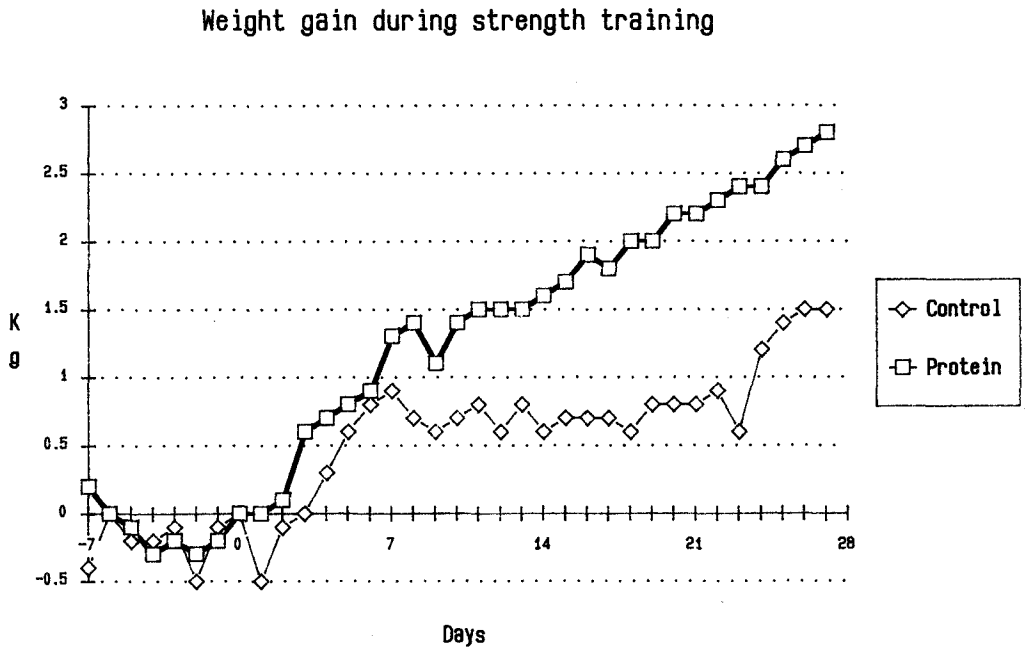

Figure 2. Difference in body weight gain during strength training between the protein supplemented group and the placebo group. (See text)

statistically significant. In contrast, in the other group, after 28 days of exercise on the high level of dietary protein there were significant changes in all three parameters. The rate of protein synthesis increased by 105%, that of protein breakdown by 107% and that of oxidation by 159%. Similar changes in protein turnover have also been observed in response to acute (as opposed to chronic) excess protein intake [11].

In summary, the results of this study, which used two independent methods to assess body composition (skinfold thickness and underwater weighing), suggest that dietary protein supplementation (in conjunction with strength training) can increase lean tissue mass. To account for the observed net nitrogen retention in the body the overall rate of whole body protein synthesis during the four-week period of supplementation must have been slightly greater than that of whole-body protein breakdown. Measurement of the rates of protein metabolism at the beginning and end of the four-week period showed that the greater part of the dietary protein supplement was in fact oxidized. Oxidation, therefore, appears to be the major homeostatic mechanism for controlling the size of the free amino acid pool within the body. Nevertheless, the rate of whole-body protein turnover (that is, synthesis and breakdown) doubled as a result of the protein supplementation. This is an important observation inasmuch as the absolute rate of protein turnover may determine the extent to which lean tissue mass can increase in adult man.

The investigation presented here has been concerned with physical training and the effects of protein supplementation on the growth of lean body mass. What level of dietary protein is needed to maintain an increase in lean tissue mass is not yet known. From the fragmentary data available at the moment it could be argued that a similar level of protein supplementation would be necessary for maintenance. However, this aspect really remains to be studied.

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Physiological importance of ω-3/ω-6 polyunsaturated fatty acids in man. An overview of still unresolved and controversial questions

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Summary. The 'essentiality' of (ω-6) and (ω-3) fatty acids in mammals is well known. Nevertheless, some important points remain unclear concerning their implication in physiology. After a short discussion about the definition of essential fatty acids deficiency, this brief overview deals with some of these points, pointing out some of the unresolved questions.
Different subjects are approached concerning the (ω-6) and (ω-3) fatty acids metabolism: desaturases, eicosanoids, production, as well as some of their metabolic effects on cell membranes, intestinal function, glucose and lipid metabolism, haemorheology.
Key words. Essential fatty acids; deficiency; metabolism; mammals.

For the past ten years, a growing interest has been focused on the unsaturated fatty acids (FA), mainly on the polyunsaturated fatty acids (PUFA) and especially on those of the (ω-3) and (ω-6) series (ω indicates positioning of double bonds). Many data have been published, mostly concerning their effects on lipid metabolism and on prevention of coronary heart disease. It is therefore not useless to remind us that some of the PUFA are essential fatty acids (EFA).
As always, the most difficult task resides in defining words. What is the meaning of 'essential'? Some FA have been called 'essential' for two reasons: firstly, because their absence provokes an impairment of the quality of health; secondly, because the organism is unable to synthesize them, and must obtain them from the diet. In fact, the word 'essential' is sometimes used wrongly. Epidemiological data have shown that, in order to prevent coronary heart disease and atherosclerosis, it is necessary to consume a certain daily amount of PUFA. However, some PUFA are not 'essential' but, like other nutrients, may be very useful in preventing illness.
'Essential' means more than 'useful'. It means that these PUFA are required to establish the physiological conditions of normal body development and to achieve physiological functioning. It also means that these PUFA have to be present in the diet. Some aspects of 'essentiality' are still questionable, as shown by Lands[45].

Obviously, it is not within the scope of this short overview to describe deeply and exhaustively the physiological effects of (ω-3) and (ω-6) PUFA, which are still under discussion. Our aim is just to point to some of them.

EFA deficiency

Clinical and biological symptoms of (ω-6) FA deficiency have been well known since the discovery of EFA by Burr and Burr[12] and their first use in human therapy by Hansen[25, 26]. A general review of EFA deficiency in humans was written by Holman[32], and experimental deficiency was studied by Collins et al.[16] and by Wene et al.[88] using either intravenous feeding with a fat-free solution, or nasogastric drip feeding with a fat-free diet.
Less is known about deficiency of the (ω-3) FA in man. In 1982, Holman[33] observed visual and cerebral disturbances in a 6-year-old girl who had an abdominal shot wound, who was fed intravenously a formula low in (ω-3) fat and high in (ω-6) fat. The symptoms quickly disappeared after a sufficient amount of (ω-3) FA was supplied. Bjerve[4] has recently reported (ω-3) FA deficiency in 9 patients who had been on drip-feeding for 2.5 to 9 years, who received only 0.02–0.09% of their energy as (ω-3) FA. He observed 'a scaly sandy haemorrhagic dermatitis, haemorrhagic folliculitis of the scalp, growth