Article to the Special Issue

Protein Turnover, Ureagenesis and Gluconeogenesis

Yves Schutz

Department of Physiology, University of Lausanne, Lausanne, Switzerland

Abstract: The major processes discussed below are protein turnover (degradation and synthesis), degradation into urea, or conversion into glucose (gluconeogenesis, Figure 1). Daily protein turnover is a dynamic process characterized by a double flux of amino acids: the amino acids released by endogenous (body) protein breakdown can be reutilized and reconverted to protein synthesis, with very little loss. Daily rates of protein turnover in humans (300 to 400 g per day) are largely in excess of the level of protein intake (50 to 80 g per day). A fast growing rate, as in premature babies or in children recovering from malnutrition, leads to a high protein turnover rate and a high protein and energy requirement. Protein metabolism (synthesis and breakdown) is an energy-requiring process, dependent upon endogenous ATP supply. The contribution made by whole-body protein turnover to the resting metabolic rate is important: it represents about 20 % in adults and more in growing children. Metabolism of proteins cannot be disconnected from that of energy since energy balance influences net protein utilization, and since protein intake has an important effect on postprandial thermogenesis – more important than that of fats or carbohydrates. The metabolic need for amino acids is essentially to maintain stores of endogenous tissue proteins within an appropriate range, allowing protein homeostasis to be maintained. Thanks to a dynamic, free amino acid pool, this demand for amino acids can be continuously supplied. The size of the free amino acid pool remains limited and is regulated within narrow limits.

The supply of amino acids to cover physiological needs can be derived from 3 sources:

1. Exogenous proteins that release amino acids after digestion and absorption
2. Tissue protein breakdown during protein turnover
3. *De novo* synthesis, including amino acids (as well as ammonia) derived from the process of urea salvage, following hydrolysis and microflora metabolism in the hind gut.

When protein intake surpasses the physiological needs of amino acids, the excess amino acids are disposed of by three major processes:

1. Increased oxidation, with terminal end products such as CO_2 and ammonia
2. Enhanced ureagenesis i. e. synthesis of urea linked to protein oxidation eliminates the nitrogen radical
3. Gluconeogenesis, i. e. *de novo* synthesis of glucose.

   Most of the amino groups of the excess amino acids are converted into urea through the urea cycle, whereas their carbon skeletons are transformed into other intermediates, mostly glucose. This is one of the mechanisms, essential for life, developed by the body to maintain blood glucose within a narrow range, (i. e. glucose homeostasis). It includes the process of gluconeogenesis, i. e. *de novo* synthesis of glucose from non-glycogenic precursors; in particular certain specific amino acids (for example, alanine), as well as glycerol (derived from fat breakdown) and lactate (derived from muscles).

   The gluconeogenetic pathway progressively takes over when the supply of glucose from exogenous or endogenous sources (glycogenolysis) becomes insufficient. This process becomes vital during periods of metabolic stress, such as starvation.

DOI 10.1024/0300–9831/a000064 Int. J. Vitam. Nutr. Res. 81 (2–3)

Key words: Proteins, peptides, protein oxidation, energy expenditure, amino acids, glucose homeostasis

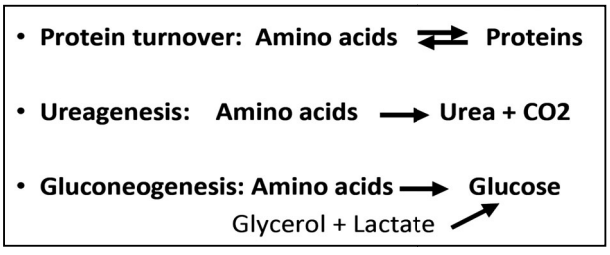

Figure 1: Schematic diagram of protein metabolism

Protein turnover

General Aspects

The maintenance of body protein stores (i. e. lean body mass) at an appropriate level forms the basis of protein homeostasis. The proteins of the body are not metabolically inert, but are continuously being broken down and replaced by new molecules. This forms the basis of the concept of protein turnover, indicating that body proteins are in a dynamic equilibrium, which largely contributes to protein homeostasis [1]. Note that the concept of nitrogen balance, which is the net difference between total N (protein) intake and total N excretion, is a different, more static, concept since it disregards the magnitude of overall protein synthesis and protein breakdown [2].

