LIFE LONG LEARNING SYSTEM FOR TRAINING MEDICAL DOCTORS AND STUDENTS IN NUTRITION

STUDY MANUAL
Educational Modules
Volume 2

Editors:

Regina Komsa-Penkova
Co-director of LLL System in Nutrition
Head of Chemistry and Biochemistry Department
University of Medicine Pleven
Pleven - Bulgaria

Remy Meier
Director of LLL System in Nutrition
Medizinische Universitätskilinik
Kantonsspital Liestal
Liestal - Switzerland

Lubos Sobotka
Chairman of Education and Clinical Practice Committee
Department of Metabolic Care and Gerontology
Charles University, Teaching Hospital
Prague - Czech Republic

Authors:

Claude Pichard
Remy Meier
Yvon Carpentier
Lubos Sobotka
Regina Komsa-Penkova
Katia Kovacheva-Kotseva
Winrich Breipohl
Olle Ljungqvist
Pierre Singer
Andre Van Gossum
Nedka Trifonova
Jean-Charles Preiser
Rene Chiolero
CHC Dejong
Bernard Messing
Francisca Joly
Yitshal Berner
Federico Bozzetti
George Paunov
Peter Soeters
Ken Fearon
Michael Staun
Mattias Soop
Nachum Vaisman
Iliana Buteva
Maya Stavreva
Varban Ganev
Agnes Namislo
Brigitte Schmitz
Louise LaJoie
Ed van Klink
Sonali Vaid
Pencho Tonchev

Acknowledgement

The authors thank Tatyana Genadieva, Ani Kamenova, Svetlana Angelova, Evgenia Docheva and Valeria Valentinova for their technical assistance in preparing this manual.

Notice

The writing of this text was accomplished through an extensive review of the literature and through the use of critiques provided by experts and content specialists. Every attempt has been made to develop a text that is current, accurate and presented in a logical manner. As this vast amount of information was processed, inaccuracies or omissions may have occurred. However, the authors, editors and publisher are not responsible for error or omissions or for any consequences from application of the information in this text and make no warranty, express or implied, with respect to the contents of this publication. The authors, editors and publisher have exerted every effort to ensure that drug and nutrient selection and dosage given in this text are in accordance with current recommendations and practice at the time of publication. However in view of ongoing changes in regulations and the constant flow of information, the reader is urged to check the package insert of drugs and nutrient supplements for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new or infrequently employed.

Life Long Learning System:
Nutrition at the Level of Molecular Medicine

Leonardo da Vinci Programme
Pilot Project: BG-03-B-F-PP-166039

ISBN-10: 954-756-053-0
ISBN-13: 978-954-756-053-6

CONTENTS

PREFACE

Life Long Learning system for the training of medical doctors and students in nutrition

The development of a Life Long Learning (LLL) system in nutrition is timely since the essential role of nutrition in human health is becoming increasingly recognized and, as a consequence, the need for a proper education in nutrition to improve patient care has arisen.
Increasing awareness of the role of nutrition in the prevention and treatment of human disease has made clinical nutrition one of the fast growing fields in medicine during the past two-three decades. The unprecedented expansion of human knowledge and education, leading to differentiation in the levels and degree courses, makes it necessary to shift educational activities to lifelong learning, and to satisfy a growing proportion of specialists including health related professionals seeking for the programmes for additional qualification in a special field.
The intent of this manual is to allow students and physicians as well as other medical specialists to learn and understand recent achievements of nutritional science and to apply them to patient care in the areas of prevention and treatment of disease.

The Study manual for users of LLL system in nutrition contains:

- A core curriculum developed with the consensus of European partners according to the requirements of modern education in nutrition;
- A module catalogue;
- The modules developed for the system.

The main objective of this manual is to present the basic principles of clinical nutrition and metabolism and their application in clinical practice. It is constructed in a way that introduces the learner step by step into a modern training content. The first part of the Study manual is the Module catalogue which contains a list of modules with the corresponding system code and credits. The modules are presented in a summarized version with key messages, contents and learning objectives, providing an overview of the training content and enabling the selection of modules of interest.
The second part of the Study manual provides the educational modules of the LLL system in nutrition printed in volumes.
The first and the second volumes developed in 2005 contain 29 modules out of 105 of the module catalogue developed by a network of European partners and ESPEN with the support of the European Commission, Directorate-General for Education and Culture Leonardo da Vinci Programme.
Publishing of the educational modules of LLL system in nutrition is planned as a periodic annual edition according to the educational programme of ESPEN and partners network.
The first volume contains the modules that were presented at live session as a LLL course related to 27th ESPEN Congress in Brussels, August 26-27, 2005.
The second volume contains the modules that were developed and presented by partners network at live sessions held at their institutions.

Chapter 1

Topic 3

Nutritional Assessment and Techniques

Nutritional Assessment and Techniques

Topic 3

Module 3.1

Nutritional Assessment

Yitshal Berner
Rémy Meier
Lubos Sobotka
Nachum Vaisman

Learning Objectives

To understand nutritional screening and assessment;
To assess a patient for general nutritional status;
To realize the signs and the symptoms of nutritional problems;
To be familiar with nutritional screening;
To understand different methods used for the nutritional assessment;
To know limitations of different method for nutritional assessment;
To know the benefits and limits of laboratory and balance-studies for nutritional assessments.

Contents

1. Nutritional screening and assessment
   - 1.1 Screening
   - 1.2 Assessment
2. Techniques used in nutritional assessment
   - 2.1 History
   - 2.2 Physical examination
   - 2.3 Functional tests
   - 2.4 Laboratory parameters
     - Serum proteins, total lymphocyte counts, vitamins and minerals
     - Creatinine height index (CHI)
     - Nitrogen balance studies
   - 2.5 Assessment of food intake

Key Messages

To understand how important to get the history taking and physical examination on the definition of nutritional problems;
Nutritional screening and assessment are important part of patient care to identify patients requiring nutritional support;
Nutrition screening is an important tool for rapid and simple evaluation of an individual nutritional status;
Nutrition assessment is important for detailed diagnosis of acute and chronic malnutrition (over- and undernutrition);
Nutrition screening and assessment are important in clinical medicine because acute and chronic malnutrition (over- and undernutrition) are prevalent.

1. Nutritional screening and assessment

Nutrition screening is an important tool for rapid and simple evaluation of an individual nutritional status.

Nutrition assessment is important for detailed diagnosis of acute and chronic malnutrition (over- and undernutrition).

Nutrition screening and assessment are important in clinical medicine because acute and chronic malnutrition (over- and undernutrition) are prevalent (1, 2).

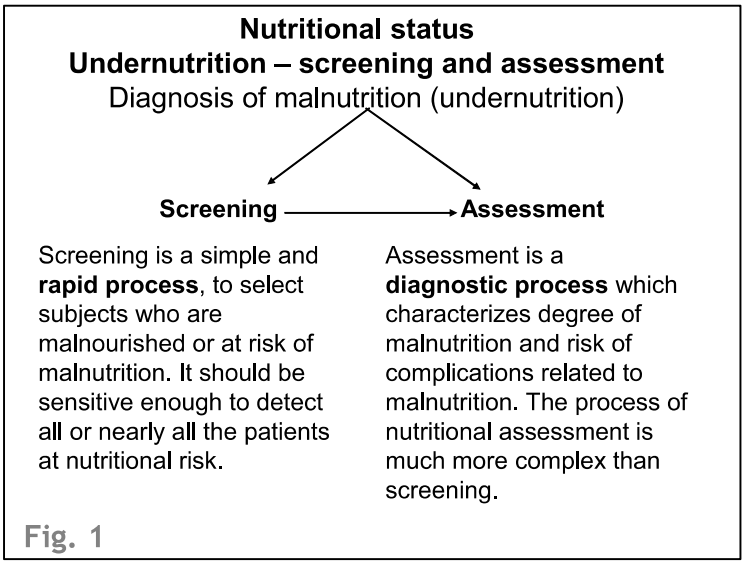


Fig. 1

1.1 Screening

Screening is a simple and rapid process, to select subject who are malnourished or at risk of malnutrition. It can be carried out by busy admitting nursing and medical staff. It should be sensitive enough to detect all or nearly all the patients at nutritional risk.
Most screening tools address four basic questions (4):

recent weight loss
recent food intake
current body mass index
disease severity.

ESPEN published guidelines for nutrition screening in the community, in the hospital and among elderly in institutions.
Screening tools recommended by ESPEN for:

Community: Malnutrition Screening tool (MUST) (5);
Hospital: Nutritional Risk Screening.

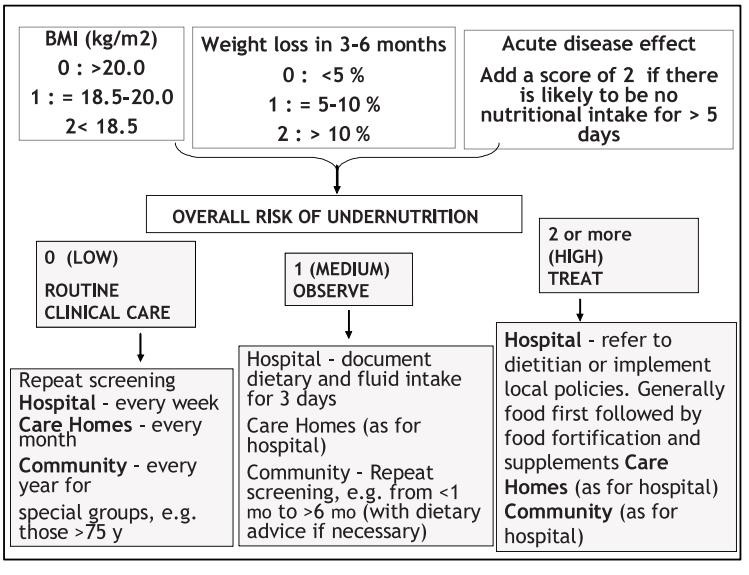


Fig. 2 Malnutrition Universal Screening Tool (MUST) for adults

Table 1 Nutritional Risk Screening (NRS 2002)

Initial screening I			
1	Is BMI < 20.5	Yes	No
2	Has the patient lost weight within the last 3 months?		
3	Has the patient had a reduced dietary intake in the last week?		
4	Is the patient severely ill ? (e.g. in intensive therapy)		

Yes: If the answer is 'Yes' to any question, the final screening is performed.
No: If the answer is 'No' to all questions, the patient is re-screened at weekly intervals.
If the patient e.g. is scheduled for a major operation, a preventive nutritional care plan is considered to avoid the associated risk status.

Table 2 Final screening II

Impaired nutritional status		Severity of disease (increase in requirements)	
Absent	Normal nutritional status	Absent	Normal nutritional requirements
Mild Score 1	Wt loss > 5% in 3 mths or Food intake below 50-75% of normal requirement in preceding week	Mild Score 1	Hip fracture* Chronic patients, in particular with acute complications: cirrhosis*, COPD*. Chronic hemodialysis, diabetes, oncology
Moderate Score 2	Wt loss > 5% in 2 mths or BMI 18.5 - 20.5 + impaired general condition or Food intake 25-60% of normal requirement in preceding week	Moderate Score 2	Major abdominal surgery* Stroke* Severe pneumonia, hematologic malignancy
Severe Score 3	Wt loss >5% in 1 mth (>15% in 3mths) or BMI <18.5 + impaired general condition or Food intake 0-25% of normal requirement in preceding week in preceding week.	Severe Score 3	Head injury* Bone marrow transplantation* Intensive care patients (APACHE>10).
Score:	+	Score:	=Total score:
Age if >70 years: add 1 to total score above = age-adjusted total score:			
Score >3: the patient is nutritionally at-risk and a nutritional care plan is initiated Score <3: weekly rescreening of the patient. If the patient e.g. is scheduled for a major operation, a preventive nutritional care plan is considered to avoid the associated risk status.			

Table 3 Elderly: Mini Nutritional Assessment (MNA) (6)

Initial Screening in Mini Nutritional Assessment (MNAr) for the Elderly		
A	Has food intake declined over the past 3 months due to loss of appetite digestive problems, chewing or swallowing difficulties? 0 = severe loss of appetite 1 = moderate loss of appetite 2 = no loss of appetite	
B	Weight loss during last months? 0 = weight loss greater than 3 kg 1 = does not know 2 = weight loss between 1 and 3 kg 3 = no weight loss	
C	Mobility? 0 = bed or chair bound 1 = able to get out of bed/chair but does not go out 2 = goes out	
D	Has suffered physical stress or acute disease in the past 3 months? 0 = yes 2 = no	
E	Neuropsychological problems? 0 = severe dementia or depression 1 = mild dementia 2 = no psychological problems	
F	Body Mass Index (BMI) |weight in kg|/|height in m|2 0 = BMI less than 19 1 = BMI 19 to less than 21 2 = BMI 21 to less than 23 3 = BMI 23 or greater	
Screening score (total max. 14 points)		
12	points or grater	Normal – not at risk no need to complement assessment
11	points or below	Possi ble malnutrition continue assessment

1.2 Assessment

Assessment is a **diagnostic process** which characterizes degree of malnutrition and risk of complications related to malnutrition. The process of nutritional assessment is much more complex than screening and it should include the following principles:

history and examination;
factors leading to malnutrition;
natural history of the patient's condition;
weight loss;
appetite;
gastrointestinal symptoms;
fever;
medical and drug history;
diet history:

- disease status,
- temperature,
- pulse rate,
- blood pressure,
- laboratory tests of inflammation,
- nutrient losses from wounds, fistulae etc.;

functional assessment;
energy expenditure;
mental and physical dysfunction;
muscle strength;
mental scoring system;

mood status;
laboratory tests;
quantifying inflammation and disease severity;
plasma protein levels (transthyretin, transferrin etc.);
plasma changes in minerals (e.g. K, Ca, Mg, P, Zn, Fe;
plasma levels of vitamins;
fluid balance.

There are many methods and indexes which are based on above mentioned assessment methods, however their interpretation and correlation is still difficult see Nutr. Rev. 2000.

2. Techniques used in nutritional assessment

2.1 History

The history is the starting point for any nutritional assessment. Specific features of note include a recent weight changes; dietary habits and alteration in dietary intake; allergies and food intolerance; medications that may effect appetite, gastrointestinal functions and symptoms; current functional capacity, including recent limitations; and previous medical conditions (any chronic or acute disease state).

2.2 Physical Examination

Physical examination is the next step in nutritional assessment. This assessment predominantly relies on subjective and descriptive information. Although not quantitative, a physical examination is may still influence the nutritional management of a patient.
The main objective of a physical examination is to establish signs and symptoms of nutrient deficiencies or toxicities, and tolerance of current nutritional support. A systems approach should be applied using the examination techniques of inspection, palpation, percussion, and auscultation.
The physical examination should include (7):

Assessment of muscle mass and subcutaneous fat stores;
Inspection and palpation for edema and ascites. These two conditions are important physical indicators of diminished visceral protein levels and hepatic dysfunction;
Inspection and evaluation for sings and symptoms of vitamin and mineral deficits, such as dermatitis, glossitis, cheilosis, neuromuscular irritability, and coarse, easily pluckable hair;
The patient's prescribed medication should be examined for potential drug-nutrient interactions, increased macro-or micronutrient requirements, and nutritionally related side effects such as constipation, diarrhea, nausea, vomiting.

The simplest validated nutritional assessment is the SGA, whish is based on patient's history and physical examination. Clinicians prefer SGA because of its simplicity, feasibility and sensitivity that is almost equivalent to objective tests.

Nutritional assessment of patients is not an easy procedure. Although, lots of the clinical and laboratory measurements are available for nutritional assessment, all of them have lots of deficiencies. Nutritional assessment is an art more than science. At the current state of the art, in addition to the physical examination and clinical history, many experienced clinicians solve this problem by using a few laboratory tests.

2.3 Functional Tests

Hand dynamometry;
Direct muscle stimulation;
Peak flow and FEV;
Immune function;
Skin responses to intradermal antigens;
Lymphocyte count;
Proportion and number of T-lymphocytes.

Immune function can be tested by lymphocyte counts and by cutaneous applied skin tests. In most hospitalized patients, delayed hypersensitivity, reactivity and total lymphocyte counts are not very useful components of a nutrition assessment profile.

2.4 Laboratory Parameters

The serum albumin;
The shorter half time proteins;
Transthyretin (formerly prealbumin) - 2 days;
Transferrin - 7 days;
Creatinine height index (CHI);
Nitrogen balance.

A complete nutritional assessment consists of a combination of subjective and objective parameters, but up to now, no single parameter has been shown to be useful in all patients. Most nutritional parameters lack sensitivity and specificity; therefore, methods of identifying malnourished patients are not entirely satisfactory.
Laboratory testing is useful for assessment of the nutritional status and monitoring of nutritional interventions.

Serum proteins, total lymphocyte counts, vitamins and minerals
Several laboratory parameters (serum proteins, total lymphocyte counts, vitamins and minerals) are used. Serum proteins have different half-life times. Serum albumin is a good predictor for outcome and reflects disease severity. On the other hand, it is a bad marker to assess nutritional status. Serum albumin can be used for long term control.
To assess short term changes, prealbumin or transferrin is more useful. Serum proteins have many limitations. The serum concentrations of visceral proteins decline with overhydration and increase with dehydration independent of nutritional status. Low serum albumin levels exacerbate ascites, lower extremity edema, and gut edema because of depressed colloid oncotic pressure. Serum transferrin is the less affected protein by other factors.

Creatinine height index (CHI)
The somatic protein compartment can be evaluated by the creatinine height index (CHI).
Creatinine excretion correlates with lean body mass and body weight. The CHI is depended on urine creatinine excretion.
Renal insufficiency, meat consumption, physical activity, fever, infections and trauma influence urine creatinine excretion

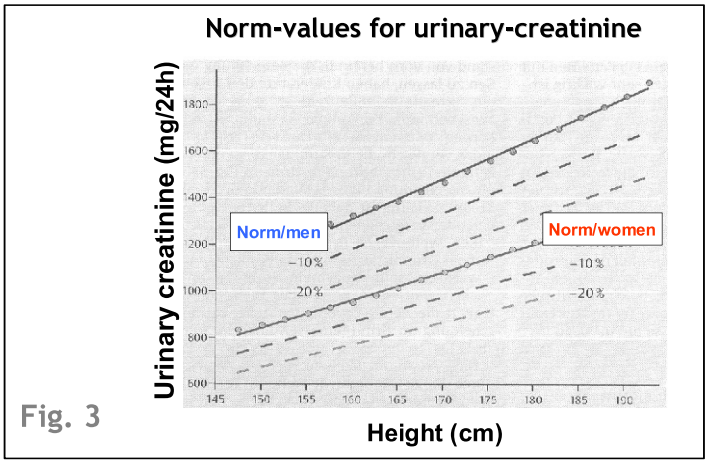


Fig. 3

Role and Limitations of Nitrogen Balance

- Nitrogen balance = N input – N output
  - *Neg. N-balance*
    ↓
    The rate of protein catabolism exceeds the role of protein anabolism
    ↓
  - *Pos. N-balance*

  Indicative of nitrogen retention
- Research tool
- In clinical conditions
  - intake is often overestimated
  - losses in urine, faeces skin, wounds is often underestimated

Fig. 4

Nitrogen-balance

$$\text{N-balance (g/d)} = \frac{\text{Protein-intake}}{6.25^{*}} - \text{UUN}^{**} + \text{fecal losses} + \text{obligatory losses}$$

2-4 g/d

*specialized enteral or parenteral formulations have often a different conversion coefficient

(** urinary urea nitrogen)
UUN x (1.25) = TUN

Fig. 5

Urea-production rate

UPR (g/24 h) =
UUN (g/24 h)+(d-serum-crea (mg/dl)x0.0099xBWxfactor*)

*? 0.55
*? 0.6

Interpretation: UPR > 30 g/24 = catabolie

Fig. 6

Nitrogen balance studies

Nitrogen balance studies are often used to assess protein catabolism. In non-stressed conditions, urea composes 30-90% of total urea nitrogen. For usual clinical purposes, nitrogen balance calculation done with urinary urea nitrogen instead of total urinary nitrogen is adequate.

It has to be considered that nitrogen excretion calculated from urinary urea nitrogen is affected by increased stress, which can alter urea production and/or increase of non-urea nitrogen by-products.

The validity of nitrogen balance is affected by severe nitrogen retention disorders, accuracy of the 24-hour-urine collection and completeness of protein or amino-acid intake data.

The calculation of nitrogen balance is shown on Fig. 5.

A simple assessment to assess catabolic states is also the urea production rate and the urea/creatinine quotient.

Nutritional monitoring and reasons of response can be measured in vivo (by weight gain, N-balance, complication rates) and in vitro measurements by plasma-serum concentration of proteins. The calculation of urea-production rate is shown on Fig. 6.

For identifying patients with pre-existing malnutrition or those at most risk, a combination with a comprehensive nutritionally focus physical exam together with carefully selected objective parameters provide the best information.

2.5 Assessment of food intake

Quantifications of food intakes and their comparison with energy expenditures can not only describe current status but also predict whether the patient's nutritional status is likely to improve or deteriorate.

Nutrient balance = intake (e.g. food intake charts) - expenditure

Food intake assessment estimates food intakes and is among the main tools for assessing nutritional status. Food intake measurements are used not only for the determination of patient's nutritional status, but also characterization of the nutritional status of a population for monitoring and surveillance.
Assessment of dietary intake has considerable challenge and prone to significant error and bias. Food balance sheets and household budget surveys are indirect methods of food consumption studies. Food records and dietary recalls measure food intake on specified periods, usually 1-7 days. Because of day to day variability, several days of records may be required to estimate usual food intake. Food frequency questionnaires are developed to describe standardized data on usual long-term diet.
The determination of the consumption of nutrients can be achieved either by analyzing the foods consumed directly or by using food composition tables. Most food composition tables are organized according to the classification of foods into food groups. Dietary reference intakes (DRI) provide standards to serve as a goal for good nutrition.
Questionnaires are in common use in the medical practice and in nutrition assessment as well as in decisions making process. Every questionnaire has to pass validation to certain “Gold Standard”, and reliability tests.
The data in questionnaire may be of different types:

- Dietary data, either before analysis or specific components after dietary analysis;
- Anthropometrics data like height, weight, BMI, or body composition;
- Laboratory results and special tests done;
- Eating habits like timing of meals and where they are taken;
- General health questions;
- Medical data like diagnoses, surgical treatments and drugs;
- Demographic and socio-economic data.

It is very important to evaluate the objective of the questionnaires. Many questionnaires are designed as epidemiological surveys, and others as clinical tools for specific purposes, some are designed for every person and some for specific populations, some are for the detection of malnutrition while other concentrate on risk evaluation due to metabolic diseases as diabetes, hyperlipidemia and obesity.
Energy expenditure can be measured (indirect calorimetry) or estimated from different formulas see module energy metabolism.
Energy intake is measured using either 37 day food diaries kept by the patient or food intake charts kept by nursing staff and used by the dietician to calculate energy and protein intake.

References

1. David E. et al.. Current concepts in nutritional assessment Archives of Surgery 2002; 137: 42
2. Basics in Clinical Nutrition, Sobotka L. (edit) 3rd edition 2004, Galen
3. Weekes et al. The development, validation and reliability of a nutrition screening tool based on the recommendations of BAPEN . Clin Nutr 2004; 23: 1104
4. Kondrup J, et al . ESPEN guidelines for nutrtion screening 2002. Clin Nutr 2003; 22: 415
5. Guigoz Y, et al, Assessing the nutritional status of the elderly: the mini nutritional assessment as part of the geriatric assessment. Nutr Rev 1996;54:S59
6. Ignatavicius et al. Medical-Surgical-Nursing, 2nd ed. WB Saunders, Philadelphia, 1995
7. Detsky et. al. What is subjective global assessment of nutritional status. JPEN 1987; 11:8

Nutritional Assessment and Techniques

Topic 3

Module 3.2

Body Composition

Yitshal Berner
Rémy Meier
Lubos Sobotka
Nachum Vaisman

Learning Objectives

Assumptions and application of techniques for the measurement of body composition;
To have knowledge on their precision and limitations;
To be informed about the two-, three- and four-compartment models for body composition.

Contents

1. Anthropometry
   - 1.1 The two-compartment model
   - 1.2 The four-compartment model
2. The methods of measurement
   - 2.1 Underwater weighing
   - 2.2 MRI and CT scan
   - 2.3 Subcutaneous fat skin folds measurement
   - 2.4 Dual energy X-ray absorptiometry (DEXA)
   - 2.5 Body water measurement
   - 2.6 Muscle mass measurement
   - 2.7 Body impedance

Key Messages

In vivo body composition measurements are always indirect, based on one or more assumptions concerning the nature of the body components fat mass and fat-free mass;
The other methods are all double-indirect, validated against indirect methods, and therefore based on more assumptions;
Whatever method is used, the starting point is the measurement of body mass with a calibrated scale;
Subsequent subdivision of body mass in components like fat mass and fat-free mass has an accuracy of 1 kg or less, especially in patients where assumptions are often violated.

1. Anthropometry

This measures the anatomical changes associated with change in nutritional status (1):

Body weight;
Involuntary weight loss over three months;
< 5% mild ; >10% severe malnutrition;
Body Mass Index (BMI): BMI = Wt (kg) / Ht^2 (m^2);
Midarm circumference (MAC);
Triceps skin fold thickness (TSF);
Densitometry;
Total body water measurement (TBW);
Total body electrical conductivity (TOBEC);
Body impedance (BI);
Dual energy X-ray absorptiometry (DEXA).

Measurement of triceps skin fold with caliper

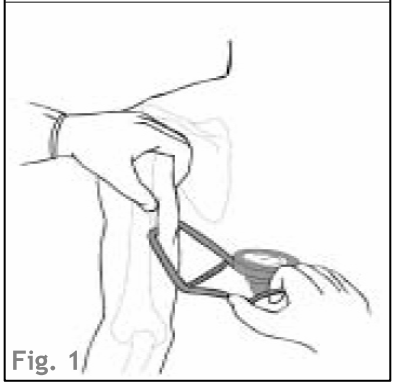


Fig. 1

Measurement of body composition is important tool for evaluating the patient's nutritional status on admission and for measurement of the efficacy of his nutritional intervention on follow-up. Measurements can be done on different levels: atomic, tissue, cellular, molecular, or the whole body level. In clinical settings, the mostly used methods include the whole body approach or the tissue-system approach. The latter can be studied in a 2-compartment model or a more detailed one; the 4-compartment approach.

1.1 The two-compartment model

The two compartments include the body fat mass (FM) and the fat-free body mass (FFBM). The latter is almost identical to the lean body mass (LBM).

1.2 The four-compartment model

The four-compartment approach further breaks down the fat-free mass into three sub-compartments: body cell, extra-cellular water and bone.

2. The methods of measurement

The methods of body composition measurement are based on biochemical and physiological characteristics. Some of these methods measure directly the specific component, but others are derived from other measurements, based on specific physiological assumptions. Understanding the background and the rationale behind a method can help in the interpretation of the results.

2.1 Underwater weighing

Underwater weighing or air displacement plethysmography can help in obtaining the two compartments: FM and FFBM.

2.2 MRI and CT scan

MRI and quantitative CT can also measure FM directly.

2.3 Subcutaneous fat skin folds measurement

Indirectly, FM can be derived from measurements of 2-4 sites of subcutaneous fat skin folds.

2.4 Dual energy X-ray absorptiometry (DEXA)

FFM and FM as well as the bone can be measured by the DEXA (method that uses two levels of X-ray energy and separates the compartments based on the different attenuation of the X-ray energy in a tissuespecific manner.