Protein synthesis, protein degradation, and amino acid oxidation are tightly regulated to preserve lean body mass in healthy individuals. Protein turnover can be considered as the sum of protein synthesis + protein degradation. A greater rate of whole body synthesis than breakdown indicates an anabolic state that results in lean tissue deposition, whereas more breakdown than synthesis indicates a catabolic state that degrades lean tissues.

Growing or reproducing cells require new protein molecules to be constantly synthesized and, as a result, need a supply of exogenous amino acids from food. In fact, in children and in the adult, body proteins are continually synthesized and degraded in all tissues. Individual endogenous proteins turn over at various different rates. Their half-lives (time interval required for the amount of protein to be degraded to half of its initial value) can vary from a few hours (some hepatic proteins) to one year or so (collagen protein in connective tissue). However, the half-life of a specific protein in different organs is generally similar. An efficient protein turnover is essential since it allows some regulatory proteins to be rapidly synthesized respectively degraded so that the cell can rapidly respond to constantly changing conditions, a typical manifestation of protein homeostasis. The proteins which have very short half-lives are specifically targeted for protein degradation, since their turnover is high; as a result they quickly respond to an acute change in protein intake.

The magnitude of daily protein synthesis in adults is 3- to 4-fold greater (5- to 6-fold greater in growing children) than the intake of protein. For example, it has been demonstrated that the rate of protein synthesis (10–12 g/kg body weight/day) in rapidly growing infants greatly exceeds that necessary for net protein gain; e. g., 2 g/kg body weight/day [3]. This indicates an efficient recycling of amino acids in the free amino acid pool, the size of which is tightly regulated [2]. In other words, the amino acids released by protein breakdown can be reutilized for protein synthesis rather efficiently for N recycling, but this has an energetic cost (see further).

The rate of protein turnover can be assessed non-invasively by using non-radioactive sTable Isotopes [4]. It is fortunate that, in nature, nitrogen possesses a heavy non-radioactive isotope; i. e. ^{15}N, present in low abundance (0.36 %). The amino acid classically used for human studies has been ^{15}N-Glycine (nonessential amino acid) as a single acute dose (or continuous infusion), since it is ubiquitous. The isotopic abundance of the two terminal end products of protein oxidation (^{15}N-urea & ^{15}N-ammonia) is measured in urine by isotopic ratio mass spectrometry. Thanks to a rather straightforward model developed by Waterlow [1], the protein (N) flux, the rate of overall protein synthesis and protein breakdown, can be calculated. This requires simultaneous measurement of total protein (N) intake and total protein oxidation (i. e. total N excretion). Note that the difference between the former and the latter represents the nitrogen (protein) balance, which is mathematically equivalent to the difference between total protein synthesis minus total protein breakdown. An identical N (protein) balance can be obtained by measuring different rates of whole body synthesis and breakdown. For example, catabolic disorders result in increased protein breakdown. Protein feeding during this condition

results in a relative increase in protein synthesis, thereby minimizing N losses. Conversely, short-term protein restriction (of a few weeks' duration) leads to an adaptation of protein metabolism in the direction of a decrease in protein turnover (synthesis and degradation), as well as amino acid oxidation. As a result, the net protein mobilization (as measured by N balance) is lower than the total reduction in protein degradation.

Kinetic models developed to assess whole body protein turnover were presented more than a decade ago [2, 5].

What factors influence protein turnover?

Endogenous factors include age and aging, growth, body composition (fat-free mass, adipose tissue), and various diseases (catabolic, hypermetabolic). For example, the rate of protein turnover is known to decrease with age in humans, particularly in muscles. The changes in body composition (in particular a fall in fat-free mass and muscle mass) with aging may explain the fall in protein turnover observed [6]. However, whether there is a decrease or not, depends upon how the results are expressed, in absolute value or per unit fat-free mass. Let's take the example of obesity in children. Whole-body nitrogen flux, protein synthesis, and protein breakdown were measured postprandially over 9 hours from ^{15}N abundance in urinary ammonia by using a single oral dose of ^{15}N-glycine [7]. Absolute rates of protein synthesis and breakdown were significantly greater in obese children than in control children (208 ± 24 compared with 137 ± 14 g/day and 149 ± 20 compared with 89 ± 13 g/day, respectively). Obesity in prepubertal children was associated with an absolute increase in whole-body protein turnover, which contributed to explain the greater energy expenditure in obese children than in control children, when expressed in absolute values. However when expressed per kilogram of body weight, all values were lower in the obese children.

Exogenous factors include the nutritional status in terms of protein and energy [8], anabolic substances, prolonged exercise, and recovery from malnutrition or "regrowth" in adults. During endurance exercise there is an increase in the utilization of protein as a metabolic fuel (i.e. increased urea excretion) and the net rate of whole body protein is depressed [9]. Note that daily rates of protein turnover are rather insensitive to protein intake over a wide range of intakes [10].

Energy-protein interaction of whole body protein synthesis and breakdown

Comprehensive reviews showing the complexity of protein-energy interaction has been published [11,12]. Energy and protein (amino acid) metabolism interacts at various levels, including both molecular and cellular: a step-change in energy intake will result in a change in net protein utilization, the magnitude of which will depend upon the magnitude of dislocation and the host condition (nutritional status). For example a drop in total energy intake (keeping protein intake constant), as encountered during a weight-loss diet, will give rise to an increase in protein needs, since there is an increase in body protein loss due to enhanced protein oxidation [13]. If a reduced energy intake results in an acute negative nitrogen balance, carbohydrates and fat are nitrogen-sparing to about the same extent. However, their mechanisms of action (hormones, substrate competition) are different.

There are a number of energy-dependent processes associated with protein and amino acid metabolism: a. protein synthesis (and breakdown) in particular activation of amino acids, and the formation of peptide bonds during the elongation of the peptides (see below); b. r-RNA and t-RNA turnovers; c. amino acid transport; d. ion pumps and channels; e. signal transduction and protein translocation; f. the glucose-alanine cycle and; g. urea synthesis (see further).

In summary, the high rate of protein synthesis costs energy primarily because it requires ATP for the synthesis of peptide bonds connecting amino acids together, but other concomitant biochemical processes also cost energy. In addition, the energetic cost of protein degradation, generally neglected, is not zero, although the exact cost related to this process remains unknown.

An estimate of this cost has been made either from a biochemical unidirectional approach, using stoichiometric calculations. Alternatively it can be assessed from *in vivo* human studies, by relating the resting energy expenditure of the subject to its rate of whole-body protein synthesis measured simultaneously. Both approaches yield different values: the former (static, *in vitro*) yielding lower values than the latter (dynamic, *in vivo*).

Estimate based on stoichiometric calculations indicate that at least 4 ATP equivalents are required in order to bind one Mole of amino acids together for the biosynthesis of polypeptides, and consequently to produce specific proteins. If this estimate includes the active transport of amino acid into cells + mRNA

synthesis, a total value of about 1 kcal/g of protein synthesized is found. This accounts for 25 % of the metabolizable energy value of exogenous protein (4 kcal/g). Assuming a whole-body protein synthesis of 250 to 350 g/day in an average adult, this indicates that 250 to 350 kcal/day of heat are produced in the intermediary metabolism because of this relatively inefficient process.

In vivo, estimates based on the relationship between whole-body protein synthesis and resting energy expenditure, suggest that, in adults, the contribution of protein turnover is roughly 20 % of the resting energy expenditure in adults; i. e. about 300 kcal/day [14,15]. This proportion can increase to 30 % in fast-growing premature babies [3].

What is the fate of exogenous (food-derived) amino acids?

Amino acids (AAs) derived from the hydrolysis of food protein, reach a highly active intracellular amino acid pool, a metabolic pool limited in size and not expandable, from which they can follow 3 major pathways:

1. AAs can be used for the synthesis of new endogenous proteins and other biological substances
2. AAs can be irremediably oxidized by the body, yielding urea (+ ammonia) and carbon dioxide (CO_2) as terminal end-products (see process of ureagenesis) and,
3. AAs can be converted into other compounds (see process of gluconeogenesis).

A schematic diagram showing these key metabolic processes that occur in the liver, i. e. elimination of nitrogen from the body and endogenous production of glucose when its availability is low, is shown in Figure 2. Catabolism of AAs by the liver is mainly regulated, on a short-term basis, by substrate availability from exogenous supply (i. e. food) or endogenous mobilization in the post-absorptive state, i. e. from protein breakdown.

In summary, protein and amino acids ingested in excess of those needed for biosynthesis cannot be stored, in contrast to fatty acids and glucose, nor are they excreted as such (aminoaciduria is negligible in healthy subjects) without prior transformation. This is explained by limited size of the intracellular free amino acid pool, which cannot be much expanded. In other words, in adulthood, protein cannot be stored in body tissue by just increasing the amount of exogenous protein…and staying in bed. The surplus of amino acids is used as metabolic fuel and is oxidized, unlike glucose and fatty acids substrates, which are stored in the liver (+ muscles) and adipose tissue, respectively. In contrast, enhancing physical exercise in chronic conditions, particularly strength exercises, can lead to an increase in skeletal muscle protein storage.