2.5 Body water measurement

Based on the assumption that cell hydration is constant (73% of the cell) measurements of total body water and its two components: extra cellular and intracellular, can be used to derive FM.
Total body water can be studied by dilution method - isotopic labeling of water ($^{3}H_2O$, $^{3}H_2O$, $H_2{}^{18}O$) or by the bioelectrical impedance method (BIA). Extracellular water can be measured by the dilution method (bromide space) or using BIA. Bone mass can be assessed directly by calcium neutron activation method or indirectly by DEXA and body cell mass can be directly assessed by whole body potassium and nitrogen neutron activation, or indirectly by measuring intracellular water.

2.6 Muscle mass measurement

Muscle mass can be estimated by creatinine excretion or 3-methyl histidine excretion in the urine.
Choosing the proper method to study body composition depends on availability, the question asked and the ability to interpret the results.

2.7 Body impedance

Body impedance is a technique that can be used for routine bedside measurement of body composition. It is based on differences in conducting properties of different tissues. Tissues containing large amounts of water and electrolytes are good conductors. Fat mass, air or bones are poor conductive materials.

At low frequencies the current is unable to penetrate the cell membrane and resistance is negatively related to the extracellular fluid;
At higher frequencies the current is able to pass through the cell membrane and the measured resistance is reflection of total body water;
Finally, the measured resistance values at low and high frequencies can be used to calculate ECW and TBW. These compartments can be used for calculation of fat mass (FM) and fat free mass (FFM).

References

1. Basics in Clinical Nutrition, Sobotka L. 3rd edition, 2004, Galen
2. Goodpaster BH. Measuring body fat distribution and content in humans. Curr Opin Clin Nutr Metab Care 2002: 5; 481
3. Wang ZM, et al. The five-level model: a new approach to organizing body-composition research. Am J Clint Nutr 1992:56:19
4. Mazess RB, et al. Dual-energy x-ray absorption for total-body and regional bone mineral and soft tissue composition. Am J Clin Nutr 1990: 51; 1106
5. Schoeller DA. Bioelectrical impedance analysis. What does it measure? Ann NY Acad Sci 2000:904; 159
6. Kyle et. al. Bioelectrical impedance analysis-part I. Clin Nutr. 2004 23:1226

Chapter 2

Topic 4

Nutritional Requirements for Health throughout Life Span

Nutritional Requirements for Health throughout Life Span

Topic 4

Module 4.1

Nutrition in Neonates

George Paunov

Learning Objectives

To understand the physiological differences, concerning growth and development that define the nutritional needs of the neonates;
To understand the differences in the balance of energy, proteins, water, electrolytes, minerals and micronutrients in different ages;
To give generally accepted recommendations for all nutrients intake.

Contents

Key Messages

Age definition of Premature and Low Birth Weight Infants (LBWI);
Physiological characteristics of Full-term and Premature/LBWI define their different nutritional needs;
The needs of protein, energy and all other nutrients are much higher than in the adults;
Most part of the nutrients is used for cellular proliferation and less for physical activity;
Undernutrition;
In the neonates and especially low birthweight, undernutrition can easily cause physical and mental retardation that may be irreversible.

The needs of neonates for macro and micronutrients estimated per unit of body weight or body surface area are considerably higher than in the older children and adults. Neonates have higher Resting Energy Expenditure (REE) and also high nutrient expenditure for growth and development (1-5). Physical activity of the neonates is not high. There are many different schemes for Recommended Daily Intake (RDI) of macro - and micronutrient. They all are similar and should be used for orientation in the individual approach to every infant.

1. Premature/Low Birthweight infants (LBWI)

Premature are considered the babies born before 36th week of gestation. Low birthweight are those born less than 2500 g. Generally, the weight at birth corresponds with the degree of prematurity.

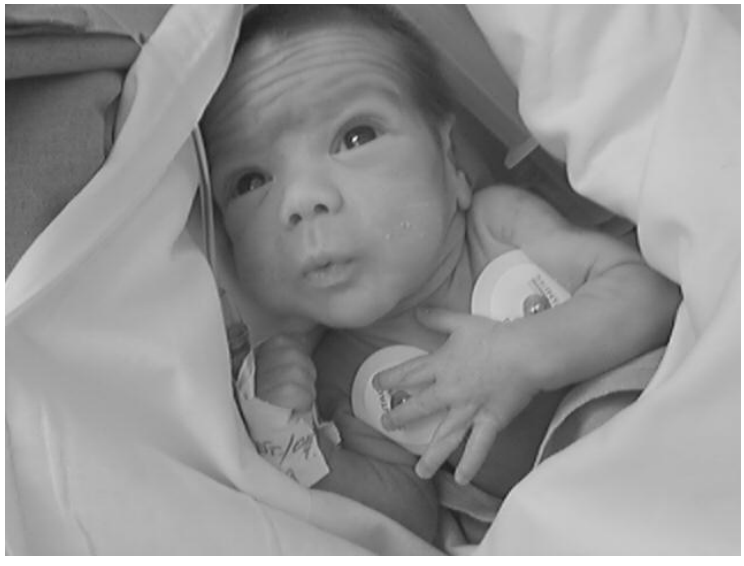


Fig.1 Shows a Premature/Low Birthweight infant

Adequate nutritional management is one of the most important factors for their survival and optimal growth and development.
Sufficient amount of energy and nutrients must be provided to maintain rates of growth that are similar to the intrauterine.
Undernutrition in that period may cause irreversible cellular deficits, especially in the central nervous system.
Adequacy of nutrition in that age period is estimated by the rate of growth. According to the recent knowledge it must be close to the intrauterine growth rates. This concept is the base of the recommended nutrient intake for LBWI, proposed by the Committee for Nutrition of the American Academy of Pediatrics (3) (Table 1).
There is still a discussion about the benefits of using human milk or special formula for feeding LBWI. The energy and protein content of human milk is lower than the recommended intake. It cannot provide the desired rate of physical growth. That fact has motivated the development and use of specialized artificial formulas rich in nutrients that provide better rates of growth.
Proponents of human milk feeding point out provision of specific factors, needed for the development of gastrointestinal tract and immune system. They suppose some important theoretical benefits like better protection from infections, lower incidence of NEC and better neurodevelopmental outcome. Special attention must be paid also to the enhanced mother-infant binding.
None of these two concepts have been accepted as a standard. Considerable insight is provided by the multicenter study of Lucas A, Gore SM, Cole TJ, et al. (6), conducted in England in the early 1980. Their results indicate worse physical development indices, but lower incidence of NEC and infections in LBWI, fed with human milk provided by their own mothers or from special milk banks. At 7- 8 years of age these children showed also some neurodevelopmental advantage.
According to the study of Hales и Ozanne (7), some degree of growth retardation in the first few months after birth is later associated with lower incidence of some serious diseases (obesity, cardiovascular, type II diabetes). They compared breast-fed LBWI, with slower than recommended rates of growth, and formula-fed LBWI with higher growth rates.

Table 1 Recommended nutrient intakes for Low Birtweight Infants

Nutrient	Recommended intake (amount/100 kcal)
Protein (g)	2.7 - 3.1
Fat (g)	4.3 - 5.4 (300 mg essential fatty acids)
Carbohydrate	-
Electrolytes and minerals	
Sodium (mEq)	2.3 - 2.7
Potassium (mEq)	1.8 - 1.9
Calcium (mg)	140 - 160
Magnesium (mg)	6.5 - 7.5
Phosphorus (mg)	95 -108
Chloride (mEq)	2 - 2.4
Iron[a]	
Zinc (mg)	0.5
Copper (μg)	90
Manganese (μg)	5
Iodine (μg)	5
Vitamins	Amount / day
Vitamin A (IU)	1400
Vitamin D (IU)	500
Vitamin E (IU)	5 - 25 (1.0 IU/g linoleic acid)
Vitamin C (mg)	35
Thiamin (μg)	300
Riboflavin (μg)	400
Niacin (mg)	6
Vitamin B_6	300 (15 μg/g protein)
Folic acid (μg)	50
Vitamin B12 (μg)	0.3
Panthotenic acid (mg)	2
Biotin (μg)	35

American Academy of Pediatrics Committee on Nutrition. Pediatrics 1985; 75:976-86.
Lower values are the recommended intakes for larger infants; higher values are the recommended intakes for smaller infants (BW < 1250 g).
[a]See text in paragraph 1.7.

1.1 Energy

It is assumed that premature/LBWI need daily about 120 kCal/kg/24 h, which include:

75 kCal/kg/24 h for REE (50 - 60 kCal Basal Metabolic Rate (BMR) plus energy for enteral absorption of food and management of cold stress);
10 kCal/kg/24 h for physical activity;
25 kCal/kg/24 h for growth;
10 kCal/kg/24 h loses of undigested food in the stools.

1.2 Proteins

Daily intake of approximately 3 g/kg is considered as adequate for LBWI. There is direct relation between protein intake and lean body mass deposition. Biological value of ingested proteins is defined by the amount of essential amino acids, which include for LBWI also histidine, tyrosine and cysteine.

1.3 Fats

The only known requirement for fats is the provision of the essential fatty acids. That needs can be covered by linoleic (2 - 4% of the total energy intake) and linolenic acid. The latest concepts support the benefits of some amounts ω-3 and ω-6 polyunsaturated fatty acids (PUFA), which precursors are linoleic and linolenic acids.

1.4 Carbohydrates

Carbohydrates are the main source of energy. Central nervous system and hematopoetic tissue use glucose as primary metabolic fuel. They are defined as glucose dependents. Glucose can be synthetized from exogenous or endogenous proteins. Thus, there must be no absolute requirements for carbohydrate intake. In practice, exogenous glucose is often needed to prevent hypoglycemia, especialy in the early neonatal period. In human milk carbohydrates are about the half of nonprotein energy sources.

1.5 Water

Several approaches are used to estimate the needs of water. They are used for both, term and LBW infants. Needs of fluids can be calculated per unit of weight (ml/kg), body surface area (ml/m^2), and energy intake (ml/100 kCal). The later is considered most relevant, because it takes in account also many nonphysiologic factors.

Structure of water balance:

Insensible losses (through the skin and respiration) at least 30 ml/100 kCal. They correspond with the degree of prematurity and are caused by the high skin permeability and respiratory rate. This is the reason for the high influence of the ambient temperature and humidity. Phototherapy increases significantly the insensible water loses. In the very low birthweight infants they can be 2 - 3 times greater.

Obligatory urine loses about 50 - 60 ml/100 kCal

Loses through gastrointestinal tract about 10 ml/100 kCal

Growth - 10 - 12 ml/100 kCal. It is considered that in the first 4 months after birth the water consists around 50 - 60% of the weight gain.

Endogenously produced water by oxydation about 12 ml/100 kCal must be included in the water balance.

Combination of all mentioned components shows that if ambient temperature and humidity are adequate for the gestational age, both, term and LBW infants, have similar minimum water requirements of 1 ml/kCal.

The most reliable index of good hydration is the urine output, which after 2 - 3 day of birth must be about 50 - 60 ml/100 kCal.

1.6 Electrolytes

See Table 1.

1.7 Minerals

Calcium daily intake of 5 mmol/kg is assumed to be sufficient.

Phosphorus - calcium : phosphorus ratio of 1.5 - 2 is considered optimal.

Iron needs depend upon the own body stores and growth rates. LBW infant's body stores of iron will be sufficient for 2 - 3 months after birth without exogenous intake (and for 4 - 5 months in the term infants). Iron supplementation increases the need of vitamin E. Iron-binding proteins lactoferin and lactoglobulin have some bactericidal properties when they are not saturated with iron. Thus, large amounts of iron can abolish their protective properties. The American Society of Clinical Nutrition (3) recommends 200 g/kg/24 h enteral and 100 g/kg/24 h parenteral intake for premature and healthy term infants.

1.8 Vitamins

There are no LBWI specific intake recommendations. Daily needs, estimated for the term infants, are assumed to be adequate for the LBW. Vitamins A, C, D and especially E supplementation is recommended. As a powerful antioxidant vitamin E prevents PUFA peroxidation in cell membranes (recommended ratio 1 IU vit E **:** 1g PUFA). Vitamin E is an important erythrocyte stability factor. Probably it can also reduce the risk of retrolental fibroplasia and bronchopulmonal dysplasia.

2. Healthy term infants 0 - 12 months

Fig. 2 Shows a Full term infant

At this age the most part of all nutrients is used for growth and development. Appropriate rates of growth and lack of sympthoms of Fe, Ca, Mg, P and vit D defficiency indicate adequate nutrition.

Special characteristics:

- High REE,
- High rates of growth,
- Low physical activity,
- Lower intestinal capacity for absorption and digestion of some dietary elements (approaches the adults around 6 months).

2.1 Energy

85 - 120 kCal/kg/day are recommended for enteraly fed, healthy infants of that age. Carbohydrates and fats are the main energy sources. There is no clear statement abot their optimal proportion. A minimal carbohydrate intake of 5g/kg/day is needed to prevent hypoglycemia and/or increased ketone production. Minimal amounts of 0.5 - 1.0 g/kg/day linoleic and 0.3 - 0.5 g/kg/day linolenic acid are sufficient to prevent defficiency.

2.2 Proteins

Protein needs of healthy infants are much higher than in the aduldts. Their nutritional value is defined by the essential:nonessential amino acid ratio. Best are considered these with amino acid pattern, close to the human milk. Protein intake recommendations change a lot in the first year of life from 2.2- 2.7 g/kg/day immediately after birth to 1.0 g/kg/day at 12 months.

Table 2 Amino acid requirements of the term infant 0-12 months

Amino Acid	Minimum requirement (mg/kg/day)
Leucine	76 - 229
Isoleucine	102 - 119
Lysine	88 - 103
Methionine (with cyst(e)ine)	33 - 45
Phenylalanine (with tyrosine)	47 - 90
Threonine	45 - 87
Tryptophan	15 - 22
Valine	85 - 105
Histidine	16 - 34

Adapted from Holt LE Jr, Snyderman SE. JAMA 1961; 175: 100-3.

2.3 Electrolytes, minerals, vitamins

Daily needs of electrolytes, minerals and vitamins are not as clear as those of protein and energy. Nonetheless, recommended daily allowances have been established and considered in the modern nutritional formula.

Iron deficiency is the most common nutrient deficiency in the age up to 12 months. It is more often in formula-fed infants, despite of low iron content of human milk.

Vitamin deficiencies are rare in infants less than 1 year if protein intake is adequate.

Nicotinic acid and choline are synthesized respectively from trypthofan and methionin. They may be in deficit if the protein intake is low.

Formula-fed infants are also at greater risk of vitamin D deficiency and special attention must be paid to it's content. Vitamin D supplementation of breast-fed infants is also advocated.

Routine administration of vitamin K is recommended for prophylaxis of hemorrhagic disease of the newborn.

Fig.3 Shows a Full term infant

Table 3 Recommended daily allowances of nutrients for term infants 0 - 12 months

	Recommended intake per day	
Nutrient	0 - 6 Months; Weight = 6 kg	6 - 12 Months; Weight = 9 kg
Energy (kcal)	650	850
Fat (g)		
Carbohydrate		
Protein (g)	13	14
Electrolytes and minerals		
Calcium (mg)	400	600
Phosphorus (mg)	300	500
Magnesium (mg)	40	60
Sodium (mg)[a]	120	200
Chloride (mg)[a]	180	300
Potassium (mg)[a]	500	700
Iron (mg)	6	10
Zinc (mg)	5	5
Copper (mg)	0.4 - 0.6	0.6 - 0.7
Iodine (μg)	40	50
Selenium (μg)	10	15
Manganese (μg)[b]	0.3 - 0.6	0.6 - 1.0
Fluoride (μg)[b]	0.1 - 1	0.2 - 1
Chromium (μg)[b]	10 - 40	20 - 60
Molybdenum (μg)	15 - 30	20 - 40
Vitamins		
Vitamin A (μg RE)	375	375
Vitamin D (μg)	7.5	10
Vitamin E (μg α-TE)	3	4
Vitamin K (μg)	5	10
Vitamin C (mg)	0.3	0.4
Thiamin (mg)	0.3	0.4
Riboflavin (mg)	0.4	0.5
Niacin (mg NE)	5	6
Vitamin B_6 (μg)	0.3	0.6
Folate (μg)	25	35
Vitamin B_{12} (μg)[b]	0.3	0.5
Biotin (μg) [b]	10	15
Panthotenic acid (mg)[b]	2	3

Data from Food and Nutrition Board, National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
[a]Minimum requirements (mg/day) rather than recommended.
[b]Estimated safe and adequate daily intake.

3. Summary

Adequate nutrition is one of the most important factors for the correct development of every child. In neonates most part of the nutrients is used for cellular proliferation and less for physical activity. All preterm and term neonates need more nutrients and energy per kilogram body weight than the older children and adults because of the higher rates of growth. Severe undernutrition or isolated nutrient deficiency disturb physical growth and/or maturation of one or more physiological systems. Neonates and especially preterm/low birth weight infants are very sensitive to undernutrition which can cause not only physical but also mental retardation. The sequences of undernutrition can be long lasting or even irreversible.

Reference

1. Shils, Maurice E.; Olson, James A.; Shike, Moshe; Ross, A. Catharine Modern Nutrition In Health and Disease. 9th Edition 1999, Lippincott Williams & Wilkins.
2. Behrman, Richard E; Kliegman, Robert M; Jenson, Hal B. Nelson Textbook of Pediatrics, 17th edition, 2003 W B Sounders.
3. American Academy of Pediatrics Committee on Nutrition. Pediatrics 1985; 75:976-86.
4. Nutrition Committee, Canadian Paediatric Society. Nutrient Needs and Feeding of Premature Infants [Clinical Practice Guidelines]. CMAJ. Vol 152 (11) June 1995: 1765-85.
5. Committee on Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and Nutrition. Nutrition and feeding of preterm infants. Acta Paediatr Scand Suppl 1987; 336: 1-14
6. Lucas A, Gore SM, Cole TJ, et al. Arch Dis Child 1984;59:72230.
7. Hales CN, Ozanne SE2003 The dangerous road of catch-up growth. J Physiol 547(Pt 1):510.
8. Thureen P, Heird WC. Protein and Energy Requirements of the Preterm/Low Birthweight (LBW) Infant. Pediatric Research, 2005 International Pediatric Research Foundation, Inc. Vol 57, No 5, Pt 2, 2005.
9. Food and Nutrition Board, National Research Council, Recommended daily allowances. 10th Edition. Washington DC: National Academy Press, 1989.
10. Forbes GB. Nutritional requirements in adolescence. In: Suskind RM, ed. Textbook of pediatric nutrition. New York: Raven Press, 1981.
11. World Health Organization. Handbook on human nutritional requirements. Monograph series no. 61. Geneva: WHO, 1974.
12. Hollinbery PW, Massey LK. (Abstract 1280) Fed Proc 1986;45:375.
13. Center for Disease Control. Ten State Nutrition Survey in the United States, 1968-1970. Atlanta, GA: Health Services and Mental Health Administration, 1972.

Nutritional Requirements for Health throughout Life Span

Topic 4

Module 4.2

Nutrition in Infancy, Childhood and Adolescence

George Paunov

Learning Objectives

To understand the physiological differences, concerning growth and development that define the nutritional needs in different ages;
To understand the differences in the balance of energy, proteins, water, electrolytes, minerals and micronutrients in different ages;
To give generally accepted recommendations for all nutrients intake.

Contents

Key Messages

Definition of age periods in infancy, childhood and adolescence, concerning nutrition;
Physiological characteristics of the different age groups define their different nutritional needs;
The needs of protein, energy and all other nutrients in infancy, childhood and adolescence are much higher than in the adults;
Most part of the nutrients is used for cellular proliferation and physical activity;
In all ages undernutrition causes growth retardation and may have long lasting sequelae.

1. Introduction

The needs of pediatric population for macro and micronutrients estimated per unit of body weight or body surface area are considerably higher than in the adults. Children have higher Resting Energy Expenditure (REE) and also high nutrient expenditure for growth and development (1-2).
The needs of nutrients change with age because of the differences in growth rate and activity. There are many different schemes for Recommended Daily Intake (RDI) of macro - and micronutrient (3, 5, 6, 9). They all are similar and should be used for orientation in the individual approach to every child.

2. Children from 1 to 10 years

This period is characterized with great changes in nutrition. They follow the physical and the mental development of the child. Gastrointestinal tract reaches adult's digestive capacity near age of 1 year.
About two years of age most children have almost the same food as their parents. Compared with the period 0 - 12 month the rates of growth keep slowing down and physical activity increases significantly. The children become more independent and build up their own style of nutrition.
In most children between 2 and 5 years feeding becomes dependent of many nonnutritional factors like weariness or strong emotions. Often eating or not eating is used as instrument of control over the adults. It's very important to provide diverse foods with sufficient content and quality of minerals, vitamins, proteins and energy. They must be separated between the three meals and 2- 3 snacks.

3. Adolescents from 11 to 24 years

Adolescence is a period of dramatic anatomical, physiological, psychological and emotional changes. Nutrient intake must be adequate to very intense physical growth, maturation and psychosocial changes, which affect nutritional needs of the adolescent.
Three aspects of growth must be emphasized:

Intensity and extent of pubertal growth,
Intersexual differences in the timing of growth and nature of changes in body composition,
Individual variations.

Physical growth intensity defines the most part of the nutrient requirements. In the recent decades most rapid linear growth is found between 10 and 13 years for the girls and 12 to 15 years for the boys. In that period, adolescents have the highest nutritional requirements.
Calorimetry data shows that energy consumption is more closely related to the linear growth than to the biological or current weight. That's why calculation of nutrient needs per centimeter of height is accepted as more precise. The main differences in body structure between the two sexes develop in that period.

Table 1 Recommended daily allowances of nutrients for healthy children between 1 and 10 years

	Recommended intake per day		
Nutrient	1 - 3 Years Weight = 13 kg	4 - 6 Years Weight = 20 kg	7 - 10 Years Weight = 9 kg
Energy (kcal)	1300	1800	2000
Fat (g)			
Carbohydrate			
Protein (g)	16	24	38
Electrolytes and minerals			
Calcium (mg)	800	800	800
Phosphorus (mg)	800	800	800
Magnesium (mg)	80	120	170
Sodium (m Eq)[a]	13	20	28
Chloride (m Eq)[a]	13	20	28
Potassium (m Eq)[a]	26	36	40
Iron (mg)	10	10	10
Zinc (mg)	10	10	10
Copper (mg)[b]	0.7 - 1.0	1.0 - 1.5	1 - 2
Iodine (μg)	70	90	120
Selenium (μg)	20	20	30
Manganese (μg)[a]	1 - 1.5	1.5 - 2	2 - 3
Fluoride (μg)[b]	0.5 - 1.5	1 - 2.5	1 - 2.5
Chromium (μg)[a]	20 - 80	30 - 120	50 - 200
Molybdenum (μg)	25 - 50	30 - 75	50 - 150
Vitamins			
Vitamin A (μg RE)	400	500	700
Vitamin D (μg)	0.10	10	10
Vitamin E (μg α-TE)	6	8	7
Vitamin K (μg)	15	20	30
Vitamin C (mg)	40	45	45
Thiamin (mg)	0.7	0.9	1.0
Riboflavin (mg)	0.8	1.1	1.2
Niacin (mg NE)	9	12	13
Vitamin B_6 (μg)	1	1.1	1.4
Folate (μg)	50	75	100
Vitamin B_{12} (μg)[a]	0.7	1.0	1.4
Biotin (μg) [a]	20	25	30
Panthotenic acid (mg)[a]	3	3 - 4	4 - 5

Data from Food and Nutrition Board, National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
[a]Minimum requirements (mg/day) rather than recommended.
[b]Estimated safe and adequate daily intake.

4. Energy

It's important to note that energy needs in adolescence have great individual variations. Increased physical activity is the other important component of the energy consumption.

Table 2 Recommended energy intake for adolescents 11- 24 years

Age (years)	Average allowance (kcal/cm height)	(kcal/day)
Males		
11 - 14	15.9	2500
15 - 18	17.0	3000
19 - 24	16.4	2900
Females		
11 - 14	14.0	2200
15 - 18	13.5	2200
19 - 24	13.4	2200

Data from Food and Nutrition Board, National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.

Table 3 Energy expenditure of selected activities (kCal/min activity)

Activity	kcal/min/kg	45 kg	55 kg	65 kg
Basketball	0.138	6.2	7.6	9.0
Cycling				
5.5 mph	0.064	2.9	3.5	4.2
9.4 mph	0.100	4.5	5.5	6.5
Dancing (twist)	0.168	7.6	9.2	10.9
Football	0.132	5.9	7.3	8.6
Running				
11.5 min/mile	0.135	6.1	7.4	8.8
8 min/mile	0.208	9.4	11.4	13.6
Sitting quietly	0.021	0.9	1.2	1.4
Walking, normal pace	0.080	3.6	4.4	5.2
Writing, sitting	0.029	1.3	1.6	1.9
Vacuuming				
Females	0.045	2.0	2.5	3.1
Males	0.048	2.2	2.6	3.1
Ironing				
Females	0.033	1.5	1.8	2.1
Males	0.064	2.9	3.5	4.2

Adapted from McArdle WD, Katch VL. Exercise physiology: energy, nutrition and human performance. Phyladelphia: Lea & Febiger, 1991.

4.1 Proteins

Recommended daily intake:
11 to 14 years - 0.29 g/cm of height
15 to 18 years - 0.34 g/cm of height
19 to 24 years - 0.33 g/cm of height

Results in some studies (4,12,13) show that average protein intake is often higher than recommended values. In this age period protein metabolism is especially sensitive to low energy

intake. Except socioeconomic conditions, desire to lose weight, or eating disorders like anorexia neurosa, can severely diminish protein and energy intake in adolescents.

4.2 Minerals

High rates of growth define the increased need of minerals. Three of them are of particular importance: calcium, iron and zinc.

Calcium
About 99% of total body calcium is stored in the skeleton. High rates of growth are associated with increasing of skeletal length and mass. That makes this process very sensitive to decreased provision of calcium. During the pubertal growth spurt the rate of calcium deposition in the body can almost double, compared with the average increment during the adolescent period.
About 900 mg is the minimal daily intake, recommended during active skeletal growth (6, 7, 8, 9, 10). Some other standards recommend different values, which change with the age.

Recommendations of WHO
11 to 15 years - 600 to 700 mg/day
16 to 19 years - 500 to 600 mg/day

USA National Institute of Health Consensus Conference
11 to 24 years - 1200 to 1500 mg/day

USA Food and Nutrition Board's 1997 dietary reference
9 to 18 years - 1300 mg/day

Individual differences in growth and the intake of proteins, vitamin D, phosphorus, fibers, caffeine and sucrose have great impact on calcium metabolism.

Iron
The need of higher intake is conditioned by the great amounts of hemoglobin and myoglobin synthesized in that period. Iron requirements of the girls increase additionally with the beginning of menstruation.
Recommended daily income is considered to be about 12 - 13 mg/day for the boys and 13 - 15 mg/day for the girls.
Except the low intake, active sport can be another important factor in developing anemia in adolescence. Acute metabolic stress response to intense exercise in training can increase the destruction of erythrocytes.

Zinc
Zinc is particularly important for protein synthesis and is essential for sexual maturation. In adolescents zinc deficiency is associated with growth retardation and hypogonadism.
Recommended daily intake is about 15 mg/day for boys and 13 mg/day for girls.