The production of urea by the liver: ureagenesis

All animals, from flatworms to mammals, have the genetic capacity to synthesize urea and therefore have a functional urea cycle [16]. The complete cycle is present in the liver of terrestrial vertebrates, and in man it represents the sole mechanism for ammonia disposal [17]. Embryological development of the urea cycle in mammalian fetal liver therefore permits use of amino acids as new sources of energy to meet oxidative demands for continuing growth.

Urea is a water-soluble substance produced by the liver, and removed from the blood by the kidneys. The role of urea is not only as a carrier of waste nitrogen. Being practically neutral, urea is a safe vehicle for the body to both transport and excrete excess nitrogen. Being highly soluble in water and easily distributed within the body by simple diffusion (no active transport across membranes required), the urea synthesized in the liver is distributed and diluted in a very large body pool considered almost equivalent to total body water (about 45 liters of intracellular + extracellular water in a 70-kg man).

The liver produces urea in the so-called urea cycle as a “waste” end product of the metabolism of protein. Urea is found dissolved in blood (from 2.5 to 7.5 mmol/L) and is excreted by the kidney as a component of urine, primarily as a function of dietary protein intake: the higher the protein intake, the higher the urea production, and vice versa. The handling of urea by the kidneys is a vital part of human metabolism. Urea serves an important role in the metabolism of nitrogen-containing compounds. It is the main nitrogen-containing substance in the urine in man (about 85 % of total N), the remaining being ammonia and creatinine, the latter being derived from endogenous, non-enzymatic creatine precursor in muscles.

A large excess of protein (2–3 g/kg body weight/day, i. e. more than 2 to 3 times the basic recommendations) modestly increases blood urea nitrogen but largely increases total urinary urea excretion (and urea in sweat along with sodium chloride and water).

The biosynthesis of urea requires ATP: less than 20 % of the energy derived from the metabolism of amino acids is required for ureagenesis [17]. This partly explains (in addition to the cost of whole-body protein turnover) the high thermogenic effect of protein ingestion.

Finally, note the role of the microflora (microbiota) in the lower hind gut (colon), which should not be neglected. It is called the "urea salvage" pathway [18]. There is a non-negligible degree of urea recycling by bacterial hydrolysis in the colon, which allows some N to be recovered in the amino acid pool, and subsequently retransformed into amino acids in the liver. The potential of this *de novo* synthesis of amino acid from urea recycling is quantitatively small but still remains an accepted phenomenon [19].

The endogenous de novo production of glucose: gluconeogenesis

Gluconeogenesis serves as an alternative source of glucose when endogenous supplies are limited (in size and rate of availability) or when exogenous supply of carbohydrates is too low or absent. It is also influenced by the nature and level of dietary intakes: a fasting diet and a low-carbohydrate diet (less than 100 g/day) stimulate this process, whereas a high-carbohydrate diet (hyperenergetic or not) blunts gluconeogenesis.

Note that from a biochemical point of view, the gluconeogenesis pathway is not simply a reversal of the glycolytic pathway: the irreversible steps of glycolysis are bypassed. Thanks to the tight enzymatic control in the glycolysis and gluconeogenesis pathways, these two processes are regulated to prevent them from being simultaneously activated, since this would result in a waste of energy (ATP); this is also known as "futile cycle." The physiological conditions stimulating the gluconeogenesis pathway also simultaneously inhibit the glycolytic pathway, and vice versa, indicating that these pathways are controlled in a reciprocal fashion [20].

Although glycolysis occurs universally, gluconeogenesis is confined to the liver and kidney. The key hormone regulating the metabolic transformation of amino acids into glucose in the liver and kidney, i. e. gluconeogenesis, is glucagon. Another hormone (cortisol) also plays an important role.

The role of glucagon (which has an opposing metabolic effect to insulin) largely concerns glucose metabolism:

It stimulates gluconeogenesis i. e. *de novo* glucose synthesis, simultaneously inhibiting glycolysis i. e. degradation of glucose in the intermediary metabolism.

It enhances glycogen breakdown, whereas glycogen synthesis is inhibited and, as a result,

It allows the release of free glucose into the circulation to maintain glycemia.