4.3 Vitamins

Requirements of all vitamins in adolescence and especially during pubertal growth spurt are much higher than in the adults. Vitamins take important part in function and growth of all physiological systems. That's why all of them must be provided in sufficient amounts.

Table 4 Recommended daily intake of vitamins

		Vitamins	
Category	Age (years)	Biotin (μg)	Pantothenic acid (mg)
Infants	0 - 0.5	10	2
	0.5 - 1	15	3
Children and adolescents	1 - 3	20	3
	4 - 6	25	3 - 4
	7 - 10	30	4 - 5
	11 +	30 - 100	4 - 7
Adults		30 - 100	4 - 7

Table 5 Recommended daily intake of trace elements

		Trace elements[b]				
Category	Age (years)	Copper (mg)	Manganese (mg)	Fluoride (mg)	Chromium (mg)	Molybdenum (mg)
Infants	0 - 0.5	0.4 - 0.6	0.3 - 0.8	0.1 - 0.5	10 - 40	15 - 30
	0.5 - 1	0.6 - 0.7	0.6 - 1.0	0.2 - 1.0	20 - 60	20 - 40
Children and adolescents	1 - 3	0.7 - 1.0	1.0 - 1.5	0.5 - 1.5	20 - 80	25 - 50
	4 - 6	1.0 - 1.5	1.5 - 2.0	1.0 - 2.5	30 - 120	30 - 75
	7 - 10	1.0 - 2.0	2.0 - 3.0	1.5 - 2.5	50 - 200	50 - 150
	11 +	1.5 - 2.5	2.0 - 5.0	1.5 - 2.5	50 - 200	75 -250
Adults		1.5 - 3.0	2.0 - 5.0	1.5 - 4.0	50 - 200	75 -250

Data from Food and Nutrition Board, National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989:284

[a]Because there is less information on which to base allowance, these figures are not given in the main table of RDA and are provided here in the form of ranges of recommended intakes.

[b]Because the toxic levels for many trace elements may be only several times usual intakes, the upper levels for the trace elements given in this table should not be habitually exceeded.

4.4 Special nutritional problems

Obesity

Some studies of obesity show increasing of cardiovascular problems, arthritis, diabetes, and colorectal carcinoma in adult, who have had BMI > 30 kg/m^2 in adolescence (14).

Eating Disorders

Two major eating disorders are anorexia neurosa and bulimia. Psychological and emotional changes that take place in adolescence and especially during puberty are very deep. That makes difficult to distinguish the “normal” eating habits from the disorders. These problems must be approached very carefully, always by qualified, multidisciplinary staff.

Pregnancy

Pregnancy and lactation in adolescence pose some special requirements to the nutrition. Nutritional requirements in that period are much higher than in pregnant adult female. Except the development of the fetus, pregnant adolescents must provide the growth of their own body. Recent studies show that adolescent mothers deliver low birthweight and very low birthweight infants more often than adult mothers.

Recommended daily intake of some nutrients for pregnant adolescents.

Energy - 300 to 500 kCal more than average daily needs for that age, which are 2400 - 2600 cKal;

Proteins - additional 14 - 26 g;

Iron - about 30 mg;

Zinc - same amounts as for pregnant adults are considered sufficient.

5. Summary

Adequate nutrition is one of the most important factors for the correct development of every child. Profound physiological changes that take place from the moment of birth to the end of adolescence define the different nutritional needs in these age periods. Most part of the nutrients is used for cellular proliferation and physical activity. That is why children of all ages need more nutrients and energy per kilogram body weight than the adults. In all ages undernutrition or isolated nutrient deficiency disturb physical growth and/or maturation of one or more physiological systems. The sequences of undernutrition in the infancy, childhood and adolescence can be long lasting or even irreversible.

References

1. Shils, Maurice E.; Olson, James A.; Shike, Moshe; Ross, A. Catharine Modern Nutrition In Health and Disease. 9th Edition ?1999, Lippincott Williams & Wilkins.
2. Behrman, Richard E; Kliegman, Robert M; Jenson, Hal B. Nelson Textbook of Pediatrics, 17th edition, 2003 W B Sounders.
3. American Academy of Pediatrics Committee on Nutrition. Pediatrics 1985; 75:976-86.
4. Lucas A, Gore SM, Cole TJ, et al. Arch Dis Child 1984;59:72230.
5. Food and Nutrition Board, National Research Council, Recommended daily allowances. 10th Edition. Washington DC: National Academy Press, 1989.
6. McArdle WD, Katch FI, Katch VL. Exercise physiology. Energy, nutrition, and human performance. Philadelphia: Lea & Febiger, 1991.
7. Forbes GB. Nutritional requirements in adolescence. In: Suskind RM, ed. Textbook of pediatric nutrition. New York: Raven Press, 1981.
8. Greenwood CT, Richardson DP. World Rev Nutr Diet 1979;33:141.
9. World Health Organization. Handbook on human nutritional requirements. Monograph series no. 61. Geneva: WHO, 1974.
10. Schuette SA, Linksweiler HM. Calcium. In: Present knowledge of nutrition. 5th ed. Washington, DC: Nutrition Foundation, 1984.
11. Hollinbery PW, Massey LK. (Abstract 1280) Fed Proc 1986;45:375.
12. Nelson M. Dietary practices of adolescents. In: Winick M, ed. Adolescent nutrition. New York: John Wiley & Sons, 1982.
13. Center for Disease Control. Ten State Nutrition Survey in the United States, 19681970. Atlanta, GA: Health Services and Mental Health Administration, 1972.
14. National Center for Health Statistics. Caloric and selected nutrient values for persons 174 years of age. First Health and Examination Survey, 19711974, DHEW publ. (PHS) 79-1657. Hyattsville, MD: National Center for Health, 1979.
15. Bray GA. J Endocrinol Metab Clin North Am 1996;25:90719.
16. Miller HS, Lesser KB, Reed KL. Obstet Gynecol 1996;87:838.

Chapter 3

Topic 5

Malnutrition

Malnutrition

Topic 5

Module 5.1

Undernutrition - Simple and Stress Starvation

Lubos Sobotka
Peter Soeters
Remy Meier
Yitshal Berner

Learning Objectives

To know how the body reacts to short-term and long-term starvation during non-stress conditions;
To understand the difference between simple and stress starvation;
To know the consequences of stress on metabolic pathways related to starvation.

Contents

1. Definition and classification of malnutrition
2. Undernutrition
3. Aetiology of undernutrition
4. Adaptation to undernutrition - non-stress starvation
5. Stress starvation
6. Summary

Key Messages

Humans adapt well to short or a longer-term starvation, using their reserve stores of carbohydrates, fat and protein;
Reduction of energy expenditure and conservation of body protein are further reaction to starvation. Energy stores are replenished during feeding period;
Long-term partial or total cessation of energy intake leads to marasmic wasting;
With the addition of the stress response, catabolism and wasting are accelerated and the normal adaptive responses to simple starvation are overridden;
Weight loss in either situation results in impaired mental and physical function, as well as poorer clinical outcome.

1. Definition and classification of malnutrition

Malnutrition can be defined as a state of nutrition in which a deficiency or excess (or imbalance) of energy, protein and other nutrients causes measurable adverse effects on tissue/body form (body shape, size, composition), body function and clinical outcome.

In broad term, malnutrition includes not only protein-energy malnutrition (both over- and under-nutrition) but also malnutrition of other nutrients, such as micronutrients.

Malnutrition of micronutrients can cause deficiency states or toxic symptoms - these are discussed in particular modules related to vitamins and trace elements.

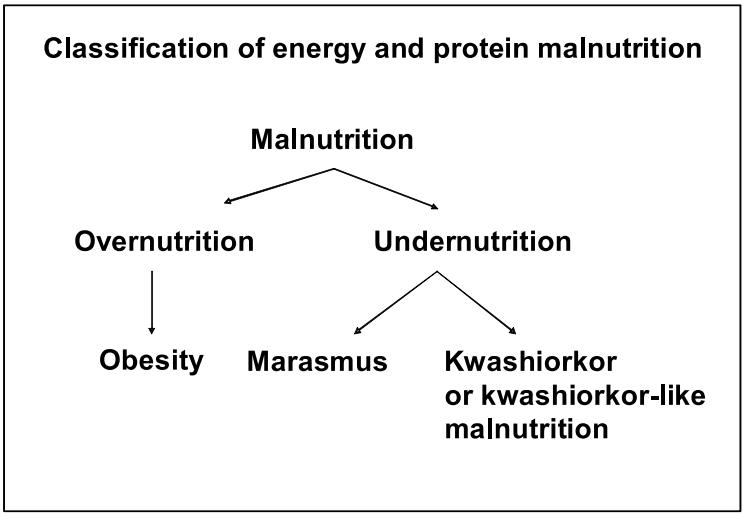


Fig. 1 Classification of energy and protein malnutrition

The most widely used classification of malnutrition is based on calculation of body mass index (BMI) - see Table 1.

Table 1 Classification of malnutrition according to body mass index (Body mass index (BMI) = weight (kg) / body height^2 (m^2))

Body mass index (kg/m^2)	Classification
Less than 18.5	Severely underweight
Less than 20	Underweight
20 to 25	Desirable or healthy range
Over 25 to 30	Overweight
Over 30 to 35	Obese (Class I)
Over 35 to 40	Obese (Class II)
Over 40	Morbidly or severely obese (Class III)

2. Undernutrition

Undernutrition can be defined as a state of nutrition deficiency, which is connected with adverse consequences on physical functions or clinical outcome. Usually BMI < 20 kg/m^2 identifies high probability of undernutrition. However, individuals with BMI > 20 kg/m^2 may be at risk of undernutrition when losing unintentionally more than 10% weight loss over 3-6 months. In opposite weight stable healthy individuals with a BMI < 20 kg/m^2 can be without any functional changes related to malnutrition.

Risk of undernutrition related clinical and functional problems can be predicted using nutrition screening tool (see module 3.1).

3. Aetiology of undernutrition

Undernutrition results from an imbalance among nutrient intake and nutrient needs. The rapidity, severity and clinical outcome of undernutrition are dependent on:

- difference between energy intake and energy expenditure;
- nutritional status and energy reserves at the onset of the undernutrition - theoretical energy reserves are shown in Table 2;

extend of adaptive processes to the undernutrition;
potential incidence of stress response (e.g. inflammation, bleeding, surgery) during a period of undernutrition;

Table 2 The theoretical reserves of a 74 kg man although survival is unusual when fat content is reduced below 3 kg and protein is depleted by more than 50% (Hill 1992)

Body substrate	Substrate weight (kg)	Energy content (kcal)
Fat	15	141.000
Protein	12	48.000
Glycogen (muscle)	0.5	2000
Glycogen (liver)	0.2	800
Glucose (extracellular fluid)	0.02	80
Total		191.880

The main factors that lead to undernutrition are:
disease;
social and psychosocial factors.

Disease-related factors which lead to undernutrition
insufficient food/nutrient intake (anorexia, taste disturbances, nausea, vomiting, treatment-induced side effects, eating and swallowing difficulties);
impaired nutrient digestion and absorption (especially in gastrointestinal diseases);
increased requirements for nutrients (sepsis, trauma, endocrine disease);
increased losses (e.g. from wounds, malabsorption and intestinal losses);
catabolism.

Social and psychological factors which lead to undernutrition
problems with shopping or preparing meals;
anxiety;
depression;
poverty;
lack of suitable or enjoyable food;
environmental factors (nursing homes etc.);
inadequate catering practices;
anorexia nervosa;
hunger strikers.

Metabolic consequences of undernutrition are dependent on clinical conditions (adaptive changes of presence of stress, sepsis or critical illness).

4. Adaptation to undernutrition - non-stress starvation

Development of adaptive mechanisms to food shortage was necessary for survival of famine periods. These adaptive changes allow to healthy subjects of normal initial body composition to survive more than two months of total starvation.

Short starvation (< 72 hours)
Short period of starvation is connected with:
diminished insulin and increased glucagon and catecholamine secretions;
increased glycogenolysis and lipolysis;
hydrolysis of triglycerides in adipose tissue releases fatty acids (FFAs) and glycerol into the circulation;
increased gluconeogenesis from amino acids after depletion of glycogen stores.

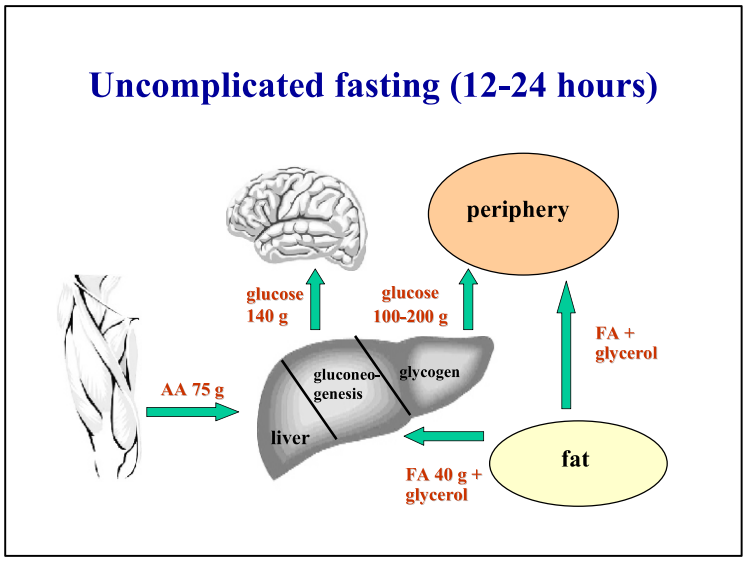


Fig. 2 Metabolic fluxes during short term simple starvation (non stress conditions).

Prolonged starvation (> 72 hours)

Beyond 72 hours starvation is connected with:

- further decrease in insulin level;
- glycogen stores depletion;
- reduction of energy expenditure related to physical activity;
- decline in resting metabolic rate by 10-15%;
- increased oxidation of fatty acids;
- increased production of ketone bodies in the liver;
- adaptation of the brain to using ketones as energy fuel;
- reduction in net tissue protein catabolism.

During simple starvation albumin concentration is unchanged, although plasma proteins with a shorter half-life may be decreased.

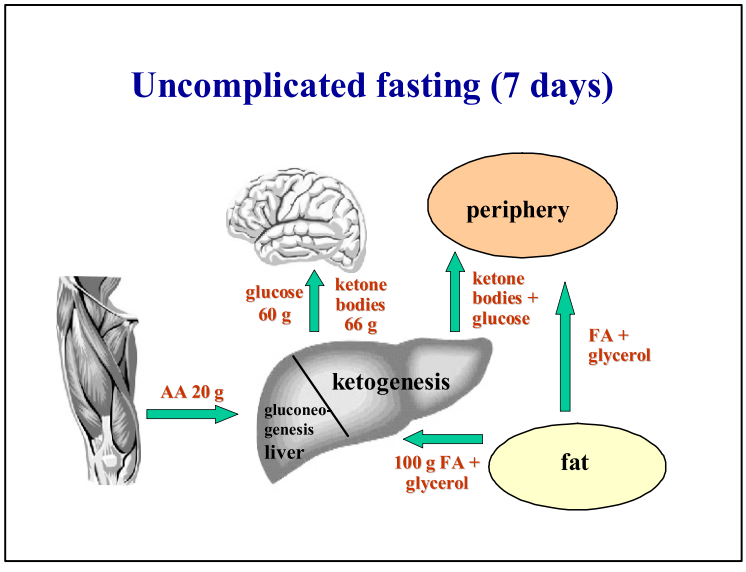


Fig. 3 Metabolic fluxes during long term simple starvation (non stress conditions).

The modifications in metabolic processes during short and long term starvation are described in the figure below.

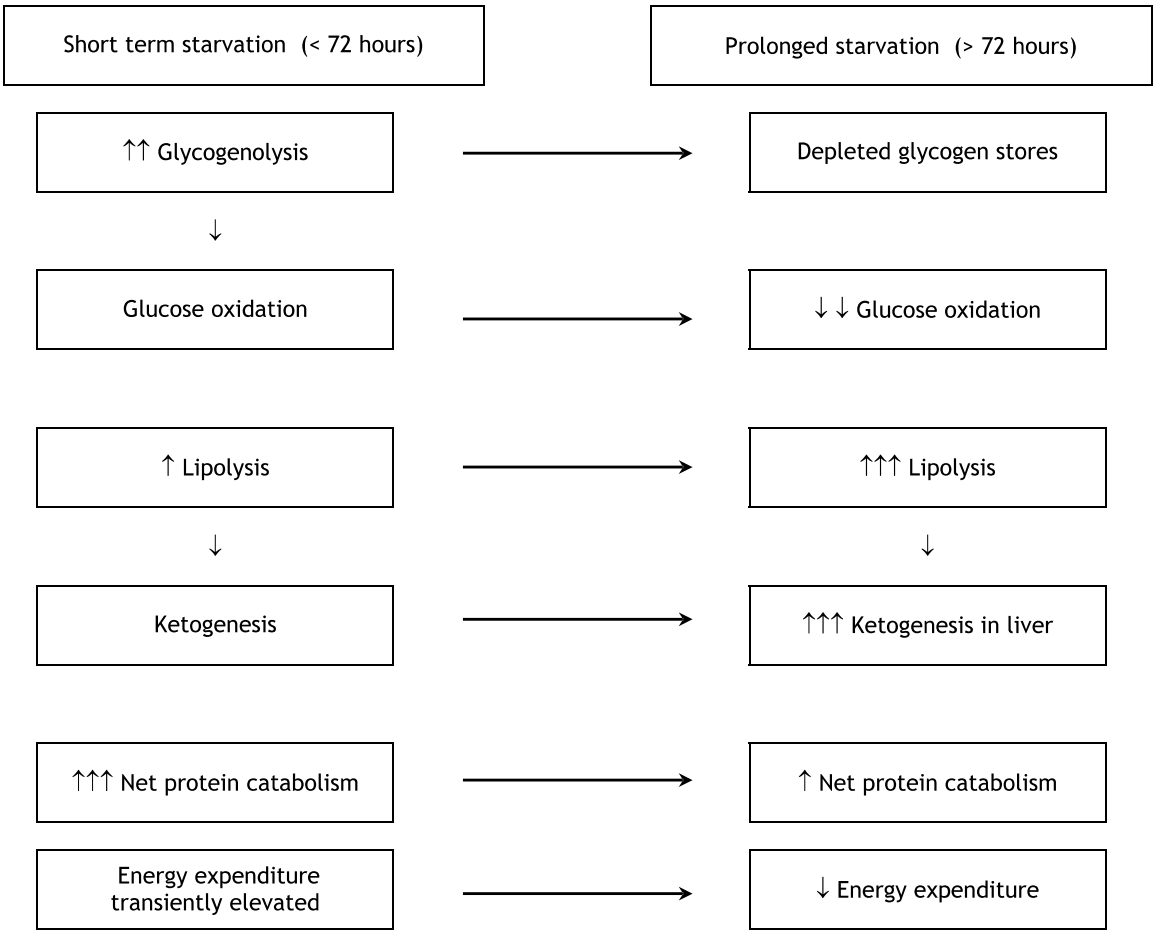


Fig. 4 The difference between metabolic reactions of short-term and long-term starvation.

5. Stress starvation

The reaction to starvation is altered during stress conditions like:

burns;
necrosis (acute pancreatitis, ischemic necrosis);
severe infection and sepsis;
penetrating and blunt injury;
the presence of tumour cells;
radiation;
exposure to allergens;
the presence of chronic inflammatory diseases;
environmental pollutants.

In these situations, the normal adaptive responses of simple starvation, which conserve body protein, are over-ridden by the cytokine and neuroendocrine effects of injury (Fig. 5, Fig. 6).

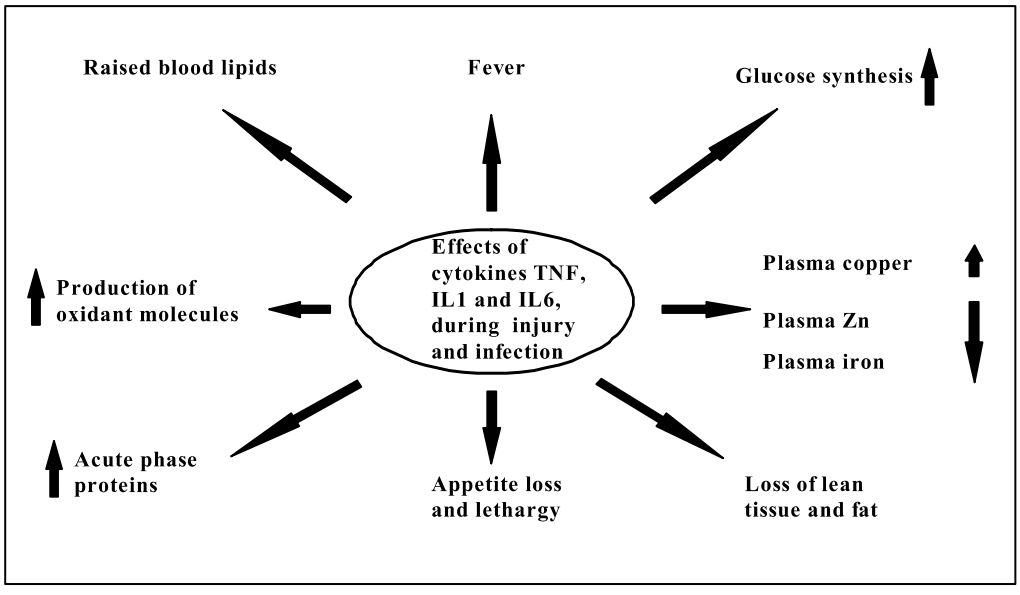


Fig. 5 Effects of pro-inflammatory cytokines in infection, injury and inflammatory disease TNF- Tumour necrosis factor, IL1- interleukin 1, IL6 - interleukin 6.

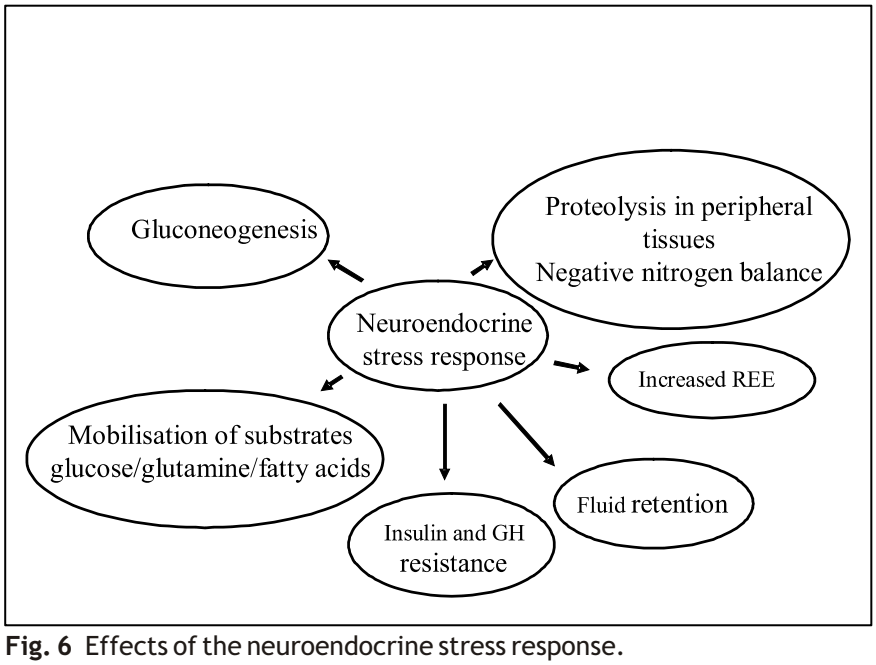


Fig. 6 Effects of the neuroendocrine stress response.

As a result of cytokine and neuroendocrine reaction to stress the metabolic rate raises rather than falls, ketosis is minimal, protein catabolism accelerates to meet the demands for tissue repair and of gluconeogenesis and there is hyperglycaemia and glucose intolerance. Salt and water retention is exacerbated and this may result in a kwashiorkor-like state with oedema and hypoalbuminaemia (Table 3). The latter may also be exacerbated by protein deficiency.

Table 3 Simple versus stress starvation

	Simple starvation (> 72 h)	Stress starvation
Metabolic rate	↓	↑
Protein catabolism (relatively)	↓	↑
Protein synthesis (relatively)	↓	↑
Protein turnover	↓	↑
Nitrogen balance	↓	↓↓
Gluconeogenesis	↓	↑
Ketosis	↑↑	-
Glucose turnover	↓	↑
Blood glucose	↓	↑
Salt and water retention	↑	↑↑↑
Plasma albumin	-	↓↓

Carbohydrate metabolism
Stress initiates a strong increase in endogenous glucose production and turnover. Glucose is an indispensable substrate in this respect because part of the glucose breakdown (glycolysis) does not require oxygen, while still furnishing energy. Lactate as result of anaerobic glucose metabolism is important precursor of gluconeogenesis in liver (Cori cycle). Glucose metabolism during prolonged starvation and stress reaction is shown in Table 4.

Table 4 Glucose metabolism during starvation and critical illness

	Postprandial state	Prolonged starvation	Stress reaction
Gluconeogenesis	↓	↑	↑↑↑
Glycolysis	↑	↓	↑↑↑
Glucose oxidation	↑↑↑	↓	↓
Glucose cycling	↑	↓	↑↑↑

Protein and amino acid metabolism
Amino acids are, together with glycerol, the main substrates for „de novo" glucose production in the liver. Moreover, particular amino acids, such as glutamine and branched-chain amino acids (BCAA), are the only substrates that can be utilized in some peripheral or wounded tissues as a source of energy and nitrogen. Muscle amino acids are also used for the synthesis of acute phase proteins, albumin, fibrinogen etc. The degree of protein catabolism in sepsis is large which can reach a daily loss of more than 1 kg of muscle tissue (Table 5).

Table 5 Protein metabolism during starvation and critical illness

	Postprandial state	Prolonged starvation	Stress reaction
Proteolysis	↓	↓	↑↑↑
Proteosynthesis	↑	↓	↑↑
Amino acid oxidation	↑	↓	↑↑↑

Lipid metabolism
Accelerated rate of lipolysis is part of the metabolic response to severe illness, the resulting fatty acid release can exceed energy requirements. Part of these fatty acids is oxidized and the remainder is re-esterified to triglycerides. Ketogenesis is suppressed during critical illness combined with starvation (Table 6).

Table 6 Lipid metabolism during starvation and a critical illness

	Postprandial state	Prolonged starvation	Stress reaction
Lipolysis in fat tissue	↓↓	↑↑↑	↑↑
Lipid oxidation	↓	↑↑↑	↑
Ketogenesis	↓↓	↑↑↑	↑
Fatty acids - triglyceride cycling	–	↓	↑↑

Quantitative aspects of metabolic fluxes during stress starvation are shown in Fig. 7.

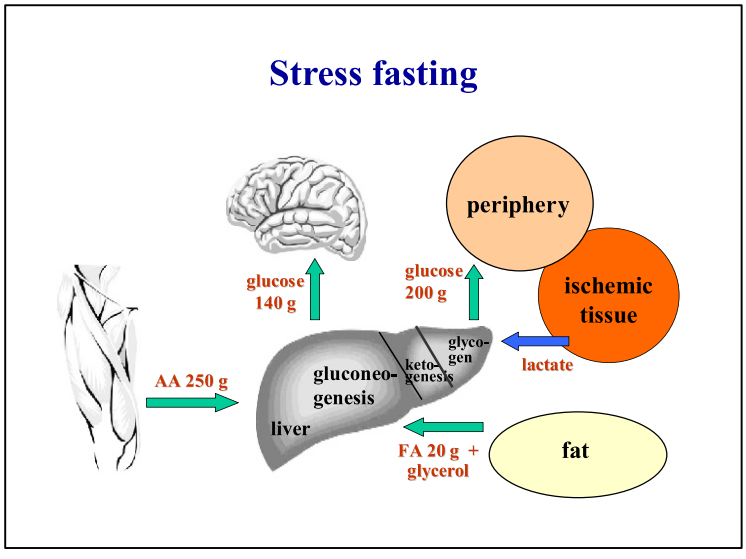


Fig. 7 Metabolic fluxes during stress starvation

6. Summary

Humans adapt well to short or a longer-term starvation, using their reserve stores of carbohydrates, fat and protein. Reduction of energy expenditure and conservation of body protein are further reaction to starvation. Energy stores are replenished during feeding period. Long-term partial or total cessation of energy intake leads to marasmic wasting.
With the addition of the stress response, catabolism and wasting are accelerated and the normal adaptive responses to simple starvation are overridden. Weight loss in either situation results in impaired mental and physical function, as well as poorer clinical outcome.
Previously malnourished subjects have fewer reserves with which to face an acute illness. They are unable to release sufficient amounts of endogenous nitrogen in response to trauma and infection with subsequent higher mortality, more complications and prolonged recovery. If surgery is planned in these patients nutritional support improves physiological functions and lessens surgical risk.

References

1. Biolo G, Toigo G, Ciocchi B, Situlin R, Iscra F, Gullo A, et al. Metabolic response to injury and sepsis: changes in protein metabolism. Nutrition 1997;13(9 Suppl):52S-57S.
2. Desborough JP. The stress response to trauma and surgery. Br J Anaesth 2000;85(1):109-117.
3. Grimble RF. Main cytokines and their effect during injury and sepsis In Basics in Clinical Nutrition, Sobotka L. (edit.) Third Edition, 2004 Galen p. 118-124
4. Hammarqvist F, Wernerman J, Allison SP Injury and sepsis - The neuroendocrine response In Basics in Clinical Nutrition, Sobotka L. (edit.) Third Edition, Galen 2004 Galen p. 114-117
5. Kehlet H, Wilmore DW. Multimodal strategies to improve surgical outcome. Am J Surg 2002; 183: 630
6. Keys A, Brozek J, Henshel A, et al. The Biology of Human Starvation. University of Minnesota Press, Minneapolis 1950
7. Melchior JC. From malnutrition to refeeding during anorexia nervosa. Curr Opin Clin Nutr Metab Care 1998;1(6):481-485.
8. Nandi J, Meguid MM, Inui A, Xu Y, Makarenko IG, Tada T, et al. Central mechanisms involved with catabolism. Curr Opin Clin Nutr Metab Care 2002; 5: 407
9. Sobotka L, Soeters PB. Metabolic response to injury and sepsis In Basics in Clinical Nutrition, Sobotka L. (edit.) Third Edition, 2004 Galen, p. 124-129
10. Soeters PB. Plasma albumin: a confusing nutritional measure. Nutrition 1990;6(3):267

Chapter 4

Topic 10

Nutritional Support in Paediatric Patients

Nutritional Support in Paediatric Patients

Topic 10

Module 10.2

Parenteral Nutrition in Paediatric Patients

Georgi Paunov

Learning Objectives

To define the principles and indications for PN in children;
To summarize the physiological characteristics of the children in different ages and their influence on PN;
To give age appropriate standard schemes for nutrient dosage;
To give age appropriate standards for metabolic monitoring;
To discus the reasons and mechanisms of possible complications. To give recommendations for prevention;
To give practical tips for drawing up age appropriate balance of nutrients, water and electrolytes.

Contents

1. Basic principles of parenteral nutrition (PN)
   - 1.1 Indications for PN
   - 1.2 When to start PN?
   - 1.3 Some technical aspects of PN
2. Elements of PN
3. Monitoring water balance
4. Monitoring of PN
5. Complications of PN
   - 5.1 Catheter related complications
   - 5.2 Metabolic complications of PN
   - 5.3 Hepatobiliary complications
6. Drawing up the plan for PN
   - 6.1 Estimation of energy needs
   - 6.2 Macronutrient dosage
   - 6.3 Vitamin and trace element dosage
   - 6.4 Water and electrolyte balance
   - 6.5 Gradual adaptation

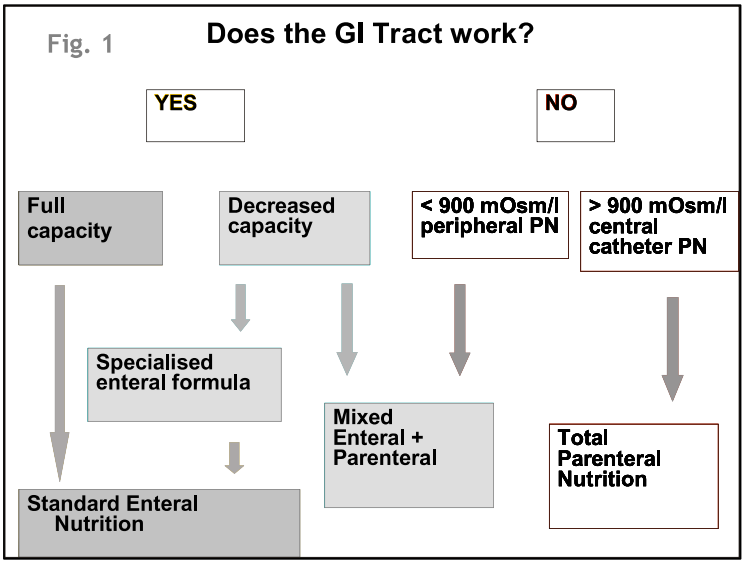
Key Messages

Parenteral nutrition of paediatric patients is based on the same principles as in adults;
Along with metabolic recovery and maintenance PN must provide suficient amounts of protein and energy for growth and development of the children of all ages;
Nutrition body stores (fat and muscle tissue) in neonates and especially premature/Low Birth weight (LBW) are much lower than in the adults. That's why they are very sensitive to the deficit of protein and energy;
Macro-and micronutrient balance is made according to standard, age appropriate schemes. Metabolic monitoring is used to personalize it for each patient;
Compared with the adults the greatest anatomical and physiological differences are found in term and preterm neonates. These differences concern all organs and systems.

1. Basic principles

Administration of all necessary nutrition elements intravenously, with no involvement of gastrointestinal tract, is defined as Parenteral Nutrition (PN).
PN of paediatric patients is based on the same principles as in the adults. The special features are determined by the physiological differences of the children in the different age periods. Along with metabolic recovery and maintenance PN must provide sufficient amounts of protein and energy for growth and development of the children of all ages.

1.1 Indications for PN



When gastrointestinal tract does not work. The whole amount of nutrients is delivered intravenously Total Parenteral Nutrition (TPN);
Nutrient needs exceed gastrointestinal capacity of the patient. It happens when gastrointestinal function is impaired, metabolic needs are increased or combination of both. Enteral nutrition must be preserved in maximal degree and parenteral nutritional support must be given;
Praematurity and/or weight at birth less than 1500 g. Parenteral nutritional support is strongly recommended parallel with gradual adaptation of the gastrointestinal function.

Reasons:

Protein and energy requirements of preterms and LBW infants, calculated per kg body weight, are much higher than in the older children;
Enteraly delivered nutrients are not sufficient due to the immaturity of gastrointestinal absorption. The gastrointestinal capacity depends on the degree of prematurity.

1.2 When to start PN?

The decision for starting PN depends on several factors:

Age of the child. It determines the protein and energy requirements and the body nutritional reserves (muscle and fat tissue). Premature infants have lower nutritional body stores, sufficient for 4 days (body weight ~ 1000 g) up to 12 days (body weight ~2000 g) in full starvation. Nutritional stores of full term infants are sufficient for 40 - 50 days of full starvation with adequate water and electrolyte provision (10, 11);
Nutritional status at the moment the presence and the degree of malnutrition;
Disease factor - includes the main disease and all medical problems that influence the nutrient balance.

Some practical guidelines can be drawn from the combination of these factors:

In LBW/preterm infants with medical problems that influence nutrition TPN must be started from the first day of admission if they will not be fed enteraly for 3 or more days, but not earlier than 24 h after birth. The exception is made for the glucose. Infusion of 7,5% to 12.5% solutions must be started immediately after birth considered with the ability of enteral provision of water and nutrients;

In healthy LBW/preterm infants less than 1500 g PN support must be started 24 h after birth considered with the enteral nutrition abilities of each patient;
In full term infants and older children TPN must be started if they will not be fed enteraly for 5 or more days;
In presence of malnutrition, regardless the age, TPN or PN support must be started immediately;
In critically ill patients TPN or PN support must be started after achieving respiratory and haemodynamical stability (including mechanical ventilation and inotropic support).

1.3 Some technical aspects of PN

Throgh central venous catheter. It is recommended for TPN especially longer than 5 days;
Through peripheral vein catheter. It is recommended when the osmolality of the nutrient solutions is less than 900 mOsm/l (usually for short term TPN or PN support of enteral nutrition);
Continuous PN - the whole amount of nutrients is infused with constant rate for 24 h;
Cyclic PN - the nutrients are infused in one or several intervals during the day and night. It can be applied only after the complete adaptation of the patient to the whole planned amount of nutrients. It is convenient for mobile patients and for home PN.

2. Elements of PN

Water,
Electrolytes: Na, K, Mg, Ca, P,
Carbohydrates,
Proteins as amino acid solutions,
Lipids,
Vitamins,
Trace elements.

2.1 Carbohydrates - glucose

Glucose is the main parenteraly used carbohydrate;
Glucose is used universally from all body cells. It is the main source of energy for the cells of CNS, bone marrow and renal medulla. These tissues are defined as glucose dependent;
Energy content ~ 4 kCal/g;
Available in different solutions from 5% to 40%. 5% to 10% solutions are suitable for peripheral vein administration;
Doses are correspondent to the energy requirements of the children in different ages. They can vary from 5 - 7 g/kg/24 h (3.5 - 5 mg/kg/min) in older children up to 14 - 18 g/kg/24 h (10 - 13 mg/kg/min) in preterm neonates (Table 1);
Hyperglycemia in the preterm infants is defined as blood glucose levels above 8 mmol/l (150 mg/dl). The risk for development is proportional to the degree of prematurity. Possible reasons might be saturation of the insulin receptors and immaturity of hepatic and pancreatic response. In very premature infants glucose infusion rates greater than 8 mg/kg/min (11.5 g/kg/24 h) are connected with high incidence of hyperglycemia. This rate is often not sufficient to cover the caloric needs of these patients and insulin may be needed for additional glucose administration. Insulin infusion of 0.01 - 0.1 U/kg/h is used to maintain blood glucose between 5.5 and 8 mmol/l (100 - 150 mg/dl). Blood glucose must be monitored at least every 2 hours (9).

2.2 Protein - amino acids

Nutritional value of the amino acid (AA) solutions is determined by the amount of the essential AA, the nonessential AA composition and total nitrogen content. Adult AA compositions can be used safely in children older than 9 - 12 months.

Table 1 Parenteral daily macronutrient requirements of patients from different ages

NUTRIENTS	DOSES - g/kg/24h					
	ADULTS	CHILDREN				
		premature	full-term infants up to 1 year	1 - 7 years	8 - 12 years	13 - 18 years
AMINO ACIDS	0.8 - 2.0	2.5 - 3.5	2.0 - 3.0	2.0 - 2.5	1.5 - 2.0	1.0 - 2.0
GLUCOSE	2 - 5	15 - 18	12 - 15	9 - 12	6 - 9	4 - 7
LIPIDS	0.5 - 2.0	to 3.0	to 3.0	to 3.0	to 2.0	to 2.0

Specialized paediatric AA compositions are recommended for patients younger than 9 months and especially for preterm/LBW infants. They have lower capacity of some liver and renal enzyme systems that are involved in AA metabolism.
Recent investigations give some recommendations for the specialized neonatal AA solutions:

Lower pH ensures better solubility of Ca and P;

Taurine addition. It is semiessential for the neonates. Taurine is essential membrane stability factor for the myocardial cells and acts as an antioxidant. Its metabolism gives lower amounts of hepatotoxic bile acids. Taurine deficiency is associated with higher risk of retinal and CNS development problems in preterm/LBW infants;

Tyrosine and histidine addition. They are semiessential for the preterm/LBW infants;

Cysteine addition and lower amounts of methionin. Capacity of renal and liver cystathyonine synthethaze that transforms methionin to cysteine is still not sufficient. In preterm/LBW and newborn infants high methionin levels may cause mental retardation;

Branched chain AA addition. Some investigations have found that addition of leucine, isoleucine and valine lowers the incidence of apnea in the premature infants. Lower plasma levels of branched-chain AA, arginine and cystine have been found in neonates with pulmonary hypertension;

Lysine addition is a factor for protein synthesis promotion;

Lower amount of phenylalanine and proline. High plasma levels impair liver functions;

Lower amount of glycine. It is hepatotoxic when conjugated with bile acids;

Lower amount of tryptophane. Concentrations in adult AA compositions are too high for the neonates and may cause cholestasis.

Energy content of the AA is ~ 4 kCal/g. They must not be included in the balance of energy. Provision of 130 - 150 nonprotein kCal/1g N is necessary for achieving positive nitrogen balance.
Doses vary with age between 1.5 g/kg/24 h for children older than 10 years and 3.5 g/kg/24 h for the preterms (Table 1).

2.3 Lipids

Lipid emulsions
Lipids are administered parenteraly in the form of 10% and 20% emulsions. They may be composed from long-chain fatty acids (Long Chain Triglycerides (LCT)) or combined with middle-chain (LCT/MCT).
Energy content of LCT is ~ 9 kCal/g. For MCT it is ~ 8 kCal/g.
Including the lipids in the PN improves the respiratory quotient. This is of significant benefit for patients with altered respiratory function and/or low physiological respiratory reserves (like neonates and infants up to 1 year).
Doses - see in Table 1.

Mixed LCT/MCT are considered to have some advantages over pure LCT emulsions. They are:

- Lower incidence of liver dysfunctions;
- Improve the immune functions;
- Maintain normal ω-3/ω-6 Polyunsaturated Fatty Acids (PUFA) ratio in the cell membranes (MCT effect);
- More stable in "All-in-one" compositions;
- MCT have better clearance. Their transport in the cells does not depend on the carnitine system;
- MCT have lower affinity to the albumin;
- LCT/MCT emulsions provide the essential linoleic and linolenic fatty acids (they both are LCT);
- Their deficiency can impair the immune function and cause growth retardation.

Parenteral use of lipids in preterm/LBW neonates

This topic remains debatable. According to some authors parenteral administration of lipids is associated with great risk of complications due to the immaturity of various metabolic systems in the preterm/LBW neonates. Others consider lipid emulsions as mandatory part of TPN regimen as a source of substances of crucial importance for growth and development of the preterm/LBW neonates.

The cons:

Limited lipoprotein lipase activity impairs the clearance of the parenteral lipids.

Administration of lipid emulsions in preterm/LBW neonates may be associated with severe lung injuries (even need of mechanical ventilation) with long-term consequences. Several mechanisms are supposed to be involved:

- Lipid deposition in the pulmonary capillaries which impairs O_2 diffusion;
- Impairment of the lymphatic drainage which may lead to pulmonary edema;
- Alteration of prostaglandin metabolism, which may lead to pulmonary hypertension;
- Direct toxicity of lipid peroxides.

Plasma lipids compete for albumin binding sites with the bilirubin and increase it's free fraction.

Larger amounts of lipids may suppress the immune functions and increase the risk of infections.

The pros:

Parenteral lipids are the only source of essential fatty acids for the patients on TPN. They provide also some lipid soluble vitamins (Vit E). Lipids are main source of energy. They allow to be covered the needs of preterm/LBW neonates avoiding the risks of high glucose administration.

Nutritional committee of American Academy of Paediatrics gives the following recommendations for parenteral use of lipids in preterm/LBW neonates:

- Parenteral lipid dose must not exceed 3 g/kg/day. It must be infused with constant rate for 24 h;
- This maximal amount must be decreased in cases of infections, impaired respiratory function and hyperbilirubinemia. The exact dose must be determined according to the patient condition;
- Individual lipid tolerance should be assessed using the plasma triglyceride levels.

Added to all-in-one solutions, heparin stabilizes lipid emulsions and improves plasma lipid clearance. If there are no contraindications doses 0.5 - 1 IU/ml of are used.

2.4 Vitamins

Vitamins and trace elements are of great importance for the children of all ages because of the intensive metabolic activity, growth and development of all organs and systems. Guidelines for paediatric PN administration of vitamins and trace elements are build up long time ago and despite the subsequent investigations have not been revised.

Classified by solubility, the lipid-soluble vitamins A, D, E, and K have the potential for storage and therefore the potential for toxicity. The water-soluble vitamins like ascorbic acid and the B-complex vitamins cannot be stored in the body. They are considered relatively nontoxic and are

excreted when administered in excess. That's why their deficiency can develop more rapidly. Vitamins addition is mandatory element in the PN regimens for the children of all ages.
The most part of the vitamins are now commercially available in modern multivitamin preparations. Preterm infant preparations should be formulated without propylene glycol or polysorbate, which may be found in adult formulations, to avoid potential toxicity related to immature metabolism.
In the neonatal period addition of some vitamins is of special importance. These are:

Vit K - The own synthesis of vit K is limited in the neonates due to the immaturity of the liver enzyme systems. They reach the adult's capacity about 1 month of age (for the full terms). This relative deficiency has no clinical expression in the healthy neonates. All critical states like severe operations, infections, cardiac and respiratory problems can aggravate it and lead to severe coagulation disorders. That's why vit K supplementation is very important for all neonates on TPN;

Vit E - It is one of the most powerful antioxidants and an important erythrocyte membrane stability factor. It is supposed that vit E supplementation in the neonates and especially in preterms decreases the risk of bronchopulmonal dysplasia and retrolental fibroplasia development. Except in standard formulations vit E can be found in the lipid emulsions.

2.5 Trace elements

Trace elements supplementation is especially important in the long-term TPN. Trace elements generally function as prosthetic groups of various enzymes. Zinc is an important factor of protein synthesis and sexual maturation. Its deficiency is associated with growth retardation and hypogonadism. Routine addition of zinc, copper, selenium, chromium, and manganese to PN solutions is recommended to avoid deficiency. Although not a conventional addition, molybdenum supplementation may be appropriate in long term TPN therapy.
Special paediatric trace elements compositions are used for infants and children up to 10 years. Trace elements preparations for adults are appropriate for children older than 10 years.
Additional zinc supplementation is necessary in conditions of increased losses, such as persistent diarrhoea excessive ileostomy drainage (21, 22). Copper and manganese should be administered cautiously, if at all, in patients with impaired biliary excretion or cholestatic liver disease (23, 24, 25, 26). Because of renal excretion of these elements, dosage reduction for selenium, molybdenum, and chromium should be evaluated in patients with renal dysfunction (21, 22). In long-term TPN patients plasma trace elements levels must be monitored periodically.
Doses see in Table 2.

Table 2 Parenteral daily trace element requirements of patients from different ages

Element (μg/kg/24 h)	Premature babies	Full-term babies and infants > 1 year	Older children
Zinc	400	250 < 3 months 100 > 3 months	50 (max. 5000 /24 h)
Copper	20	20	20 (max. 3000 /24 h)
Selenium	2.0	2.0	2.0 (max. 30 /24 h)
Chrome	0.20	0.20	0.20 (max. 5.0 /24 h)
Manganese	1.0	1.0	1.0 (max. 50 /24 h)
Molybdenum	0.25	0.25	0.25 (max. 5.0 /24 h)

2.6 Electrolytes

Water and electrolyte balance of paediatric patients is based on the same principles as in the adults. There are two components:

Physiological needs;
Pathological loses.

Sodium
Sodium administration can be started 24 h after birth. Physiological needs are considered to be 2 - 4 mmol/kg/24 h. It is provided with crystalloid, AA solutions and blood products.

Potassium
Potassium administration can be started at the second or third day after birth. Plasma levels below 5.5 mmol/l, stable diuresis and normal pH may be used as criteria for K^+ administration. Physiological needs are considered to be 2 - 2.5 mmol/kg/24h.

Calcium
In preterm and term neonates calcium is an important factor for myocardial contractility and nerve impulse conduction. Body stores are limited and plasma concentration is strongly dependant on the external intake. Ca^{2+} provision must be started from the first day after birth. Neonates and infants have higher rates of growth and great amounts of Ca^{2+} that are deposed in the bones. That's why their daily requirements of Ca^{2+} are higher than in the older children and adults.

Magnesium
Its administration can be started at the second or third day after birth. The needs are about 0.3 - 0.4 mmol/kg/24 h for neonates and infants up to 1 year and 0.2 - 0.3 mmol/kg/24 h for older children. Persistent diarrhoea and long-term medication with diuretics, aminoglycosides and amphoteracine-B can cause magnesium deficiency. Hypomagnesaemia can be expected in renal insufficiency and in newborn babies of preeclamptic mothers, treated with $MgSO_4$. Hypermagnesaemia is usually associated with elevated parathormone levels.

Phosphorus
Phosphorus is a key factor in various physiological processes like ATP synthesis and bone mineralization. Usually its administration can be started at the second or third day after birth. Neonates and infants need 0.5 - 1.0 mmol/kg/24 h (up to 1.34 mmol/kg/24 h in critical illness (8)). Older children need 0.3 - 0.5 mmol/kg/24 h. Doses see also in Table 3.

Table 3 Parenteral daily electrolyte requirements of patients from different ages

Electrolytes	0 – 10 years	11 – 18 years
Cl^-	3 – 4 mEq/kg/24 h	3 – 4 mEq/kg/24 h
PO_4^{3-}	1 – 2.0 mmol/kg/24 h	0.5 – 2 mmol/kg/24 h
Ca^{2+}	1 – 3 mEq/kg/24 h	0.25 – 0.5 mEq/kg/24 h
Mg^{2+}	0.25 – 0.5 mEq/kg/24 h	0.25 – 0.5 mEq/kg/24 h

3. Monitoring water balance

3.1 Physiological needs

Like in the adults they include urine, insensible and stool loses. Some important physiological characteristics of the preterm and term neonates must be kept in consideration in the balance of water and electrolytes.

3.2 Renal function

Urine water loses consist about 60% of the physiological needs.

At birth full term babies have the full number of nephrons, which is completed about 32-34 week of gestation;

At birth about 6% of the cardiac output is distributed to the kidneys (versus 20% in the adults). This part increase significantly from 24 to 48th hour. This is the reason of the low urine output in the first 24 hours after birth. In that period oliguria must not be treated aggressively with infusions and diuretics;

Dilution segment in the distal part of Henle's loop is well developed in full term neonates and they can successfully eliminate water;

The ability of urine concentration at birth is about a half of the adult's. That's why neonates cannot excrete sodium so well and are at risk of overload. Concentration ability of the kidneys approaches the adult's capacity between 2 and 6 moths of age;

In preterm neonates the immature Na^+ reabsorption mechanism keeps them in the state of isostenuria (about 500 - 600 mOsm/l) and makes them equally sensitive to Na^+ overload and deficiency;

Potassium preterm and term neonates are at risk of hyperkalemia due to the low glomerular filtration. In contrast with the adults they tolerate it quite well and plasma concentrations of 6 - 8 mmol/l have no clinical expression. In such cases no active treatment except intake limitation is needed;

Calcium is very important in the first months of life. The reasons were discussed above. That's why the daily-required amounts must be provided regularly in all neonates and infants up to 1 year of age.

3.3 Perspiratio insensibilis

In all neonates this mechanism is responsible for significant water loses, consisting 30 - 40% of the physiological needs. The amount of water loses through the skin and respiratory system is correspondent to the degree of praematurity. It is determined by several physiological and anatomical characteristics of neonates:

The amount of total body water and structure of its distribution (Table 4);

Small size;

Larger body surface area related to 1 kg of body weight;

Thinner skin with better perfusion, less adipose tissue and higher heat conductivity;

High respiratory rate;

Water loses with the stools up to 5 - 10% of the physiological needs.

Table 4 Total body water amount and distribution in children from 0 to 14 years

AGE	CARDIAC OUTPUT ml/kg/min	ECF AS % OF BODY WEIGHT	ICF AS % OF BODY WEIGHT	TOTAL BODY WATER AS % OF BODY WEIGHT	BODY FAT AS % OF BODY WEIGHT
Premature babies	> 200	~ 40 - 60	~ 25 - 30	~ 80 - 90	0 - 10
Full-term babies	~ 150 - 200	~ 40	~ 30	~ 80	~ 15 - 17
~ 2 - 3 months		~ 35	~ 35	~ 70	~ 20
~ 9 - 10 months		~22 - 25	~ 40	~ 60 close to the adult's	~ 22 - 25
6 - 14 months	~ 90 - 100	~ 20	~ 45		
adults	~ 70 - 80				

3.4 Pathological losses

Febrility - ~1ml/kg/h for each degree above 38°C axilary temperature;
Gastrointestinal drainage;
Drainage of body cavities;
Diarrhea and vomiting. Special attention must be paid to these two mechanisms of water and electrolyte loses in paediatric patients. According to WHO statistics severe dehydration due to gastrointestinal infections causes the death of 7 to 20 millions of children all over the world each year. A severe viral or bacterial gastroenteritis can lead to 10 - 20% dehydration in a small child for several hours;
Third space loses;
Abdominal wall defects (omphalocelle, gastroschisis). Water and electrolyte loses in such cases cannot be directly measured. Rehydration must be adjusted to the clinical criteria of normovolemia;
Blood loses;
Burns.

3.5 Evaluation of water loses

Most accurate and useful for practice remain the clinical signs of hydration (Table 5):
Signs of hemodynamic (heart rate, capillary refill, diuresis);
Skin, mucous membranes, tongue;
Fontanelle;
Ophthalmic bulbs;
Mental status.

Table 5 Dehydration assessment in neonates and children up to 3 years

CLINICAL SIGNS	MILD DEHYDRATATION	MODERATE DEHYDRATATION	SEVERE DEHYDRATATION
ACTIVITY	normal	lethargy or agitation	lethargy or coma
SKIN COLOUR	pale	gray	marbeled
DIURESIS	< 2 – 3 ml/kg/h	oliguria < 1 ml/kg/h	anuria
FONTANELLE	flat	slightly below parietal bones	below parietal bones
MUCOUS MEMBRANES	dry	very dry	cracked
SKIN TURGOR	slightly decreased	decreased	wrinkled
HEART RATE	normal or slightly elevated	elevated	significantly elevated
BLOOD PRESSURE	normal	normal	low
WEIGHT LOSS	5 %	10 %	15 %

4. Monitoring of PN

Metabolic monitoring is the main instrument for adjustment of PN according to individual patient needs and condition. Monitored parameters:

4.1 Blood glucose

It must be kept in physiological limits. Insulin addition is rare necessary predominantly in children older than 12 - 13 years. In neonates blood glucose must be measured at least 2 - 3 times a day or more often if necessary. A single measurement is sufficient for older children.

4.2 Plasma triglyceride concentration

It can give information about the tolerance to provided lipid emulsions. It may be also a sign of glucose overload.

4.3 Urine analysis

Presence of glucose is a sign of glucose overload.