It has also an effect on ureagenesis: hyperglucagonemia in normal man induces mild nitrogen losses by stimulation of hepatic ureagenesis.

The process of gluconeogenesis ultimately leads to a mobilization of muscle tissue to produce glucose. Fortunately, other precursor substrates are involved in this gluconeogenesis process, namely glycerol, which comes from the breakdown of fat (under the condition of accelerated lipolysis), and also a very important intermediate substrate viz. lactate, which is produced at a low rate in resting post-absorptive conditions, and at a high rate during intense exercise.

Figure 2 shows the importance of the liver as a central metabolic carrefour, where the above metabolic processes (ureagenesis and gluconeogenesis) take place.

The energy cost of gluconeogenesis still remains a matter of debate. A recent study investigated in healthy humans the extent to which a high-protein (carbohydrate-free) diet increases gluconeogenesis and whether this can explain the increase in energy expenditure [21]. The increase in resting energy expenditure was found to be significantly related to the increase in gluconeogenesis. Forty-two percent of the increase in energy expenditure after the high-protein diet was explained by an increase in gluconeogenesis. The energy cost of gluconeogenesis was estimated to be 33 % of the energy value of the produced glucose.

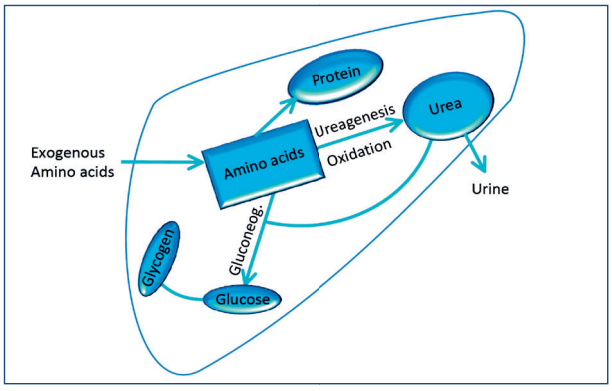

Figure 2: Ureagenesis, gluconeogenesis, and amino acid oxidation in the liver. Oxidation of amino acids is incomplete and leads mainly to urea, in addition to CO_2 and H_2O. Urea is water soluble with residual energy (2.5 kcal/g) and is excreted in urine.

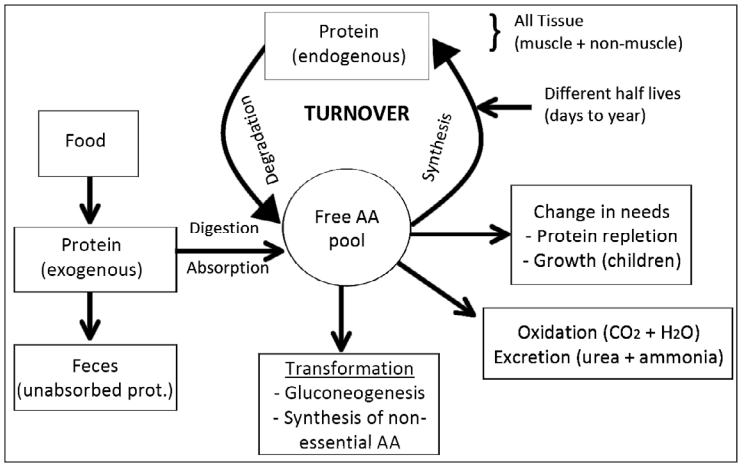

Figure 3: Protein turnover results from synthesis and degradation of proteins. The rate of protein oxidation, which primarily depends on protein intake, the process of protein transformation, and the excretion of end products are shown. AA = amino acids.

Conclusions

A simplified schematic diagram of overall protein metabolism, which includes the three processes discussed above, is shown in Figure 3. The key component of the metabolism of protein is without any doubt protein turnover, which allows moderating total protein needs and minimizing protein losses. Adequate day-to-day exogenous protein/amino acid supply will "feed" the small free amino acid pool and allows replacing amino acids which have not been reutilized via the recycling process.

Protein turnover is not a futile cycle. Life depends upon the export of nitrogenous compounds (urea and ammonia) from the body, both being excreted in urine. Amino acids from muscles can be mobilized to produce glucose in situations where glucose is scarce, maintaining plasma glucose levels, although at the expense of muscle proteolysis.

Finally one final consideration to highlight is the capacity of the organism to adapt to different intakes of proteins, resulting in rapid changes in N excretion and protein turnover as seen in chronically malnourished individuals [8].

References

1. Waterlow, J.C. (1995) Whole-body protein turnover in humans–past, present, and future. Annu. Rev. Nutr. 15, 57–92.

2. Waterlow, J.C. (1999) The mysteries of nitrogen balance Nutr. Res. Rev. 12, 25–54.

3. Micheli, J.L. and Schutz, Y. (1993) Protein Metabolism and Postnatal Growth in Very Low Birthweight Infants In: Nutritional Needs of the Preterm Infant, Scientific Basis and Practical Guidelines. (Tsang, R.C., Lucas, A., Uauy, R. and Zlotkin, S., eds.) pp. 29–46, Pawling: Caduceus Medical Publishers, Inc.

4. Duggleby, S.L. and Waterlow, J.C. (2005) The end-product method of measuring whole-body protein turnover: a review of published results and a comparison with those obtained by leucine infusion. Br. J. Nutr. 94, 141–53.

5. Slevin, K., Jackson, A.A. and Waterlow, J.C. (1991) A model for the measurement of whole-body protein turnover incorporating a protein pool with lifetime kinetics. Proc. Biol. Sci. 243, 87–92.

6. Yarasheski, K.E., Zachwieja, J.J. and Bier, D.M. (1993) Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. Am. J. Physiol. 265, E210–4.

7. Schutz, Y., Rueda-Maza, C.-M., Zaffanello, M. and Maffeis, C. (1999) Whole-body protein turnover and resting energy expenditure in obese, prepubertal children. American Journal of Clinical Nutrition, 69, 857–862

8. Soares, M.J., Piers, L.S., Shetty, P.S., Jackson, A.A. and Waterlow J.C. (1994) Whole body protein turnover in chronically undernourished individuals. Clin. Sci. (Lond) 86, 441–6.

9. Rennie, M.J., Bohé, J. and Wolfe, R.R. (2002) Latency, duration and dose response relationships of amino acid effects on human muscle protein synthesis. J. Nutr. 132, 3225S–7S.

10. Garlick, P.J., McNurlan, M.A. and Patlak, C.S. (1999) Adaptation of protein metabolism in relation to limits to high dietary protein intake. Eur. J. Clin. Nutr. 53 Suppl 1, S34–43. Review.

11. IDECG (1991) Protein-Energy Interactions (International Dietary Energy Consultative Group) – IDECG publication, Lausanne.

12. Young V.R. (1991) Protein and Energy Interactions Throughout Life Acta Pædiatrica 80, s373, 5–24.

13. Calloway, D.H. (1975) Nitrogen balance in men with marginal intakes of protein and energy, J. Nutr.105, 914–923.

14. Welle, S. and Nair, K.S. (1990) Relationship of resting metabolic rate to body composition and energy expenditure Am. J. Physiol. Endocrinol. Metab. 258, E990–E998.

15. Robinson, S.M., Jaccard, C., Persaud, C., Jackson, A.A. and Schutz, Y. (1990) Protein turnover and thermogenesis in response to high-protein and high-carbohydrate feeding in men. American Journal of Clinical Nutrition 52, 72–80.

16. Withers, P.C. (1998) Urea: diverse functions of a 'waste' product. Clin. Exp. Pharmacol. Physiol. 25, 722–7.

17. Shambaugh, G.E. (1977) Urea biosynthesis I. The urea cycle and relationships to the citric acid cycle. Am. J. Clin. Nutr. 30, 2083–7.

18. Jackson, A.A. (1998) Salvage of urea-nitrogen in the large bowel: functional significance in metabolic control and adaptation. Biochem. Soc. Trans. 26, 231–6, Review.

19. Metges, C.C., Petzke, K.J., El-Khoury, A.E., Henneman, L., Grant I., Bedri, S., Regan, M.M., Fuller, M.F. and Young, V.R. (1999) Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. Am. J. Clin. Nutr. 70, 1046–58.

20. Frayn, K.N. (2010) Metabolic regulation: A human perspective. 2nd edition, Blackwell Publ., London.

21. Veldhorst, M.A.B, Westerterp-Plantenga, M.S. and Westerterp, K.R. (2009) Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet Am J. Clin. Nutr. 90, 519–526.

Dr. Yves Schutz

University of Lausanne
Dept. of Physiology
Rue du Bugnon 7
CH-1005 Lausanne
Switzerland
Tel.: +41 (0)21 692 55 63
E-mail: yves.schutz@unil.ch