4.4 Nitrogen balance

Different equations can be used for calculation of nitrogen balance, but it cannot provide useful information for the daily practice due to several reasons:

Absolute amount of nitrogen loses can never be measured exactly. It is estimated with certain degree of assumption. It's proven that nitrogen excretion can show great day-to-day variations even in a same patient;

Nitrogen loses are measured by the urea excretion in the urine, which is assumed to be about 85% of total nitrogen loses. The other 25% are lost by the free ammonia (which can have great variation in burns and other catabolic states) and by the stools.

4.5 Urea, creatinine, AST, ALT, AP and bilirubin

Urea and creatinine can give information mainly about renal function, but not for the degree of catabolism. Rapid elevation of blood urea in neonates and especially in preterms on PN, despite the adequate diuresis, can be a sign of AA overload;

AST, ALT, AP and bilirubin can refer to developing liver complications. Their changes must be interpreted in consideration of overall patient condition. In general they can be measured once a week.

4.6 Plasma total protein and albumin

They don't give information about the efficiency of provided PN. Albumin half-life of degradation is about 18-20 days. The rapid decrease of albumin plasma levels in critical conditions is a result of distribution through the capillary walls towards the extracelular space. In such cases hypoalbuminemia cannot be corrected with PN or albumin supplementation. That's why albumin must be considered as negative acute phase protein.

5. Complications of PN

Complications of PN in children can be divided in three groups similar to the adults: metabolic, catheter related and others (intestinal mucose atrophy, thrombocytopenia, anemia). Generally metabolic complications are caused by insufficient administration or overload of certain nutrients.

5.1 Catheter related complications

Similar with the adults. Special attention must be paid to the technical difficulties of central vain canulation, caused by the small size of the preterm and term neonates and the need of general anesthesia for catheter insertion in al children from 0 to 10 - 12 years of age. Peripherally inserted central catheters are widely used in the neonatal practice in the recent years. Usually they are instilled through some of the cubital veins. Their placement is connected with lower risks. Higher incidence of occlusion is due to the small diameter. This complication can be avoided with more accurate care and control of the catheters. Heparin addition to the PN solutions (0.5 - 1 UI/ml) is almost routine for the neonatal patients.

5.2 Metabolic complications of PN

Glucose overload

Hyperglycemia with osmotic diuresis, which can cause dehydration;

Elevated production of CO_2 with respiratory quotient change, which can aggravate imminent ventilatory insufficiency (preterm/LBW infants) or may delay weaning of ventilator;
Induction of lipogenesis with elevated plasma triglycerides. Lipid infiltration of the liver and cholestasis.

Rapid complications of parenteral lipid administration are rare. They do not depend on the infused amounts and have allergic genesis. Such can be:

Erythema and eruptions;
Fever;
Back pain;
Anaphylactic reactions.

Lipid overload can develop due to large doses of lipid emulsions or high infusion rate more than 0.2 g/kg/h (similar to the adults). It can lead to:

Hyperlipidemia;
Hepato and splenomegalia;
Coagulation disorders;
Competitive displacement of direct bilirubin from albumin binding sites;
Impaired function of neutrofil granulocytes. It can also be caused by essential fatty acid deficiency;
Large amounts of phospholipids decrease lipoprotein lipase activity thereby lowering the plasma lipid clearance. Phospholipids are added to stabilize the lipid emulsions. Their concentrations in 10% and 20% emulsions are close therefore the use of 10% emulsion can easily cause elevated plasma levels of phospholipids, cholesterol and triglycerides (phospholipids content in 10% emulsion - 8 g/l, in 20% - 12 g/l). Phospholipids reduced (PLR) 10% emulsions are recently developed of avoid these problems.

Complications of parenteral AA administration
A lot of publications in the 80s of the last century report high incidence of PN-associated cholestasis with elevated direct bilirubin and AP in neonates on TPN who received high doses of AA (above 4 g/kg/24 h). Lack of taurine and high glycine concentrations are supposed to be involved in these complications. Administration of 3 - 4 g/kg/24 h AA is well tolerated even from VLBW infants (2, 3). Some authors report development of azotemia, hyperthermia and neurodevelopment retardation in preterm neonates who received 6g/kg/24 h AA.

Hepatobiliary fatty infiltration
Systematic overadministration of macronutrients (glucose, AA and lipids) are considered as the main reason of this complication. All of them can be metabolized to triglycerides and except in adipose tissue are stored in the hepatocytes.

Cholestasis
Exact reasons of cholestasis are not absolutely clear. Similar to the adults, several mechanisms are supposed to take part:

Absence of food in the intestinal lumen decreases the production of gastrointestinal cholecystokinines;
Venous stasis in the GI tract impairs the liver perfusion;
Trace element contamination of PN solutions (especially aluminum in the AA solutions);
Some guidelines are developed to decrease the risk of hepatobiliary complications;
Avoid the overfeeding;
Minimal enteral nutrition should be started in earliest possible moment;
When possible cyclic PN should prefer to continue 24 h administration;
Ursodesoxyholic acid can be used to decrease serum level of bilirubin.

6 Drawing up the plan for PN

6.1 Estimation of energy needs

Energy needs estimation is the first step of the PN planning. Against the expectations energy requirements in severely ill paediatric patients on TPN are not much elevated.

Numerous clinical trials report great difference between predicted and measured energy expenditure. Energy needs based on the common equations and corrected considering severity of the illness, activity, body temperature and growth proved to be much higher than the actual, measured by indirect calorimetry. The real energy consumption turned out to be close (±10%) to the age related Resting Energy Expenditure. In common children on TPN are in thermoneutral conditions in PICU and have low physical activity (some are sedated or ventilated).
We offer two schemes with age appropriate equations for energy needs estimation. Table 6 (13) and Table 7 (15).

Table 6 Basal Metabolic Rate equations for patients from 0 to 30 years (13)

GENDER	AGE (years)	kCal/day (T = body weight in kg)
MALE	0 - 3	60.9 x T - 54
	3 - 10	22.7 x T + 495
	10 - 18	17.5 x T + 651
	18 - 30	15.3 x T + 670
FEMALE	0 - 3	61.0 x T - 51
	3 - 10	22.5 x T + 499
	10 - 18	12.2 x T + 746
	18 - 30	14.7 x T + 496

Table 7 Basal Metabolic Rate equations for patients from 0 to 30 years (MJ/day*). (15)

AGE (years)	MALE	FEMALE
< 3	0.249 x T - 0.127	0.244 x T - 0.133
< 3	0.0007 x T + 6.349 x L - 2.584	0.068 x T + 4.281 x L - 1.730
3 - 10	0.095 x T + 2.110	0.085 x T + 2.033
10 - 18	0.074 x T + 2.754	0.056 x T + 2.898
18 - 30	0.063 x T + 2.896	0.062 x T + 2.036

T - body weight in kg
L - body length in meters (for children less than 3 years)
* to transform the results in kCal/day, multiply by 239

6.2 Macronutrient dosage

There are a lot of schemes and tables developed for macronutrient dosage. They are all similar and should be used for orientation. They also give the maximal safe doses of nutrients. The approach to every patient must be individual, based on his age, overall condition, main disease and coexisting medical problems.

It's more appropriate to calculate the energy in non-protein calories, based on glucose and lipids. They can be combined in different ways. In common practice glucose: lipid ratio varies from 70:30 to 50:50.

6.3 Vitamin and trace element dosage

Vitamin and trace elements dosage recommendations for paediatric patients are less clear than for the adults. Micronutrient provision is better made with commercial polivitamin and trace elements compositions. They are usually designed in two types for children from 0 to 10 years and for older children and adults. Following the producer's dose recommendations will provide the required amounts of micronutrients with a minimal risk of overdosage. Vitamin and trace mineral supplementation recommendations are provided below for stable paediatric patients and do not account for conditions of catabolic stress, ventilatory support, or organ dysfunction.
In such cases supplementation must be designed individually according to the patient condition. Recommended intakes for preterm infants are different than those for the older children and the adults.

6.4 Water and electrolyte balance

Special physiological characteristics of the children in different ages are summarized in one approved and widely used scheme for calculation of physiological daily requirements of water (Table 8).

Healthy full term infants, older children and adults

2 - 10 kg	4 ml/kg/h (100 ml/kg/24 h)
10 - 20 kg	(40 ml+2ml/kg)/h
above- 20 kg	(60ml+1ml/kg)/h

Healthy preterm/LBW infants

800 - 1000 g	5.5 ml/kg/h
1000 - 1250 g	5.0 ml/kg/h
1250 - 2000 g	4.5 ml/kg/h

Estimated pathological loses for the day are added to the calculated physiological needs. Correction of existing deficits must be performed gradually for 24 - 48 h.

Table 8 Parenteral daily electrolyte requirements of premature babies

Days after birth	WATER (ml/kg/d)				SODIUM (mEq/kg/d)				POTASSIUM (mEq/kg/d)
	Less than 1kg	1 to 1.5 kg	1.5 to 2.5 kg	Over 2.5 kg	Less than 1kg	1 to 1.5 kg	1.5 to 2.5 kg	Over 2.5 kg	For all weights
1	100	80	60	60	0	0	0	0	0
2	120	100	90	90	5	4	3	1	0 - 2
3	150	130	120	110	5	4	3	1	2 - 3
4	180	150	150	130	5	4	3	1	2 - 3
5	200	180	170	150	5	4	3	2	2 - 3
6	200	180	170	150	5	4	3	2	2 - 3
7	200	180	170	150	5	4	3	2	2 - 3
8 - 13	200	180	170	150	5	4	3	2	2 - 3
14 - 20	180	160	150	150	4	3	2	1	2 - 3
21 - 27	160	160	150	150	3	2	2	1	2 - 3
> 28	160	150	150	150	2	2	2	1	2 - 3

6.5 Gradual adaptation

Adaptation of all metabolic systems to the parenteral intake of nutrients is a gradual process that needs time. At least 5 days are considered as sufficient for children younger than one year. For the older children this period is 3 days. Entire calculated amounts of nutrients are considered as 100%. We offer a model scheme of gradual 3 and 5 days adaptation of macronutrient provision (Table 9) during the adaptation period and few days after; following blood glucose, triglycerides, electrolytes, urea, creatinine, urine glucose and acid/base balance.

Table 9 Parenteral daily vitamin requirements of children from different ages

VITAMIN	PREMATURES	INFANTS	CHILDREN
Vitamin A (μg)	75 - 300	300 - 750	450 - 1000
Vitamin D (g)	200 - 500	100 - 1000	200 - 2500
Vitamin E (mg)	3 - 15	3 - 10	10 - 15
Vitamin K (μg)	5 - 80	50 - 75	50 - 70
Vitamin B_1 (mg)	0.1 - 0.5	0.4 - 0.5	1.5 - 3
Vitamin B_2 (mg)	0.15 - 0.30	0.4 - 0.6	1.1 - 3.6
Vitamin B_5 (mg)	0.4 - 1.5	2 - 5	0.5 - 5
Vitamin B_6 (mg)	0.08 - 0.35	0.1 - 1.0	1.5 - 2
Vitamin B_{12} (μg)	0.3 - 0.6	0.3 - 3	3 - 100
Vitamin C (mg)	20 - 40	25 - 35	20 - 100
Folic acid (μg)	50 - 200	20 - 80	100 - 500
Biotin (μg)	5 - 30	35 - 50	150 - 300
Niacin (mg)	0.5 - 2	6 - 8	5 - 40

Table 10 Gradual adaptation to TPN for children of different ages (the values represent % of total calculated daily requirements)

AGE	AA	GLUCOSE	LIPIDS
Neonates and infants up to 1 year			
I day	30	50	20
II day	50	60	30
III day	70	70	50
IV day	85	85	75
V day	100	100	100
Older children			
I day	50	50	50
II day	75	75	75
III day	100	100	100

References

1. Acra SA, Rollins C. Principles and guidelines for parenteral nutrition in children. Paediatric Annals; Feb 1999; 28; 2.

2. Porcelli PJ, Sisk PM. Increased Parenteral Amino Acid Administration to Extremely Low-Birth-Weight Infants During Early Postnatal Life. Journal of Pediatric Gastroenterology and Nutrition. Feb 2002; 34:174179.
3. Thureen PJ, Melara D, Fennessey PV, and Hay WW, Jr. Effect of Low versus High Intravenous Amino Acid Intake on Very Low Birth Weight Infants in the Early Neonatal Period. Pediatric Research 2003. Vol. 53, No 1.
4. Griffiths RD. Is parenteral nutrition really that risky in the intensive care unit? Curr Opin Clin Nutr Metab Care 7:175181. 2004 Lippincott Williams & Wilkins.
5. Hughes D, Mather S, Wolf A. Handbook of Neonatal Anesthesia. 1996. W. B. Saunders Company Ltd. London.
6. Arnold, CJ et Al. Parenteral nutrition - assotiated cholestasis in neonates: the role of Aluminum, Nutritional Reviews. Washington: Sep 2003.ASPEN Board of Directors and the Clinical Guidelines Task Force Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN. Jan-Feb 2002. Vol. 26, Iss. 1; pg. 1SA.
8. Vileisis RA: Effect of phosphorus intake in total parenteral nutrition infusates in premature neonates. J Pediatr 110:586589, 1987.
9. Copyright American Society for Parenteral and Enteral Nutrition. SECTION XII: ADMINISTRATION OF SPECIALIZED NUTRITION SUPPORT ISSUES UNIQUE TO PEDIATRICS. JPEN, Silver Spring: Jan/Feb 2002. Vol. 26, Iss. 1; pg. SA97.
10. Pereira GR, Zeigler MM. Nutritional care of the surgical neonate. Clin Perinatol 16:233, 1989.
11. Heird WC. Nutritional support of the pediatric patient including the low birth weight infant. In: Winters RW, Greene HC, eds. Nutritional support of the seriously ill patient. New York: Academic Press, 1983;15779.
12. Suita S, Masumoto K, Yamanouchi T, Nagano M, Nakamura M. Complications in neonates with short bowel syndrome and long-term parenteral nutrition. JPEN. 1999, Vol 23, No 5.
13. Irving SY, Simone SD, Hicks FW, Verger JT. Nutrition for the Critically Ill Child: Enteral and Parenteral Support In: Nutrition. AACN Clinical Issues. Vol 11(4) Nov 2000 pp 541-558, 637-638.
14. Sasbon JS, Gardini G. How much energy must we spend to assess the energy expenditure in the critically ill pediatric patient? Pediatr Crit Care Med 2004 Vol. 5, No. 1.
15. Shulman RJ, Phillips S. Parenteral Nutrition in Infants and Children. JPGN 36:587607, 2003.
16. Schofield W. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39C S1;541.
17. Simmer K, Morris S. Lipid infusion rates for parenterally-fed preterm infants [Protocol] Cochrane Neonatal Group Cochrane Database of Systematic Reviews. 2, 2005.
18. Sobotka L. Basics in Clinical Nutrition. 2000 ESPEN. Second Edition.
19. Bistrian BR. Clinical aspects of essential fatty acid metabolism: Jonathan Rhoads lecture. JPEN. Silver Spring: May/Jun 2003. Vol. 27, Iss. 3; pg. 168.
20. Durward A, Mayer A, Skellett S, Taylor D, et al. Hypoalbuminaemia in critically ill children: Incidence, prognosis, and influence on the anion gap. Archives of Disease in Childhood. London: May 2003. Vol. 88, Iss. 5; pg. 419.
21. Greene HL, Hambidge M, Schanler R, et al: Guidelines for the use of vitamins, trace elements, calcium, magnesium, and phosphorus in infants and children receiving total parenteral nutrition: report of the Subcommittee on Pediatric Parenteral Nutrient Requirements from the Committee on Clinical Practice Issues of The American Society for Clinical Nutrition. Am J Clin Nutr. 1988, 48:1324-1342.
22. Leung FY: Trace elements in parenteral micronutrition. Clin Biochem. 1995, 28(6):561-566.
23. Krachler M, Rossipal E, Micetic-Turk D: Concentrations of trace elements in sera of newborns, young infants, and adults. Biol Trace Element Res 1999, 68(2):121-135.
24. Reynolds AP, Kiely E, Meadows N: Manganese in long term pediatric parenteral nutrition. Arch Dis Child. 1994, 71:527.
25. Fell JME, Reynolds AP, Meadows N, et al: Manganese toxicity in children receiving long-term parenteral nutrition. Lancet. 1996, 3471218.
26. Kafritsa Y, Fell J, Long S, et al: Long term outcome of brain manganese deposition in patients on home parenteral nutrition. Arch Dis Child. 1998, 79(3):263-265.

Chapter 5

Topic 24

Nutrition in Metabolic Syndrome

Nutrition in Metabolic Syndrome

Topic 24

Module 24.5

Molecular Aspects of the Metabolic Syndrome

Agnes Namislo
Winrich Breipohl
Brigitte Schmitz
Louise LaJoie

Learning Objectives

What is meant by the metabolic syndrome;
Which function does leptin have;
How does insulin resistance develop;
Which diabetic complications could occur and what are the causes for their development;
How to synthesize O- and N-linked glycoproteins;
Which functions and special characteristics do the enzymes for O-GlcNAc addition and removal have.

Contents

1. Definition and prevalence of the metabolic syndrome
2. Pathogenesis
3. Diseases of the metabolic syndrome - Adiposity and Leptin
4. Diseases of the metabolic syndrome - Hypertension
5. Diseases of the metabolic syndrome - Dyslipidemia
6. Diseases of the metabolic syndrome - Diabetes mellitus type 2 and insulin resistance
7. Diabetic complications and Advanced glycation end products (AGEPs)
8. Glycosylation
9. O-GlcNAc

Key Messages

The metabolic syndrome is a polygenetic disease;
Obesity (Body Mass Index is higher than 30) is the common cause of high triglycerides;
Leptin regulates energy balance;
Renin-angiotensin-system is more active, if subjects are obese;
Hyperglycaemia causes diabetic complications by increased formation of Advanced glycation end products (AGEPs);
Glycosylation is the process of addition of saccharides to proteins and lipids;
Most of proteins synthesized in the rough ER undergo glycosylation;
O-GlcNAcylation occurs in the nucleus and cytoplasm;
Altered O-GlcNAcylation may contribute to the development of metabolic syndrome.

1. Definition and prevalence of the metabolic syndrome

Metabolic syndrome - definition:
A clustering of cardiovascular risk factors that include elevated blood pressure, dyslipidemia (high triglycerides and low high-density lipoproteins cholesterol (HDL-C)), impaired glucose metabolism with insulin resistance and obesity (1).

In 1988 defined by *Reaven;* called variously "syndrome X", "insulin resistance syndrome", "dysmetabolic syndrome" and "Reaven-syndrome".

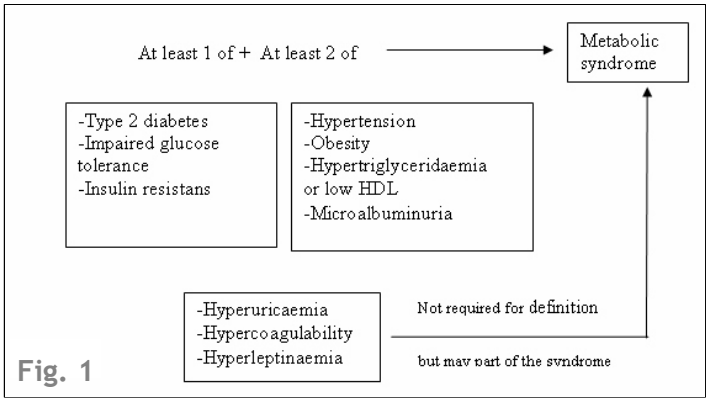


Fig. 1

For the **diagnosis** one disease in the carbohydrate metabolism and two other diseases are necessary. An impaired effect of the hormone insulin is always present.

The WHO and NCEP definitions of the metabolic syndrome

Modified WHO definition	NCEP definition
Hyperinsulinemia (upper quartile of the non-diabetic population) OR fasting plasma glucose ≥ 7.0 mmol/l AND at least **two** of the following: •Abdominal obesity •Dyslipidemia (serum triglycerides ≥ 1.70 mmol/l OR Men: HDL cholestrol < 0.9 mmol/l, Women: HDL cholesterol < 1.1 mmol/l) •Hypertension (blood pressure ≥ 140/90 mmHg or on medication)	At least **three** of the following: •Fasting plasma glucose ≥ 6.1 mmol/l •Abdominal obesity •Serum triglycerides ≥ 1.70 mmol/l •HDL cholesterol: Men: < 1.0 mmol/l Women: < 1.3 mmol/l •Blood pressure ≥ 130/85 mmHg or on medication
Abdominal obesity *Definition 1:* Men: WHR > 0.90 or BMI ≥ 30 Women: WHR > 0.85 or BMI ≥ 30 *Definition 2:* Men: waist girth ≥ 94 cm Women: waist girth ≥ 80 cm	Abdominal obesity *Definition 1:* Men: waist girth > 102 cm Women: waist girth > 88 cm *Definition 2:* Men: waist girth > 94 cm

Fig. 2 **WHO 1999 and NCEP 2001; JAMA; 285; 2486-2497**

WHO = World Health Organization; NCEP = National Cholesterol Education Program;
In the original WHO definition, insulin resistance in the top 25% of the population as measured by the euglycemic hyperinsulinemic clamp was used instead of hyperinsulinemia. Microalbuminuria was also included in addition to abdominal obesity, dyslipidemia and hypertension.

The prevalence of the metabolic syndrome ranges depend on countries and ethnic groups from 1% to 39%. But in all countries the prevalence increases rapidly with age. The following table gives an overview of studies which investigated the prevalence of the metabolic syndrome (24).

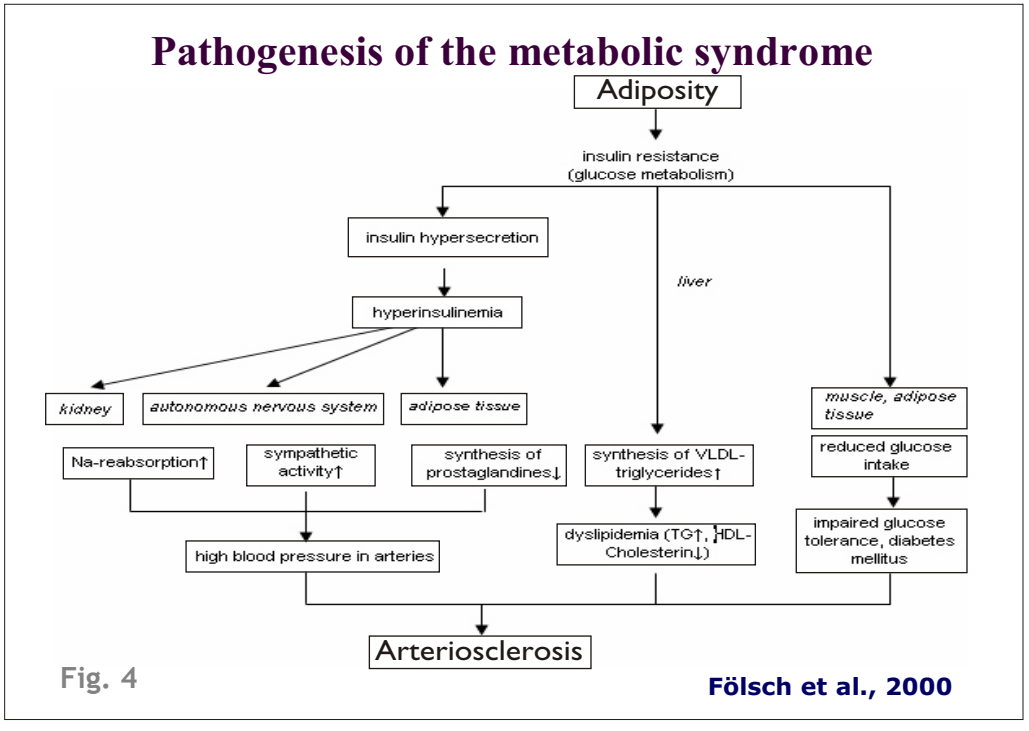
Fig. 3 Prevalence of the metabolic syndrome

Study and Area	Study Population	Prevalence
Hulthe et al., 2000; Arterioscler Thromb Vasc Biol; 20; 2140-7 ***Gothenborg area Sweden***	**362 58-year-old men free of cardiovascular disease and hypertensive medication**	**WHO definition: 16%**
The Third National Health and Nutrition Examination Survey, 2002; JAMA; 287; 356-9 ***United States***	**8608 men and women age ≥ 20 years, studied 1988-1994**	**WHO definition: 24%** **NCEP definition: 25%**
European Group for the Study of Insulin Resistance, 2002; Diabetes Metab; 28; 364-76 ***8 European countries***	**8200 men and 9363 women**	**WHO definition: 7%-36% for men 40-55 years old depending on the country; for women of the same age: 7%-22%**
Kuopio Ischaemic Heart Disease Risk Factor Study, 2002; Am J Epidemiol; 156; 1070-7 ***Eastern Finland***	**1005 42-60 year-old men studied 1984-1989**	**WHO definition: 21%** **NCEP definition: 14%**
Turkish Adult Risk Factor Study, 2002; Artherosclerosis;165; 285-92 ***Turkey***	**2398 men and women, mean age 49.1 ± 13 years**	**NCEP definition: 27% of men and 39% of women**
Women`s Health Study, 2003; Circulation; 107; 391-7 ***United States***	**14719 apparently healthy women age 45 and older participating in an ongoing trial of aspirin and vitamin E in primary CVD prevention**	**NCEP definition: 24%**
Bruneck study, 2003; Diabetes Care; 26; 1251-7 ***Northern Italy***	**888 40-79-year-old men and women**	**WHO definition: 34%** **NCEP definition: 18%**
Al-Jawati et al., 2003; Diabetes Care; 26; 1781-5 ***Oman***	**1419 urban men and women age 20 years and more**	**NCEP definition: 19.5% of men and 23% of women**
Grupta et al., 2003; Diabetes Res Clin Pract; 61; 69-76 ***India***	**1091 urban dwellers age 20 years and more**	**NCEP definition: 7.9% of men and 17.5% of women**
West Of Scotland Coronary Prevention Study, 2003; Circulation; 108; 414-9 ***West Scotland***	**6595 non-diabetic men without CVD (age 55.1± 5.5) who participated in a trial of pravastatin in cardiovascular primary prevention**	**NCEP definition: 26.2%**

Approximately 47 million (24%) of adult Americans have the metabolic syndrome. Over age 60 years, the prevalence raises to 44% (25).

2. Pathogenesis

Insulin resistance is the key factor for the pathogenesis of the metabolic syndrome. This condition is influenced by a complex interplay between multiple genetic variations interacting with numerous environmental factors (27).



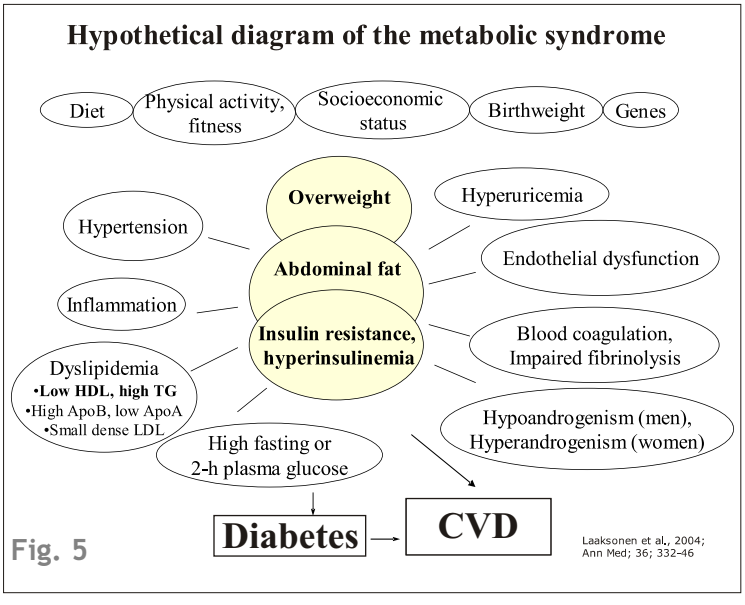
On the top of the following diagram, environmental and genetic factors and their interactions contribute to the pathogenesis of the metabolic syndrome.

Overweight, especially in the presence of environmental and genetic risk factors, leads to abdominal obesity and ectopic fat deposition with consequent insulin resistance.

At the bottom you see end-stage consequences of the metabolic syndrome (24).

Also abnormalities in serum uric acid concentration are found in patients with insulin resistance.

Maybe insulin enhances renal tubular sodium reabsorption, which results in reduced uric acid clearance. This suggests that uric acid levels may also provide insight into identifying individuals with the metabolic syndrome (26).



Obesity is also associated with over expression of tumour necrosis factor-alpha (TNF-α). TNF-α can in turn cause insulin resistance in obese subjects. Insulin has an anti-inflammatory action. These interactions remain to be elucidated (28).

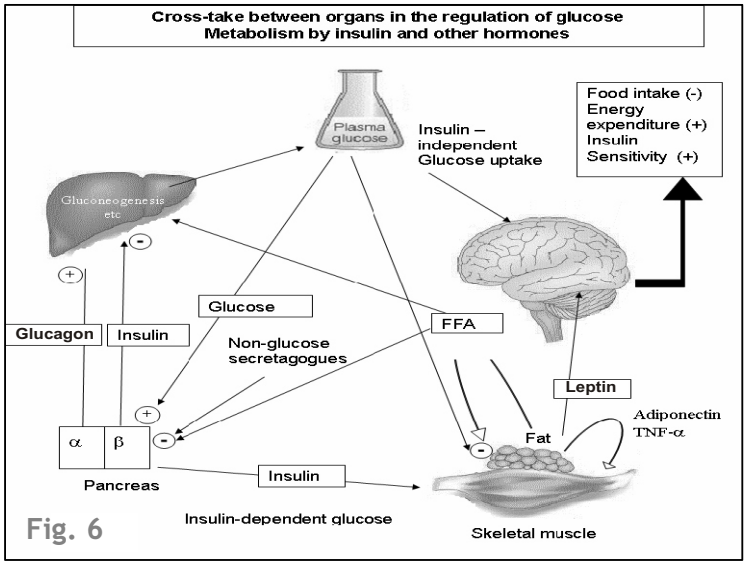


Fig. 6

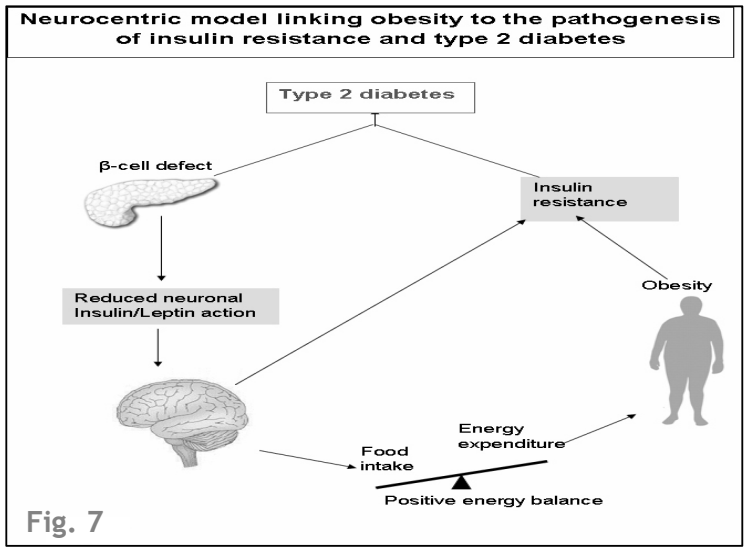


Fig. 7

3. Diseases of the metabolic syndrome - Adiposity and leptin

Definition: An abnormal increase of body fat mass (BMI ≥ 30)

Obesity is a causative factor in the development of metabolic syndrome.

Health risks of obesity:

High blood pressure and stoke are twice as common in obese people;

Evidence is strong that obesity increases the risk of breast cancer (after meno-pause), womb cancer and kidney cancer;

Obesity may also increase the risk of colon cancer;

Gall bladder disease is three times as likely to occur in middle-aged obese women;

Diabetes is four times more common in middle-aged obese people than in middle-aged people of normal weight;

Coronary heart disease is twice as common in obese men under 45;

Osteoarthritis is more painful and less easily treatable if the person is obese;

Severe obesity may cause shortage of breath, varicose veins, backache and even psychological problems.

Leptin:

is a small 16 kDa protein (167 amino acids);

mainly produced in adipocytes;

encoded by the ob-gene (ob = obese), isolated in 1994 by Zhang et al.;

regulates energy balance by acting on the hypothalamus to reduce food intake and to increase energy expenditure via sympathetic activation;

acts through the leptin receptor, a single-trans-membrane-domain receptor of the cytokine receptor family, which is found in many tissues in several alternatively spliced forms;

in the hypothalamus there is a leptin receptor with high affinity for leptin;

the long form of leptin receptor is expressed in pancreatic -cells;

subjects with heterozygous leptin gene mutations have low circulating leptin levels and increased body adiposity;

defects in leptin production cause severe hereditary obesity in rodents and humans;

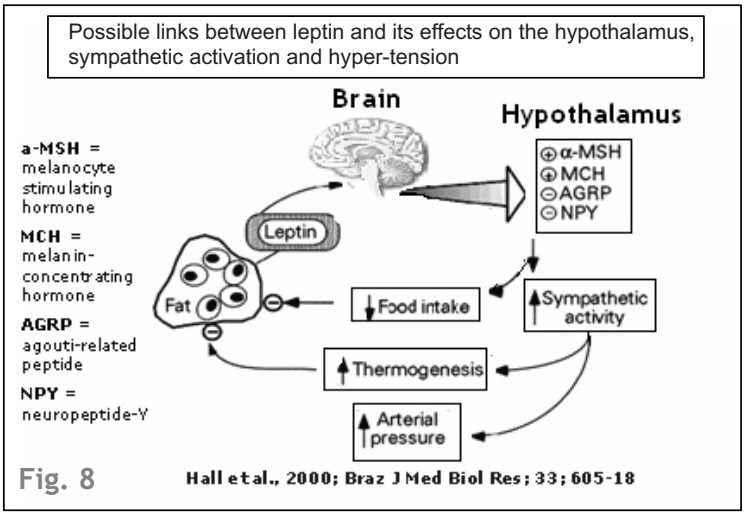


Fig. 8

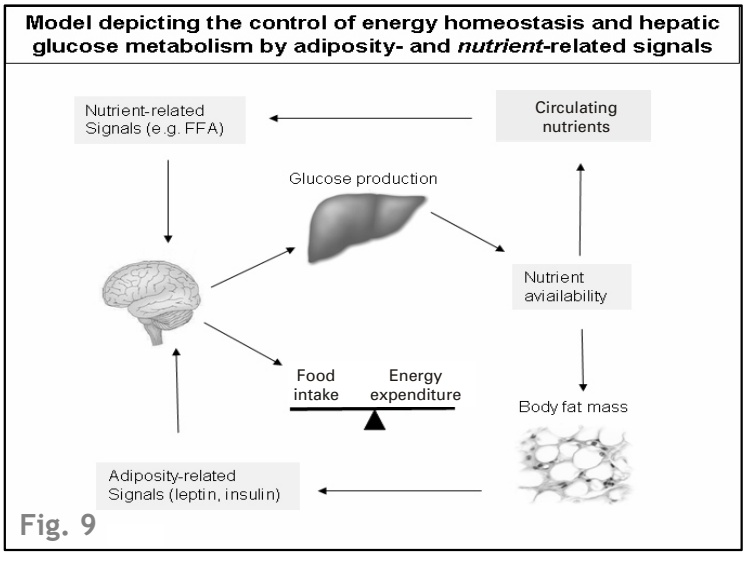


Fig. 9

leptin insensibility in brain can also cause adiposity; leptin concentration has to be higher then, so that the signal can be recognized by the hypothalamus; it is not always a deficiency of leptin which causes obesity;

can increase insulin sensitivity, this action appears to be mediated by direct and indirect (CNS) effects to activate AMP kinase and increase muscle fatty acid oxidation;

can inhibit insulin secretion by activating with ATP-dependent potassium channels or via interactions with the cAMP protein kinase A signalling pathway perhaps by activating phosphodiesterase B3.

4. Diseases of the metabolic syndrome - Hypertension

Definition:
systolic blood pressure > 140 mmHg;
diastolic blood pressure > 90 mmHg

normotension:	systolic blood pressure	< 140 mmHg
	diastolic blood pressure	< 90 mmHg
hypotension:	systolic blood pressure	< 100 mmHg
	diastolic blood pressure	< 60 mmHg

15% of the people living in industrialized countries have hypertension. 90% of these have the essential form. Obesity is the most common cause of essential hypertension. It is suggested that excess renal sodium reabsorption and a hypertensive shift of pressure natriuresis play a major role. Sympathetic activation appears to mediate at least part of the obesity-induced sodium retention and hypertension since adrenergic blockade or renal denervation markedly attenuates these changes.
Recent observations suggest that leptin and its multiple interactions with neuropeptides in the hypothalamus may link excess weight gain with increased sympathetic activity. Transgenic mice overexpressing leptin also develop hypertension. Maybe the renal sympathetic effects of leptin may depend on interactions with other neurochemical pathways in the hypothalamus, including the melanocortin-4 receptor.
The **Renin-Angiotensin system** is more active if subjects are obese and have hypertension.
There is also a direct association between hyperinsulinemia and hypertension because insulin causes increased reabsorption of sodium in the tubules of kidney. An increased supply of sodium causes increased blood pressure because the intracellular higher concentration of sodium inhibits the calcium-sodium-exchange. The result is an increase in the calcium-concentration in the vascular muscle tissue that leads to the increased muscle tonus (3). Furthermore high insulin levels stimulate the sympathetic nervous system and increase angiotensin II production (25).

5. Diseases of the metabolic syndrome - Dyslipidemia

Definition: HDL-C (high density lipoprotein-cholesterol) low and LDL-C (low density lipoprotein-cholesterol) and triglycerides high.

Obese people have generally a higher intake of saturated fatty acids and cholesterol. Saturated fatty acids cause a lower activity of the LDL-receptor, what leads to a slower intake of LDL in cells. LDL-C in serum is then higher so that LDL binds the scavenger-receptor. This receptor mediates the storage of cholesterol in skin, in walls of blood-vessels and in macrophages. This is a risk factor for aterosclerosis.

Obese people have increased levels of free fatty acids. This has the following effects:

- Free fatty acids (FFA) can reduce the hepatic insulin clearance;
- FFA can increase the synthesis of glucose in the liver;
- FFA can lead to an impaired glucose utilization in the skeletal muscle;
- FFA can increase the synthesis of VLDL (4).

6. Diseases of the metabolic syndrome - Diabetes mellitus type 2 and insulin resistance

Non-insulin-dependent diabetes mellitus (NIDDM), because insulin treatment is not always needed.
Common cause: Insulin resistance with a relative lack of insulin; 90% of the patients are obese
A lack of insulin causes hyperglycaemia, glucosuria and increased gluconeogenesis.
Main characteristics:

hyperinsulinemia
↓
insulin resistance
↓
the amount of insulin receptors decreases and the binding of the hormone is impaired

Insulin resistance - the tissues such as muscles don't respond fully to the actions of insulin, so can not make use of glucose in the blood. The pancreas response by producing more insulin and the liver releases more glucose to try to increase the amount of glucose available. The pancreas is then not able to produce enough insulin and the tissues become resistant to insulin;
Usually develops over 40 years of age;
Obese people are more at risk of type 2 diabetes and also higher risk for people who are an "apple-shape" - with lots of fat around the abdomen;
Ketones are in urine, ketones indicate there is not enough insulin to prevent the mobilization of fat;
Hyperlipidemia, because of the greater synthesis rate of lipoproteins;
Hyperglycaemia seems to cause raised levels of atherogenic cholesterol-enriched apolipoprotein B-containing remnant particles (5).

More than 19 million adults in the United States and 150 million worldwide have diabetes; by the year 2025 the WHO projects more than 300 million cases worldwide (23).

7. Diabetic complications and AGEP

Diabetes-specific microvascular pathology in the retina, renal glomerulus and peripheral nerve;
Arteriosclerotic macrovascular disease affecting arteries that supply the heart, brain and lower extremities;
Higher risk of myocardial infarction, stroke and limb amputation.

Hypothesis: Hyperglycaemia causes diabetic complications by increased advanced glycation end products formation (AGEPs). AGEPs are found in increased amounts in diabetic retinal vessels and renal glomeruli. They were originally thought to arise from non-enzymatic reactions between extracellular proteins and glucose. Accumulation of AGEP-cross linked proteins throughout life is a general phenomenon of ageing. They are markers of protein ageing. AGEPs are protein modifications: they are formed by a complex cascade of dehydration, oxidation and cyclisation reactions, subsequent to a non-enzymatic reaction of sugars with amino group of proteins. AGEPs are Maillard-products. Intracellular hyperglycaemia is the primary initiating event in the formation of intracellular and extracellular AGEPs because the rate of AGEP formation from glucose is orders of magnitude slower than the rate of AGEP formation from glucose-derived dicarbonyl precursors generated intracellularly.

Effects of AGEP precursors on cells:
AGEP precursors bind the AGEP receptors (RAGE - Receptor for advanced glycation end products") on

endothelial cells

mesangial cells;
macrophages;
inducing receptor-mediated production of reactive oxygen species.
Inhibitors of Maillard-reaction are currently being assessed in clinical trials for the treatment of diabetic complications (6).

Increased flux of glucose through the hexosamine pathway mediates insulin resistance

Increased flux through the hexosamine pathway

- → **counteracts AKT activation by insulin**
- → **inhibits GLUT4 translocation**
- → **triggers hyperglycemia- and fat-induced changes in gene transcription (e.g. of TGFα, TGFβ1, PAI-1, NFχB, leptin)**
- → **enhances O-GlcNAcylation of endothelial NO-Synthase at a serine which is otherwise phosphorylated by AKT leading to inactivation of NOS**

Fig. 10

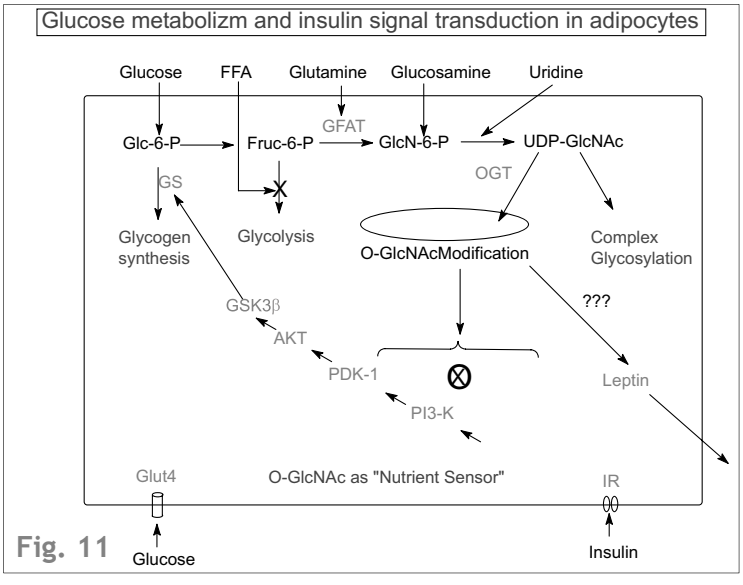


Fig. 11

There is a growing evidence of a link between aberrant O-GlcNAc modification and diabetes. One of the hallmarks of type 2 diabetes is hyperglycaemia associated with an inability of insulin to trigger appropriate glucose uptake (insulin resistance).
Glucose flux through the hexosamine pathway has been linked to the onset of insulin resistance (7).
Increased levels of extracellular glucose and glucosamine lead to elevated intracellular O-GlcNAc modification of proteins in skeletal muscle and in pancreatic β-cells (8).
In muscle cells several postreceptor insulin signalling events are dampened under hyperglycaemic conditions and reduced IRS-1 and IRS-2 (insulin receptor substrate) signalling are associated with their increased O-GlcNAc modification and decreased phosphorylation (9).
Thus it is proposed that hyperglycaemia-induced O-GlcNAc modifications perturb normal signalling events required for insulin mediated homeostasis.
Because O-GlcNAc levels on proteins appear to be sensitive to flux through the hexosamine biosynthetic pathway, a role as a general sensor of glucose availability can be hypothesized for O-GlcNAc (10).

8. Glycosylation

Definition: Process of addition of saccharides to proteins and lipids
one of four principal post-translational modification steps in the synthesis of membrane and secreted proteins;
most of the proteins synthesized in the rough ER undergo glycosylation;
it is an enzyme-directed site-specific process;
the donor molecule is an activated nucleotide sugar;
two different forms exist.

N-linked glycosylation

All N-linked carbohydrates are linked through N-acetyl-glucosamine and the amino acid asparagine;

The N-linked amino acid consensus sequence is Asn-X-Ser or Thr. The middle amino acid can not be proline (Pro), but any other;

After attachment once the protein is correctly folded, the three glucose residues are removed from the chain and the protein is available for export from ER;

The glycoprotein is then transported to the Golgi where removal of further mannose residues may take place;

Further removal of mannose residues leads to a core structure containing 3 mannose and 2 N-acetyl-glucosamine residues which may then be elongated with a variety of different monosaccharides including galactose, N-acetylglucosamine, N-acetylgalactosamine, fucose and sialic acid.

N-linked glycosylation

to the amide nitrogen of asparagine side chains

- is required for some proteins for proper folding
- occurs in eukaryotes, widely in archea and very rarely in prokaryotes
- two major types of N-linked saccharides: high-mannose oligosaccharides and complex oligosaccharides
- **SYNTHESIS:**
  - a **14-sugar** precursor is first added to the asparagines in the polypeptide chain of the target protein
  - this precursor is common to most eukaryotes and contains **3** glucose, **9** mannose, **2** N-acetylglucosamine molecules
  - a complex set of reactions attaches this branched chain to a carrier molecule called **dolichol**
  - it is then transferred to the appropriate point on the polypeptide chain as it is translocated into the ER lumen

Fig. 12

O-linked glycosylation to the hydroxy oxygen of serine and threonine side chains by the enzyme UDP-N-acetyl-D-Galactosamine:polypeptide N-cetylgalactosaminyltransferase

- is important for some protein such as proteoglycans
- occurs at a later stage during protein processing, probably in the Golgi apparatus
- **SYNTHESIS:**
- addition of N-acetyl-galactosamine followed by the addition of other carbohydrates such as sialic acid and galactose

Fig. 13

O-linked glycosylation

Most O-linked carbohydrate covalent attachments to proteins involve a linkage between the monosaccharide N-Acetylgalactosamine and the amino acids serine or threonine;

Currently there is not an O-linked amino acid consensus sequence.

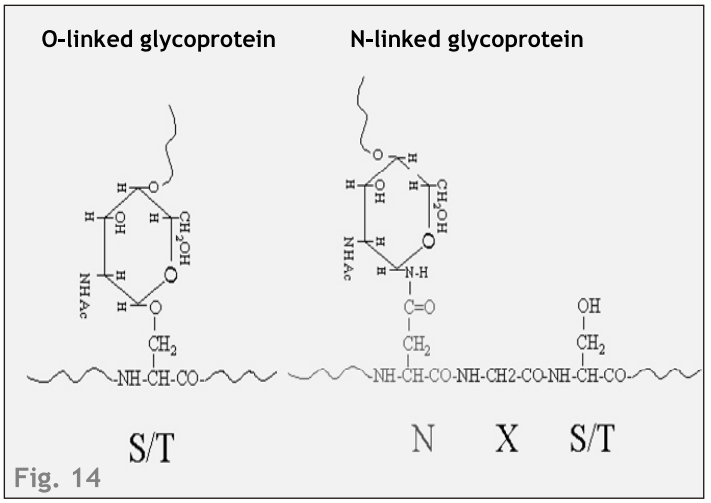


Fig. 14

Posttranslational modifications like glycosylation play a major role in many biological processes, for example: signal transduction, gene expression and metabolism.

Glycosylation is one of the most common posttranslational modifications of proteins in eukaryotes. It affects a wide range of protein functions:

Protein folding
Protein secretion
Serum half-life
Biomolecular recognition (11).

O-GlcNAc interferes with phosphorylation, e. g. in the insulin signaling pathway:

Hyperglycemia enhances

- → **O-GlcNAc transferase (OGT) expression and activity**
- → **O-GlcNAcylation of IRS-1 and IRS-2**
- → **O-GlcNAc-modificaton of the transcription factor SP1 which is involved in the expression of glucose-responsive genes (e.g. TGF-ß, PAI-1)**

- ❖ **Insulin resistance of glycogen synthase depends on its O-GlcNAc modification.**
- ❖ **Transgenic mice overexpressing OGT in muscle and adipose tissue exhibit hyperleptinemia and insulin resistance.**

O-GlcNAc is implicated in the pathogenesis of insulin resistance

Fig. 15

Multiple states of O-GlcNAc posttranslational modification

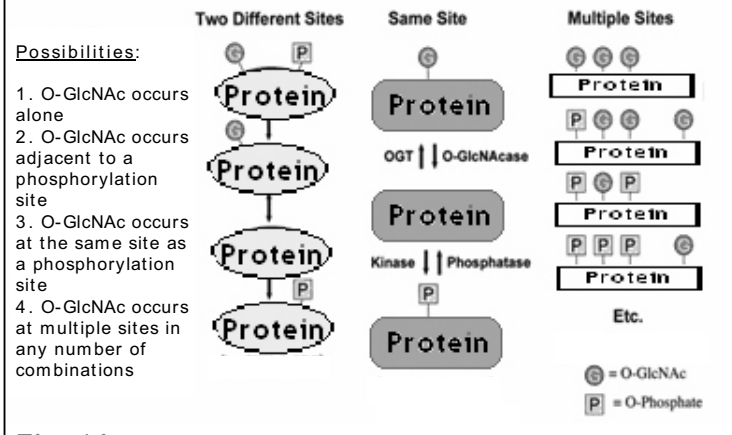


Fig. 16

Whelan and Hart, 2003; Circulation Research; 93; 1047-58

9. O-GlcNAc

O-GlcNAc was discovered by Torres and Hart in 1984. 51 years ago the phosphorylation was already get to know (12). The addition of a single O-linked N-acetylglucosamine - the Ser(Thr)-O-GlcNAcylation is dynamic and abundant analogous to phosphorylation (13).

O-GlcNAcylation occurs in the nucleus and cytoplasm. Altered O-linked GlcNAc metabolism may occur in the development of neurodegenerative disorders, diabetes mellitus, and cancer.

All of the O-GlcNAcylated proteins are also phosphoproteins. A reciprocal relationship between these modifications exists (14). Both are dynamic: O-GlcNAc and phosphate are added or removed in minutes. The O-GlcNAc half-life is much shorter than that of the modified polypeptide chain.

The sites of O-GlcNAc modification are often the same or adjacent to sites of phosphorylation, suggesting a role in regulation analogous to or competition with phosphorylation.

Thus, it is hypothesized that O-GlcNAc regulates the functions of proteins, either exclusively or in concert with phosphorylation.

Reciprocity between O-GlcNAc and phosphorylation, this so-called "yin-yang" relationship has been shown at both the global cellular protein level and at specific sites on particular proteins.

For example inhibitors of kinases increase the overall level of O-GlcNAc modified proteins (15). Furthermore the enzymes that catalyze the cycling of O-GlcNAc onto and of proteins are analogous to those that add and remove phosphates (kinases and phosphatases) (16). O-GlcNAcylation and phosphorylation can compete for the same site or adjacent sites like in the case of RNA Pol II (17).

Enzymes for O-GlcNAc modification and removal

Uridine diphospho-N-acetyl glucosamine: polypeptide β-N-acetylglucosaminyltransferase (OGT)

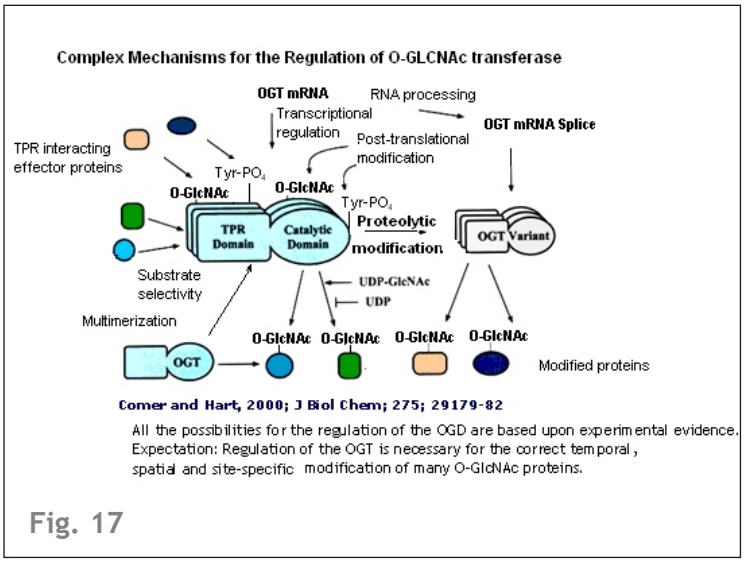


Fig. 17

Catalyzes the addition of O-GlcNAc on proteins;
Was originally purified in 1992 from rat liver;
Molecular weight: 340 kDa approximately (18);
Human and rat sequence are nearly 100% homologous;
The gene of OGT resides on the X chromosome and is necessary for stem cell viability (19);
The protein is composed of two 110 kDa polypeptides and one 78 kDa polypeptide;
Each polypeptide appears to be composed of two domains;
N-terminus contains 11.5 tetratricopeptide repeats (TPR). These repeats are thought to be involved in mediating protein-protein interactions (20);
C-terminus appears to be the catalytic domain with a putative UDP-GlcNAc binding site;
There are multiple isoforms of OGT, one splice variant is targeted to mitochondria;
OGT is O-GlcNAc modified and tyrosine-phosphorylated;
A deletion of 100 amino acids from the C-terminus results in a catalytic inactive enzyme;
Understanding the regulation of OGT will be key to future investigation of O-GlcNAc modification (21).

O-GlcNAcase

Removes O-GlcNAc from proteins;
Consists of 916 amino acids;
Purified and characterized by Dong et al., 1994 (22);
Localization primarily to the cytosol and to a lesser extent to the nucleus;
Specifically catalyzes the removal of O-GlcNAc from proteins and not GalNAc;
Is a cytosolic neutral -N-acetylglucosaminidase;
Molecular weight: 130 kDa, native O-GlcNAcase activity migrates at 600 kDa, indicating that in the cell, the enzyme may be complexed with proteins like hsp110 for example;
The gene for O-GlcNAcase resides on the chromosome 10 (10q24);
C-terminal half contains the O-GlcNAcase activity;
Very little is known about the regulation of the O-GlcNAcase (16).

O-GlcNAc and its functions

This figure shows that O-GlcNAc is implicated in many cellular processes in the cytosol and in the nucleus (10).

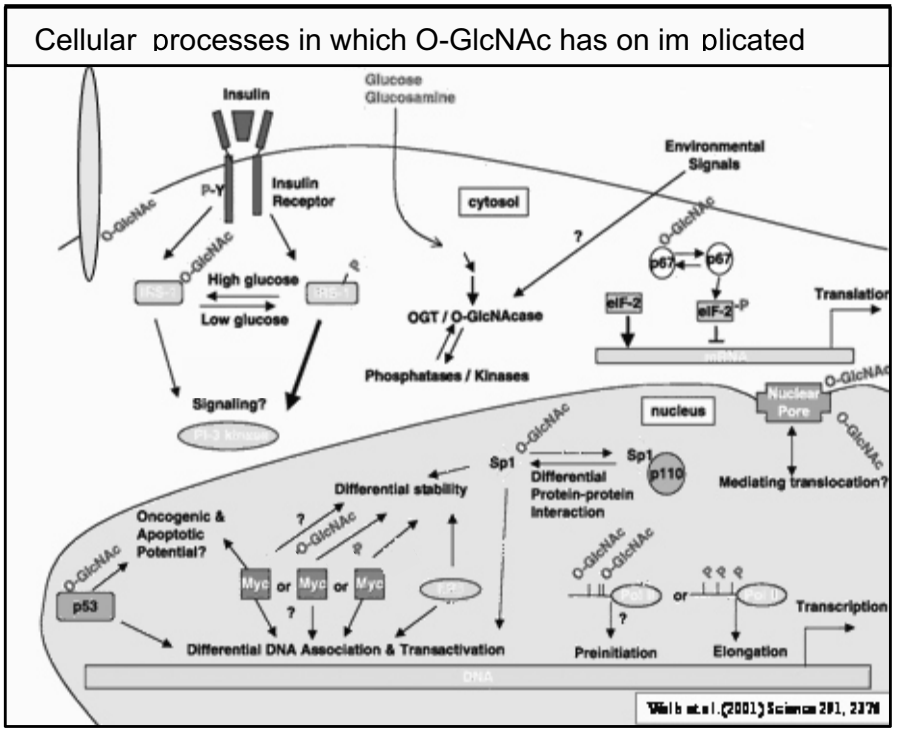


Fig. 18 Abbreviations: phosphatidylinositol-3 kinase (PI-3 kinase), TATA-binding protein-associated factor (p110), c-myc (myc), estrogen receptor b (ERb), and RNA polymerase II (Pol II). Numbers in parentheses are reference numbers. Question marks represent unpublished work and/or speculation on the part of the authors.

The current list of O-GlcNAc-modified proteins is decidedly incomplete, as detection of O-GlcNAc proteins present in soluble cells extracts reveals thousands of proteins that contain this modification.
The following table gives an overview of O-GlcNAcylated proteins:

Table 1 O-GlcNAcylated proteins

Functional Subgroup	Protein
Chaperones	Heat shock protein 27 (HSP27) Heat shock cognate 70 (HSC70) Heat shock protein 70 (HSP70) Heat shock protein 90 (HSP90)
Chromatin	Chromatin-associated proteins
Cytoskeleton Actin-based	Ankyrin G Cofilin E-cadherin Myosin Protein band 4.1 Synapsin Talin
Intermediate filaments	Keratins 8, 13, 18 Neurofilaments H, M, L
Microtubule-based	a-Tubulin Dynein LC1 Microtubule-associated proteins 2 and 4 (MAP 2 and 4) Tau
Other	Adenovirus type 2 and 5 fibre proteins Assembly protein 3 and 180 (AP-3 and AP-180) b-Amyloid precursor protein (b-APP) b-Synuclein Piccolo Plakoglobin
Kinases and adaptor proteins	Casein kinase II (CKII) Glycogen synthase kinase-3b (GSK-3b) Insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) PI3 kinase (p85)
Metabolic enzymes	Enolase Endothelial nitric oxide synthase (eNOS) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Glycogen synthase (GS) Phosphoglycerate kinase (PGK) Pyruvate kinase (PK) UDP glucose pyrophosphorylase (UGP)
Nuclear hormone receptors	Estrogen receptor-a and -b (ER-a and ER-b) V-erb A
Nuclear pore proteins (NUP)	Nup 62 Nup 153, 214, 358

	Nup 180 Nup 54, 155
Phosphatases	Nuclear tyrosine phosphatase p65 Phosphatase 2a inhibitor (i2pp2a)
Polymerases	RNA Pol II
Proto-oncogenes	c-Myc
RNA-binding proteins	40S ribosomal protein S24 (40SrpS24) Elongation factor 1a (EF-1a) Eukaryotic initiation factor 4A1 (EIF 4A1) Ewing sarcoma RNA-binding protein (EWS) RNA-binding protein G (hnRNP G; La-antigen)
Tumour suppressors	Retinoblastoma protein (Rb)
Viral proteins	Baculovirus gp41 tegument protein HCMV UL32 (BPP) tegument protein NS26 rotavirus protein SV-40 large T antigen Virion basic phosphoprotein
Transcription factors	AP-1 (c-fos and c-jun) b-Catenin CAAT box transcription factor (CTF, NF-1) Cyclic AMP response element-binding protein (CREB) ELF-1 (Ets transcription factor) Enhancer factor 2D (EF-2D) Hepatocyte nuclear factor 1 (HNF-1) KIAA0144, Oct1 NF-kB OGT-interacting protein 106 (OIP-106) p53 Pancreatic/duodenal homeobox-1 protein (PDX-1, IPF-1, STF-1) PAX-6 Pancrease-specific transcription factor (PTF-1) Human C 1 transcription factor (HCF) Serum-response factor (SRF) Sp1 and Ying yang 1 (YY1)
Other	Annexin 1 Collapsin response mediator protein-2 (CRMP-2) Elongation initiation factor-2 associated 67 kDa (EIF2a p67) GABA receptor interacting protein-1 (GRIF-1) and splice variants Glut-1 and Glut-4 Nucleophosmin Peptidyl prolylisomerase (PPI) Proteosome component C2 O-GlcNAc transferase (OGT) Q04323, UCH homologue Sec23, human homologue (hhSec23) Ran Rho GDP dissociation inhibitor 1 (Rho-GDIa) Ubiquitin carboxy hydrolase (UCH)

Abbreviations

HSP	hexosamine pathway
HSC	heat shock cognate
GFAT	glutamine: fructose-6-phosphate-amidotransferase
OGT	uridine diphospho-N-acetylglucosamine: polypeptide -N-acetyl-glucosaminyl-transferase
STZ	steptozotocin
ALX	alloxan
PUGNAc	O-(2-acetamido-2-desoxy-D-glucopyranosylidene) amino-N-phenylcarbamate
UDP-GlcNAc	N-acetyl UDP-glucose
IRS	insulin receptor substrate
PKC	protein kinase C
GlcNAc	N-acetyl glucose
DON	6-diazo-5-oxo-L-norleucine
ROS	reactive oxygen species

References

1. Cook, S. (2004): "The metabolic syndrome: antecedent of adult cardiovascular disease in paediatrics", The Journal of Pediatrics, 145, 427-430
2. Wells, L.; Vosseller, K.; Hart, G. W. (2003): "A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance", Cellular and Molecular Life Sciences, 60, 222-228
3. Hennes, M. M.; O'Shaughnessy, I. M.; Kelly, T. M.; LaBelle, P.; Egan, B. M.; Kissebah, A. H. (1996): "Insulin-Resistant Lypolysis in Abdominally Obese Hypertensive Individuals", Hypertension, 28, 120-126
4. Biesalski, H. K.; Fürst, P.; Kasper, H.; Kluthe, R.; Pölert, W.; Puchstein, Ch.; Stähelin, H. B. (1999): "Ernährungsmedizin", 2. Aufl., Georg Thieme Verlag, Stuttgart, New York
5. Brownlee, M. (2001): "Biochemistry and molecular cell biology of diabetic complications", Nature, 414, 813-820
6. Münch, G.; Schinzel, R.; Loske, C.; Wong, A.; Durany, N.; Li, J. J.; Vlassara, H.; Smith, M. A.; Perry, G.; Riederer, P. (1997): "Alzheimer's disease synergistic effects of glucose deficit oxidative stress and advanced glycation endproducts", Journal of Neural Transmission, 105, 439-461
7. Marshall, S.; Bacote, V.; Traxinger, R. (1991c): "Discovery of a metabolic pathway mediating glucose induced desensitization of the glucose transport system: role of hexosamine biosyntheses in the induction of insulin resistance", Journal of Biological Chemistry, 266, 4706-4712
8. Liu, K.; Paterson, A. J.; Chin, E.; Kudlow, J. E. (2000): "Glucose stimulates protein modification by O-linked GlcNAc in pancreatic b cells: Linkage of O-linked GlcNAc to b cell death", Proceedings of the National Academy of Sciences of the United States of America, 97(6), 2820-2825
9. Patti, M. E.; Virkamaki, A.; Landaker, E. J.; Khan, C. R.; Yki-Jarvinen, H. (1999): "Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle", Diabetes, 48(8), 1562-1571
10. Wells, L.; Vosseller, K.; Hart, G.W. (2001): "Glycosylation of nucleocytoplasmic proteins: Signal transduction and O-GlcNAc", Science, 291, 2376-2378
11. Zhang, Z.; Gildersleeve, J.; Yang, Y.-Y.; Xu, R.; Loo, J. A.; Uryu, S.; Wong, Ch.-H.; Schultz, P. G. (2004): "A New Strategy for the Synthesis of Glycoproteins", Science, 303: 371-372
12. Torres, C.-R.; Hart, G. W. (1984): "Topography and Polypeptide Distribution of Terminal N-Acetylglucosamine Residues on the Surfaces of Intact Lymphocytes", Journal of Biological Chemistry, 259(5), 3308-3317
13. Hanover, J. A. (2001): "Glycan-dependend signaling: O-linked N-acetylglucosamine", FASEB Journal, 15, 1865-1876
14. Hart, G. W., Greis, K. D., Dhong, L. Y., Blomberg, M. A., Chou, T. Y., Jiang, M. S., Roquemore,

E. P., Snow, D. M. et al. (1995): "O-linked N-acetylglucosamine: "yin-yang" of Ser/Thr phosphorylation? Nuclear and cytoplasmic glycosylation." Adv. Exp. Med. Biol., 376, 115-23
15. Comer, F. I.; Hart, G. W. (2000): "O-glycosylation of nuclear and cytosolic proteins“, Journal of Biological Chemistry, 275, 29179-29182
16. Iyer, S. P. N.; Hart, G. W. (2003a): "Dynamic nuclear and cytoplasmic glycosylation: enzymes of O-GlcNAc cycling", Biochemistry, 42(9), 2493-2499
17. Whelan, S. A.; Hart, G. W. (2003): "Proteomic Approaches to Analyze the Dynamic Relationships between Nucleocytoplasmic Protein Glycosylation and Phosphorylation", Circulation Research, 93, 1047-1058
18. Haltiwanger, R. S.; Blomberg, M. A.; Hart, G. W. (1992b): "Glycosylation of nuclear in cytoplasmic proteins. Purification and characterization of a uridine diphospho-N-acetylglucosamine: polypeptide beta-N-acetylglucosaminyl transferase", Journal of Biological Chemistry, 267(13), 9005-9013
19. Shafi, R.; Iyer, S. P.; Ellies, L. G.; O'Donnell, N.; Marek, K. W.; Chui, D.; Hart, G. W.; Marth, J. D. (2000): "The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny", Proceedings of the National Academy of Science of the United States of America, 97(11), 5735-5739
20. Blatch, G. L.; Lassle, M. (1999): "The tetratricopeptide repeat: a structural motif mediating protein-protein interactions", Bioessays, 21(11), 932-939
21. Wells, L.; Hart, G. W. (2003): "O-GlcNAc turns twenty: functional implications for post-translational modification of nuclear and cytosolic proteins with a sugar", FEBS Letters, 546, 154-158
22. Dong, L.-Y.; Hart, G. W. (1994): "Purification and charakterization of an O-GlcNAc selective N-acetyl-beta-D-glucosaminidase from rat spleen cytosol", Journal of Biological Chemistry, 269(30), 19321-19323
23. King, H.; Aubert, R.E.; Herman, W.H. (1998): " Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections", Diabetes Care, 21, 1414-1431
24. Laaksonen, D.E.; Niskanen, L.; Lakka, H.-M.; Lakka, T.A.; Uusitupa, M. (2004): "Epidemiology and treatment of the metabolic syndrome", Annals of Medicine, 36, 323-346
25. Abuissa, H.; Bell, D.S.H.; O`Keefe Jr, J.H. (2005): "Strategies to prevent type 2 diabetes", Current medical research and opinions, 21 (7), 1107-1114
26. Cohn, G.S.; Kittleson, M.M.; Blumenthal, R.S. (2005): "Toward an improved diagnosis of the metabolic syndrome", American journal of hypertension, 18, 1099-1103
27. Reaven, G.M. (1988): "Role of insulin resistance in human disease", Diabetes, 37, 1595-1607
28. Daskalopoulou, S.S.; Mikhailidis, D.P. (2004): "Prevention and treatment of the metabolic syndrome", 55, 589-612

Nutrition in Metabolic Syndrome

Topic 24

Module 24.6

Frontiers in Research of Metabolic Syndrome

Agnes Namislo
Winrich Breipohl
Brigitte Schmitz
Louise LaJoie

Learning Objectives

What is the statement of the HSP hypothesis;
Why do GFAT and OGT play a so important role;
What about the effects of STZ, ALX and PUGNAc;
Mechanisms through which FFAs can upregulate the HSP.

Contents

1. HSP hypothesis
2. Effects of overexpression/inhibition of GFAT
3. Role of OGT in β-cells and effects of overexpression
4. Effects of STZ, Alloxan and PUGNAc on β-cells and O-GlcNAcylation? Experimental results
5. Regulation of leptin synthesis
6. Effects of FFA on the biosynthesis of hexosamines
7. Oxidative stress
8. How to prevent metabolic disorders?

Key Messages

HSP flux regulates leptin secretion;
In human with NIDDM GLUT4 function and translocation is impaired;
GFAT can be inhibited by UDP-GlcNAc;
Elevation of O-GlcNAc levels attenuate insulin signaling;
Increased fatty acids upregulate the HSP;
Probably at the level of trancription and translation leptin production is regulated by hexosamines.

Preface

Western societies have shifted to a higher caloric diet and more sedentary lifestyle, the incident of type 2 diabetes has increased to epidemic proportions. Type 2 diabetes has been described as a disease of "chronic overnutrition". While the genesis of type 2 diabetes is still unclear, certain genetic traits predispose individuals for development of the disease when exposed to certain environmental factors, namely chronic nutrient excess and low energy expanditure. The increase in prevalence of metabolic syndrome parallels the increased prevalence in obesity.
There is a growing evidence of a link between aberrant O-GlcNAc modification and diabetes. One of the hallmarks of type 2 diabetes is the hyperglycemia associated with an inability of insulin to trigger appropriate glucose uptake (insulin resistance). Glucose flux through the hexosamine pathway (HSP) has been linked to the onset of insulin resistance. Increased levels of extracellular glucose and glucosamine lead to elevated intracellular O-GlcNAc modification of proteins in skeletal muscles and in pancreatic β-cells. In muscle cells several postreceptor insulin signaling events are dampened under hyperglycemic conditions. Reduced insulin receptor substrate 1 and 2 signaling is associated with his increased O-GlcNAc modification and decreased phosphorylation. Thus it is proposed that hyperglycemia-induced O-GlcNAc modifications perturb normal signaling events required for insulin-mediated homeostasis. Because O-GlcNAc levels on proteins appear to be sensitive to flux through the hexosamine biosynthetic pathway, a role as a general sensor of glucose availability can be hypothesized for O-GlcNAc.

1. HSP hypothesis

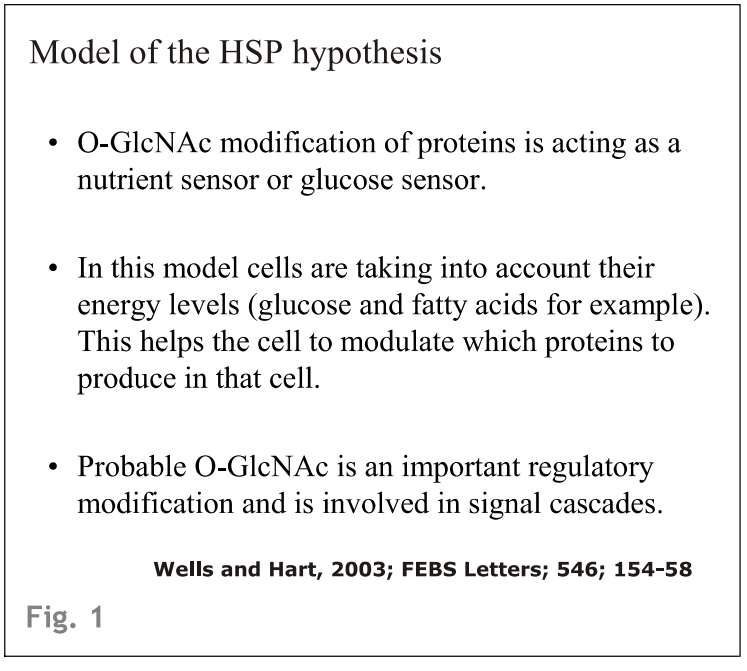


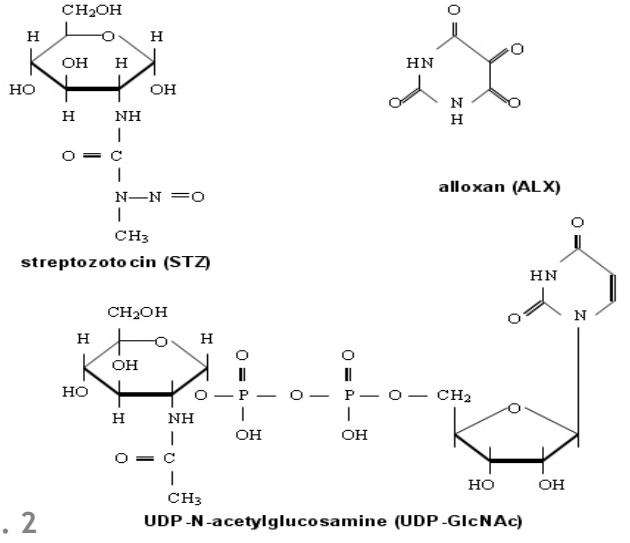
Fig. 1

Excessive concentrations of glucosamine lead to insulin resistance. It is known that suppression of expression of glutamine-fructose-6-phosphate-amidotransferase (GFAT) can block insulin resistance (1);
Increased free fatty acids can inhibit glycolysis and can increase fructose-6-phosphat levels;
Hypercaloric intake can be positively correlated with increaseed flux through the HSP (2);
Leptin alters nutrient flux such that energy expanditure is favored over energy storage;
HSP flux regulates leptin secretion: increased levels of hexosamines lead to an increase in leptin release from adipocytes (3);
In type 2 diabetic patients hyperglycemia and hyperinsulinemia lead to elevated UDP-GlcNAc levels;
Increased free fatty acids (FFA) upregulate the HSP presumbly by inhibiting glycolysis and increasing glucose-6-phosphate levels;
HSP plays a role in regulating insulin resistance and serving as an **energy sensor** (increased flux through the HSP resulting in elevated UDP-GlcNAc levels). Hypercaloric intake correlates positively with increased flux through the HSP (2);
Elevation of O-GlcNAc levels in 3T3-L1 adipocytes, including the O-GlcNAc modification of several key proteins in the insulin signaling pathway (insulin receptor substrate-1 (IRS-1) and β-catenin) directly causes insulin resistance, the hallmark of type 2 diabetes (4).

2. Effects of overexpression/inhibition of GFAT

An overexpression of the rate limiting enzyme of the HSP-GFAT in skeletal muscle and adipose tissue of transgenic mice lead to weight-depended hyperinsulinemia and insulin resistance. This lead to decreased levels of the insulin-stimulated GLUT4 especially in older transgenic mice. In human with NIDDM GLUT4 function or translocation is impaired (5). These results show that the GFAT plays an important role in the development of insulin resistance. The defects in glucose transport seen in the transgenic mice are very similar to those seen in human type 2 diabetes (6).
The GFAT can be inhibited by UDP-GlcNAc in rat adipocytes (end product inhibition) (7). An increased activity of the GFAT caused by glucose and insulin was observed in cultured human skeletal muscle cells. This suggests an important relationship between GFAT activity and the regulation of glucose homeostasis and support the hypothesis that the HSP is a major pathway used by tissues to sense and respond to changes in glucose flux (8).
The cDNA of GFAT was cloned in 1992 and gene coded the enzyme is localized on chromosome 2 (p13). ob/ob mice have a twofold increase in GFAT activity in comparison to lean mice (9). An overexpression of GFAT was also observed in diabetic glomeruli (10). It is suggested that angiotensin II regulates GFAT promoter activity by modulating signaling pathways that include calcium, PKC and tyrosine kinase cascades. Angiotensin II increases the activity of GFAT. Thus it is also suggested that angiotensin II plays an important role in the development of diabetic complications such as vascular and glomerular injury (11).

The chemical structures of the -cell-specific toxins alloxan and streptozotocin, and their common analog UDP-N-acetylglucosamine (UDP-GlcNAc).



ALX is an analog of uracil, while STZ is an analog of GlcNAc.

Konrad and Kudlow, 2002; Int J Mol Med; 10; 535-39

Fig. 2

3. Role of OGT in β-cells and effects of overexpression

In the presence of high intracellular glucose concentrations the activity of OGT (uridine diphospho-N-acetylglucosamine: polipeptide β-N-acetylglucosaminyltransferase) is higher than the activity of O-GlcNAcase. Using an in situ hybridization it is observed that transcripts encoding OGT are present at a very high level in β-cells of the pancreas. Thus it could be suggested that in β-cells the O-GlcNAc metabolism is higher than in other cells and the OGT might have a unique function in β-cells (12).
It has been demonstrated that the ob gene and the levels of leptin are regulated by hexosamines. Some data support the hypothesis that the regulation occurs through the O-glycosylation pathway. OGT transgenic mice have higher serum leptin levels. It is obviously that OGT like GFAT presumbly plays a similar important role in insulin- and leptin signal transduction cascades (13).

4. Effects of STZ, Alloxan and PUGNAc on β-cells and O-GlcNAcylation Experimental results

Alloxan and streptozotocin are used to create animal models of diabetes. Alloxan is an analog of uracil and steptorotocin (STZ) is a GlcNAc analog.
Both cause diabetes by interfering with proteins that bind UDP-GlcNAc or O-GlcNAc.
STZ is an irreversible inhibitor of O-GlcNAcase. When isolated islets were exposed to STZ alone, O-GlcNAcase was inhibited and β-cells were destroyed. When STZ-induced O-GlcNAcase inhibition was prevented by GlcNAc, β-cells remained viable. GlcNAc blocks STZ toxicity because it blocks the entering of STZ in β-cells. Glucose, glucosamine and GalNAc cannot prevent β-cell death.
Alloxan block STZ-induced increases in β-cell O-glycosylation. This supports the hypothesis that alloxan is an inhibitor of OGT.
Hyperglycemia has the same effect on the β-cells as STZ, however, the effect is reversible and thus less acutely toxic.
The chronic β-cell toxicity caused by hyperglycemia-induced (reversible) O-glycosylation exacerbated the very hyperglycemia itself, which in turn causes more β-cell toxicity, resulting in a chronic downhill spiral developing over a period of years. Since the OGT-rich β-cell is the cell type most sensitive it should also be the cell type most responsive to therapeutic manipulation of the pathway (14).
Another inhibitor called PUGNAc (O-(2-acetamido-2-desoxy-D-glucopyranosylidene)amino-N-phenylcarbamate) is an inhibitor of O-GlcNAcase. PUGNAc treatment increases levels of O-GlcNAc and causes insulin resistance in 3T3-L1 adipocytes. PUGNAc inhibition of O-GlcNAcase affects phosphorylation of AKT at Thr-308 and GSK3β at Ser-9. The phosphorylation is inhibited by PUGNAc. PUGNAc-induced insulin resistance is associated with increased O-GlcNAc modification of several proteins including IRS-1 and β-catenin, two important effectors of insulin signaling. These results suggest that elevation of O-GlcNAc levels attenuate insulin signaling and contribute to the mechanism by which increased flux through the HSP leads to insulin resistance (in adipocytes) (4).

5. Regulation of leptin synthesis

It was observed that hexosamine biosynthesis results in increased leptin release and inhibition of hexosamine biosynthesis with DON (6-diazo-5-oxo-L-norleucine), a competitive inhibitor of GFAT, results in a decrease in ob gene expression and thus in a reduced leptin production. Also a significant positive correlation was observed between BMI and UDP-GlcNAc concentration in human sc (= subcutaneous) adipose tissue and between leptin and UDP-GlcNAc in humans.
These findings support the hypothesis that leptin production is regulated by the hexosamine production in human adipose tissue probably at the level of transcription and translation.
Maybe there exist transcription factors specific for the ob gene promoter which are O-GlcNAc modified. This could be the mechanism through which hexosamines regulate leptin production (15).

6. Effects of FFA on the biosynthesis of hexosamines

Increased free fatty acids upregulate the HSP presumably by inhibiting glycolysis and increasing fructose-6-phosphate levels (16).
In a study was investigated through which molecular mechanism free fatty acids induce activation of the HSP. Subjects were stimulated with different fatty acids for 20 hours. The results depended on the degree of unsaturation. Palmitate and stearate (saturated fatty acids) resulted in a three- to fourfold increase in mRNA expression of GFAT. Palmitate increased the amount of O-GlcNAc 1.3-fold. Unsaturated fatty acids had little or no effect. Skeletal muscle insulin resistance has been correlated to the increased availability of FFAs. The molecular mechanism for this upregulation is currently unknown but it is clear that there are transcription factors and signaling pathways through which saturated fatty acids induce GFAT gene activation (17). In addition to this increased fatty acids availability generates increased acetyl-CoA which inhibits pyruvate dehydrogenase and

thus ultimately the rate of glycolysis. This results in increased accumulation of fructose-6-phosphate and hence increased substrate for the GFAT (2).

7. Oxidative stress

Fat accumulation correlated with systemic oxidative stress in humans and increased oxidative stress underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular wall cells.
Fatty acids stimulate the ROS production via NADPH oxidase activation (18).
Characteristics of subjects presenting metabolic syndrome are:

elevated plasma levels of oxidized lipids;
subnormal levels of low-molecular-weight antioxidants.

8. How to prevent metabolic disorders?

Avoidance and management of overweight, especially central obesity;
Promoting the consumption of diets that are low in fat;
Reduced consumption of saturated fat to less than 7% of total calories;
Reduced consumption of foods with high glycemic index. Metabolic consequences of carbohydrates depend not only on their quantity but also on their quality. The glycemic response of a given carbohydrate load depends on the food source, which has led to development of the glycemic index, ranking foods by their ability to raise blood glucose levels. Furthermore effects on blood glucose depend on fiber content and type. Lipid-lowering properties were observed for grain products which are rich in soluble fiber, like oat, barley, rye, psyllium. Insoluble fiber, in wheat and corn for example, was found to be inversly associated with diabetes risk (22, 23, 24);
Reduction in the consumption of refined carbohydrates and sugar (22);
Reduced consumption of cholesterol to less than 200 mg/day;
Promoting the consumption of diets that are high in fish to increase the uptake of polyunsaturated fatty acids (especially omega-3 and omega-6 fatty acids) to decrease LDL cholesterol and increase HDL cholesterol;
Promoting the consumption of unsaturated fats from natural liquid vegetable oils and nuts at the expense of saturated and trans fats;
Promoting the consumption of diets that are high in fruits and vegetables to enhance the intake of vitamines and to decrease LDL cholesterol;
Promoting the consumption of diets that are high in starchy carbohydrates;
Regular physical activity (can also decrease LDL cholesterol);
Smoking is a risk factor. Smoking adversely affects glycemic control and increases micro- and macrovascular complications;
In the first year of life breast feeding is very important, because the protein intake per kg body weight is some 55-80% higher in formula fed than in breast fed infants. The high early protein intake can increase later obesity risk (early protein hypothesis). Also the energy supplies of formula fed infants are 10-18% higher (19).

Childhood obesity:

Reduced television viewing;
Increased physical activity;
Increased vegetables and fruits intake;
Low fat diet;
Reduced consumption of sugar-sweetened drinks at home and at school (25).

Abbreviations

HSP	hexosamine pathway
HSC	heat shock cognate
GFAT	glutamine: fructose-6-phosphate-amidotransferase
OGT	uridine diphospho-N-acetylglucosamine: polypeptide -N-acetyl-glucosaminyl-transferase
STZ	steptozotocin
ALX	alloxan
PUGNAc	O-(2-acetamido-2-desoxy-D-glucopyranosylidene) amino-N-phenylcarbamate
UDP-GlcNAc	N-acetyl UDP-glucose
IRS	insulin receptor substrate
PKC	protein kinase C
GlcNAc	N-acetyl glucose
DON	6-diazo-5-oxo-L-norleucine
ROS	reactive oxygen species

References

1. Marshall, S.; Bacote, V.; Traxinger, R. (1991a): "Complete Inhibition of Glucose-induced Desentization of the Glucose Transport System by Inhibitors of mRNA Synthesis", Journal of Biological Chemistry, 266(16), 10155-10161
2. Hawkins, M.; Barzilai, N.; Liu, R.; Hu, M.; Chen, W.; Rossetti, L. (1997a): "Role of the Glucosamine Pathway in Fat-induced Insulin Resistence", Journal of Clinical Investigation, 99, 2173-2182
3. Wang, J.; Liu, R.; Hawkins, M.; Barzilai, N.; Rossetti, L. (1998): "A nutrient-sensing pathway regulates leptin gene expression in muscle and fat", Nature, 393, 684-688
4. Vosseller, K.; Wells, L.; Lane, M. D.; Hart, G. W. (2002): "Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin restistance associated with defects in Akt acitvation in 3T3-L1 adipocytes", Proceedings of the National Academy of Science of the United States of America (Biochemistry), 99(8), 5313-5318
5. Hebert, L. F. Jr.; Daniels, M. C.; Zhou, J.; Crook, E. D.; Turner, R. L.; Simmons, S. T.; Neidigh, J. L.; Zhu, J. S.; Baron, A. D.; McClain, D. A. (1996): "Overexpression of glutamine:fructose-6-phosphate amidotransferase in transgenic mice leads to insulin resistance", Journal of Clinical Investigation, 98, 930-936
6. Cooksey, R. C.; Hebert, L. F.; Zhu, J.-H.; Woffort, P.; Garvey, W. T.; McClain, D. A. (1999): "Mechanism of Hexosamine-induced insulin resistance in transgenic mice overexpressing Glutamine:Fructose-6-phosphate amidotransferase: decreased glucose transporter GLUT4 translocation and reversal by treatment with tiazolidinedione", Endocrinology, 140, 1151-1157
7. Traxinger, R. R.; Marshall, S. (1991): "Coordinated regulation of glutamine:fructose-6-phosphate amidotransferase activity by insulin, glucose and glutamine", Journal of Biological Chemistry, 266, 10148-10154
8. Daniels, M. C.; Ciaraldi, T. P.; Nikoulina, S.; Henry, R. R.; McClain, D. A. (1996): "Glutamine:Fructose-6-Phosphat Amidotransferase Activity in Cultured Human Skeletal Muscle Cells", 97(5), 1235-1241
9. Buse, M. G.; Robinson, K. A.; Gettys, T. W.; McMahon, E. G.; Gulve, E. A. (1997): "Increased activity of the hexosamine synthesis pathway in muscles of insulin-resistant *ob*/*ob* mice", American Journal of Physiology, 272, E1080-E1088
10. Nerlich, A. G.; Sauer, U.; Kolm-Litty, V.; Wagner, E.; Koch, M.; Schleicher, E. D. (1998): "Expression of glutamine: fructose-6-phosphate amidotransferase in human tissues: evidence for high variability and distinct regulation in diabetes", Diabetes, 47, 170-178
11. James, L. R.; Ingram, A.; Ly, H.; Thai, K.; Cai, L.; Scholey, J. W. (2001): "Angiotensin II activates the GFAT promoter in mesangial cells", American Journal of Physiology: Renal Physiology, 281, F151-F162

12. Hanover, J. A.; Lai, Z.; Lee, G.; Lubas, W. A.; Sato, S. M. (1999): "Elevated O-linked N-Acetylglucosamine Metabolism in Pancreatic b-Cells", Archives in Biochemistry and Biophysics, 362(1), 38-45
13. McClain, D. A. (2002): "Hexosamines as mediators of nutrient sensing and regulation in diabetes", Journal of Diabetes Complications, 16(1), 72-80
14. Konrad, R. J.; Kudlow, J. E. (2002): "The role of O-linked protein glycosylation in ?-cell dysfunction", International Journal of Molecular Medicine, 10, 535-539
15. Considine, R. V.; Cooksey, R. C.; Williams, L. B.; Fawcett, R. L.; Zhang, P.; Ambrosius, W. T.; Whitfield, R. M.; Jones, R. M.; Inman, M.; Huse, J.; McClain, D. A. (2000): "Hexosamines Regulate Leptin Production in Human Subcutaneous Adipocytes", Journal of Clinical Endocrinology & Metabolism, 85(10), 3551-3556
16. Wells, L.; Vosseller, K.; Hart, G. W. (2003): "A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance", Cellular and Molecular Life Sciences, 60, 222-228
17. Weigert, C.; Klopfer, K.; Kausch, C.; Brodbeck, K.; Stumvoll, M.; Häring, H. U.; Schleicher, E. D. (2003): "Palmitate-Induced Activation of the Hexosamine Pathway in Human Myotubes", Diabetes, 52, 650-656
18. Furukawa, S.; Fujita, T.; Shimabukuro, M.; Iwaki, M.; Yamada, Y.; Nakajima, Y.; Nakayama, O.; Makishima, M.; Matsuda, M.; Shimomura, I. (2004): "Increased oxydative stress in obesity and its impact in metabolic syndrome", Journal of Clinical Investigation, 114, 1752-1761
19. Koletzko, B.; Broekaert, I.; Demmelmair, H.; Franke, J.; Hannibal I.; Oberle, D.; Schiess, S.; Baumann, B.T.; Verwied-Jorky, S. (2005): "Protein intake in the first year of life: A risk for later obesity?", Adv Exp Med Biol, 569, 69-79
20. Anderson, A.S. (2000): "How to implement dietary changes to prevent the development of metabolic syndrome", British Journal of Nutrition, 83 (1), S165-S168
21. Berra, K. (2003): "Treatment options for patients with the metabolic syndrome", Journal of the American Academy of Nurse Practitioners, 15 (8), 361-370
22. Schulze, M.B.; Hu, F.B. (2004): "Dietary approaches to prevent the metabolic syndrome", Diabetes Care, 27, 613-614
23. Jenkins, D.J.; Kendall, C.W.; Augustin, L.S.; Vuksan, V. (2002): "High complex carbohydrate or lente carbohydrate foods?", American Journal of Medicine, 113 (9B), 30S-37S
24. Meyer, K.A.; Kushi, L.H.; Jacobs, D.R. Jr.; Slavin, J.; Sellers, T.A.; Folsom A.R. (2000): "Carbohydrates, dietary fiber, and incident type 2 diabetes in older women", American Journal of Clinical Nutrition, 71, 921-930
25. Ludwig, D.S.; Ebbeling, C.B. (2005): "Overweight children and adolescents", New England Journal of medicine, 353 (10), 1070-1071

Chapter 6

Topic 32

Food Safety

Food Safety

Topic 32

Module 32.1

Food Safety. Exposure to Toxic Environment

Maya Stavreva
Ed van Klink
Iliana Buteva
Nedka Trifonova

Learning Objectives

To define food safety;
To know basic terms on food safety;
To discuss food hazards;
To know food safety assessment criteria;
To understand basic principles of food safety;
To be familiar with global strategy on food safety - 5 keys to safer food (WHO).

Contents

1. Introduction
2. Definitions
3. Food safety criteria
4. Biological and chemical hazards
5. Food safety assessment procedure
6. Toxicological data surveying
7. Regulations concerning food safety
8. Conclusion
9. Five keys to safer food

Key Messages

Food safety;
Consumers;
Hazards;
Risk assessment;
Nutritional toxicology;
Five keys to safer food.

1. Introduction

Food safety has become a global problem. There are a number of papers, revealing the negative effect of diseased caused by food on business, global economy and life quality. Every year hundred thousands of people become sick as a result of food intoxication and every year food-manufacturing companies pay off lots of money and so bear great losses.
This is why, in regard to free movement of goods and foods in the EU, special attention is being paid to food quality and food safety.
Food consumed by people can cause a number of alimentary or infectious diseases, provided that it has been contaminated in any way. As a rule, contamination of food may occur in different ways contact with dirty surfaces; contamination caused by workers who take part in food processing and transportation and who do not meet the hygiene requirements for food, etc.
Food safety has become a major issue since 1960 in the industrial farms (consolidation in massive farms, increased use of antibiotics in feed, mechanization, etc.). Thereafter, agriculture and food have become more interrelated with the use of pesticides, herbicides, insecticides and fertilizers. Clear relation has been found between certain diseases and the industrial animal-growing, such as: mad cow disease crisis (also known as BSE bovine spongiform encephalopathy) in the 80's and 90's, as well as a number of diseases which have evoked fear about the food safety in Europe. Salmonellosis has also been unknown to people since 1940, while it is widespread nowadays. Food intoxications have increased 4 times in the last decades and they cost 1 - 3 billion Euro to the Europeans per year at present.
Lately there was another food safety issue, concerning the dioxin intoxication of eggs and chickens in Belgium. Other problems, which are attracting great attention at present, concern the use of pesticides and herbicides, as well as genetically modified food. These demonstrate the effects of industrial agriculture and have the potential for great damage on food safety.
This is why, the consumers' trust in food quality and food safety has been lost in the recent years, as a result of accumulating influence of food and health-threatening crises. In order to manage the situation, the European Union worked out clear strategy, called “farm to fork”, which aims to restore consumers' trust in food quality and food safety.
With regard to this, food safety has become an inseparable part of the EU legislation policy, concerning consumers' health protection. All new EU members also have to follow this approach in order to ensure food safety “from farm to fork”. This is a great challenge to the EU as a whole. Issues on food safety follow two main directions, concerning food production and food consumption.
They are:

Free movement of food deals with food legislation;
Agriculture deals with animal and plant-related problems, as well as animal feed.

Legislation, concerning food, includes general orders on hygiene and control, food classifying, food additives, food packaging, genetically modified food.

2. Definitions

Having regarded to the European parliament and the European council (EC) regulation No 178/2002:

Food safety defines as lack of danger for human and offspring's life and health caused by food consumption and based on public health requirement and criteria regarding food, regulations and legislation.

Foods or food products define any product or substance, regardless of whether processed or raw, aimed to or is therefore expected to be consumed by people. The term food includes also beverages chewing gums and any substances including water, which are deliberately included in food during its manufacture, processing and preparation. Food does not include feed, live animal stock (except those which are to be consumed by people), plants before gathering the crops, medical products, cosmetics, tobacco, drugs and stimulants, contaminants.

Danger defines existence or precondition for existence of biological, chemical or physical agent in food or feed, which has the potential to cause harmful effects on human health.

Dangerous food defines food which contains physical, chemical, biological and radiological contaminants and additives above defined certain level, which as a result of normal and regular consumption may lead to toxic, carcinogenic, mutagenic, allergic and other harmful effects on human health.

Misleading food is food, which does not meet regulatory requirements for contents quality and characteristics though its look, labelling, presentation and advertising claim that it does meet the requirements.

Food production, processing and distribution include:
(http://www.who.int/foodsafety/fs_management/en/)

Primary production (defines production and growing primary products including gathering crops, milking and farming before butchering. It also includes hunting fishing and wild plants gathering) or **import**,

Storage of food,

Transportation,

Trade:

- **Retail** defines food processing, storage and end-customer supply and includes distribution terminals, public restaurants, buffets and such food related services, shops, supermarkets' distribution centres and wholesale stations;
- **Introduction to the market** defines owning food or feed for sale including supplying or any form of transfer free or paid, retail, distribution and other forms of transfer;
- **Customer delivery-customer** defines the final user of certain food who is not going to use the food as a part of a company process or activity in order to produce food.

White paper - http://www.efsa.eu.int/about_efsa/legislation/catindex_en.html;

Tracking defines the ability to track certain food, feed, and animal, grown for food production, or substance, which is aimed or is expected to be included in food or feed at any stage of production, processing and distribution.

It is of major importance that the food consumed by people should be safe and should not cause negative effects on the organism or diseases.

3. Food safety criteria

In order to ensure food to be permitted for consumption, and correspondingly safe for people, it should meet certain criteria as follows:

Food should possess acceptable organoleptic properties (look, colour, taste, smell, consistency etc.);

Food should possess nutrition value defined by containing nutrients (concerning nutrients which can be utilized by human organism);

Food should posses the necessary technological properties (it should be amenable to certain processing heat processing, freezing, liofilisation, etc.);

Food should be epidemiologically and toxicologically safe.

http://www.paho.org/nglish/ad/dpc/vp/alimentos.htm

It is of major importance that the food should not contain toxic substances, since they are potentially dangerous to human health and can cause significant economical social and political consequences. Food technologies development has lead to food safety related problem development consecutively food toxicology as an important part of food safety criteria and evaluation.

4. Biological and chemical contaminants

(http://w3.whosea.org/EN/Section23/Section1001/Section1110.htm)

If contaminated food reaches people a number of infectious or alimentary diseases can be caused. Food contamination may occur in different ways, such as:

Food contamination caused as a result of contact with contaminated surfaces;

The workers taking part in the processing or transportation of food and not meeting the hygiene requirements may contaminate food and thus cause infectious diseases to the consumers;

Sources of food contamination can be: contaminated containers, used for storage and processing of food; food which comes in contact with flies or other insects; parasites; domestic animals; dust; chemical contaminants such as pesticides contaminants, vet chemicals etc.

Raw food materials themselves can also be source of contamination in case that they have been stored and transported in a contaminated medium, thus becoming location for microorganism development. Those, which are pathogenic to human organism, cause number of diseases, classified as food intoxications (Table 1). In case that contaminated food products are consumed by a large number of people, outbreak of an epidemic may occur.

At present, it is accepted that alimentary carcinogenic chemical contaminants and additives, mycotoxins and substances formed as a result of cooking and storing food, cause nearly 50% of the cancer.

Table 1 Diseases caused by food contaminants

Fresh milk	Brucellosis, salmonellosis, enterohaemorrhagic E.coli infection
Cheese	Brucellosis, salmonellosis, listeriosis, S. aureus intoxication
Cream	Salmonellosis, S. aureus intoxication
Meat and meat products	salmonellosis, enterohaemorrhagic E.coli infection, listeriosis, staphylococcal infection, clostridium perfringens - gastroenteritis, botulism, taenioses (T. solium, T. saginatus), trichinelloses (T. spiralis); mad cow disease http://whyfiles.org/012mad_cow/
Poultry	Salmonellosis
Eggs and egg products	Salmonellosis, mycointoxications
Fish and sea food	Botulism, salmonellosis, viral gastroenteritis, histamine intoxication
Rice, pasta and cereals	B. cereus and S. aureus intoxication
Fruit and vegetables	Shigellosis, amoebiasis
Ice-cream	Salmonellosis, S. aureus intoxication
Sweets	Salmonellosis, S. aureus or B. cereus intoxication, clostridium perfringens - gastroenteritis
Chocolate	Salmonellosis
Fresh water	Cryptosporidiosis, lambliosis, amebiasis, E. coli infection, shigellosis, abdominal typhus, hepatitis type A and E
Baby food	Salmonellosis, B. cereus intoxication

Chemical contaminants define substances, which are unnatural to the food contents. They can be toxic by nature or can acquire toxic properties under certain circumstances. They can get into the food from outside or can be formed in the food product during its production, processing and storage. Food may contain **food additives and food contaminants:**

Food additives define substances that normally are not used independently as food or its ingredient and which, after being added to the food during its production, processing, packaging, transportation or storage, remain included in the food, even in changed state.

Food contaminants. These types of chemical substances, also known as food contaminants, comprise substances, which are not deliberately added to the food.

They can be found in the food as a result of the nature pollution. They can also get in the food during the agricultural process production, processing, storage, packing, and transportation.

Some substances though, are deliberately included in food. This refers to pesticide contaminants in plants and animal products; vet chemicals in animal products, etc. Chronic and acute poisoning may occur as a result of consumption of food, which contains contaminants in amounts exceeding the legally defined limits.

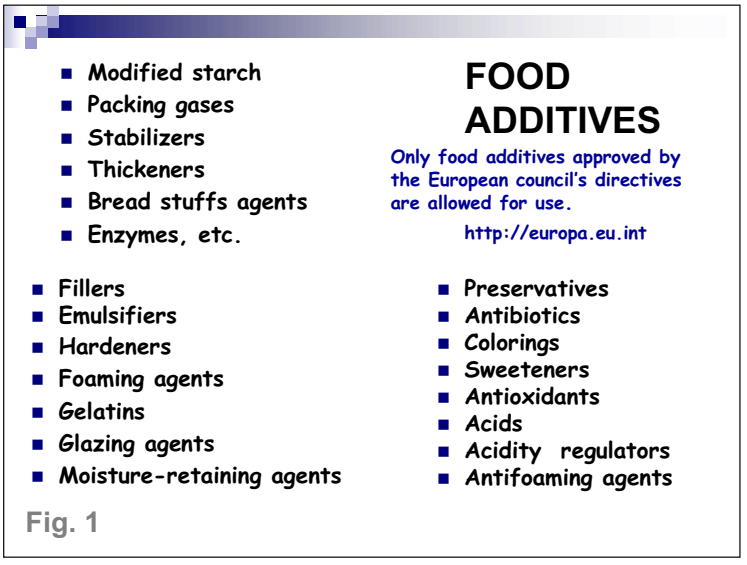


Fig. 1

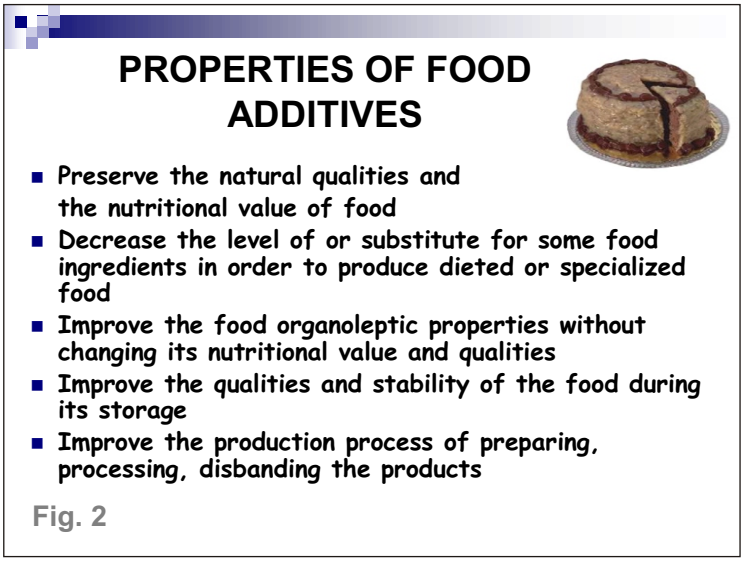


Fig. 2

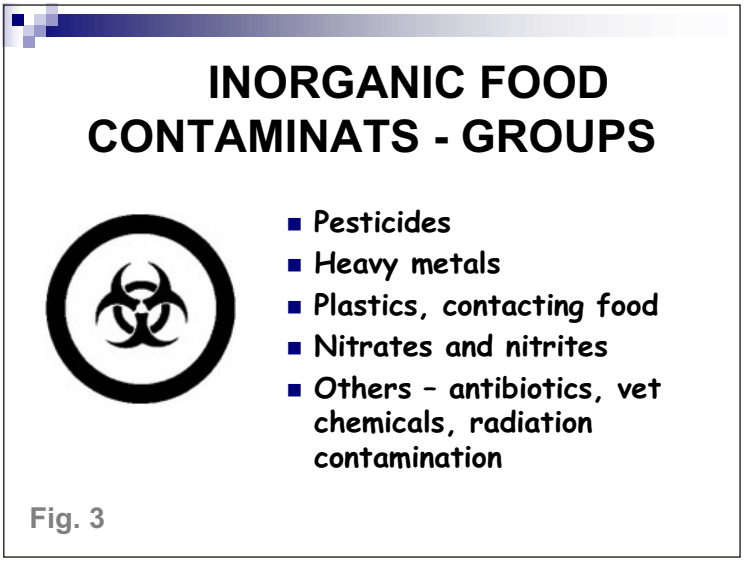


Fig. 3

In order to produce and distribute safe food, it is of major importance to clarify, which types of contaminants influence negatively on human organism.
These are:

Chemical substances, used in the food production process, such as:

- Pesticide contaminants. Stimulants, used in plant-growing and animal-growing (in cases that non-certified feed, medicines and hormones are used);
- Ferments, smoke and such chemical substances used in the food companies toxic substances can be formed as a result of not observing the regulations, concerning heat processing, smoking of meat (benzpirens and nitrosamines are formed), ionizing radiation, etc.

Chemical substances from outside, coming in contact with food:

- Heavy metals and benzpirens;
- Plastics used to contact food;
- Detergents and disinfectants;
- Radioactive substances;
- Additives.

According to their origin, contaminants can be divided into the following categories:

Antropogenous result of human activities leading to contamination anywhere in the food chain;

Natural contamination, e.g. as a result of high natural concentration of toxic elements in the soil;

Substances, formed as a result of food cooking.

5. Food safety evaluation procedure

In order to prevent the toxicological risk from chemically contaminated food, world organizations such as FAO, WHO, EU and their committees JECFA, as well as FAO/WHO together, have brought in resolutions and directives, determining the basic requirements for food safety. European Food Safety Authority (EFSA) has also been established with its particular responsibilities for both risk assessment and communication on food safety issues. The principle objective of EFSA is to contribute to a high level of consumer health protection in the area of food safety, trough which consumer confidence can be restored and maintained.

http://www.who.int/foodsafety
http://www.efsa.eu.int/about_efsa/legislation/catindex_en.html
http://www.centerforfoodsafety.org/
http://www.foodsafety.gov/
http://europa.eu.int
http://www.fao.org/index_en.htm

It is important to clear out that the EU requirements on food safety are always based on risk analysis. Risk analysis helps for the free movement of food before taking preventive measures, and also helps to avoid obstacles for free movement of food. This is in power when food legislation aims to decrease and avoid health hazard. Risk analysis comprises of three interrelated components: risk evaluation, risk management, information exchange on risk.

Risk defines the function of the probability of negative effects on health and the dimension of these effects, as a result of present hazard;

Risk analysis defines a process, comprising of three interrelated components: evaluation, risk management and information exchange on risk;

Risk evaluation defines scientifically based process, comprising of 4 stages: definition of hazard; characteristics of hazard; evaluation of possible influences of exposure to hazard; risk definition (description);

Risk management defines a separate process of considering different strategies and consultations with interested parties, discussions on risk evaluation, etc., and, if necessary, choosing suitable opportunities of control and prevention;

information exchange on risk defines interactive exchange of information and possibilities during the risk analysis process, concerning hazard, risk and the corresponding risk factors and the estimation by people who are in charge of the evaluation and the management of risk, the consumers, the food and feed companies, the academic society and other interested parties, including clarifying the conclusions, concerning the risk and the reasons for taking particular decisions on management.

Risk analysis gives solid base for defining effective and aimed measures and actions which will protect the consumers' health. In certain situations, provided that there is health or life hazard and lack of relevant scientific basis, protective measures give way for risk management or actions which must guarantee for public health protection.
The food safety insurance process comprises three basic steps:

Food safety evaluation procedure;
Toxicological data surveying (risk identification and characteristics);
Regulations to insure food safety

6. Food safety evaluation

Legislation: According to WHO, the toxicological properties of food additives and contaminants is defined after testing them in order to calculate "Maximum" daily dose;
Testing plan while conducting the toxicological evaluation of food, it is important to establish the dose-response effect. Further on, the latter serves as basis for calculation of limitation for concentration which does not cause toxic effects.
Food safety evaluation tests are always based on:

A) Hygiene-toxicological experiment, which needs to define:
   a) Acute Toxicity:
   Includes toxokinetics, metabolism and possible chemical reactions with food components. Experiments are conducted using laboratory animals (rats, mice, guinea pigs, rabbits, etc.), which take a single high dose of a toxic substance. The animals are being observed for 14 days. The average lethal dose LD50 is defined;
   b) Subacute Toxicity:
   The experiment uses 2 species at least. The substance is introduced in lower doses and its effects are observed on laboratory animals for 40-90 days. FAO/WHO propose testing periods as follows: 90 days for rats and 1 year for dogs. The experiment gives information on cumulative properties of different substances (cummulation coefficient can be calculated), the toxic effect properties, sensitivity of species, target organs, dose-response effects;
   c) Chronic Toxicity:
   Includes reproduction, embryotoxicity, teratogenicity, carcinogenicity, mutagenicity and allergic effects. The experiment requires multiple introduction of the toxic substance in low doses on 2 kinds of animals at least. The animals are being observed for 10 months to 1 year. The experiment gives information on maximum inefficient dose:
      Genotoxicity - the experiments are conducted in vitro or in vivo for short periods of time; probable mutagenous effects of the substance are followed;
      Carcinogenicity the experiments last for 2 years and uses animals which belong to one species, both male and female. The aim is to establish and follow probable neoplasm growth in the laboratory animals;
      Reproductive toxicity single doses of the test substance are applied to both male and female animals (at certain stages of pregnancy in female). Fetotoxicity is observed in the lab animals, also lactation changes and postnatal changes are followed;
      Provided that the test substance gives indications for being toxic in any way, further experiments are conducted. They are aimed to determine probable teratogenic effects, immunotoxicity; neurobehavioral toxicity, etc ;
   d) Maximum daily dose: it is calculated after gathering results from the tests.

B) Granting the data on possible negative effects on the food industry employees' health and working out rules for labour protection;

C) Data and requirements in other countries;

D) Evaluation of an acceptable daily consumption of certain additives and contaminants in all food, including consumers with special needs.

7. Toxicological data surveying

After gathering toxicological data, the maximum daily dose is calculated (MDD). MDD of different contaminants and additives in food defines the amount of the substance, presented in mg/kg, which can be consumed every day for the whole life without appreciable health hazard.

8. Regulations concerning food safety

It is of major importance to all countries that the food safety measures, undertaken by the EU-countries, should be based on risk analysis, except for the cases when this is inexpedient because of the situation or the measure itself. Using risk analysis before accepting such measures eases the free movement of food to escape baseless obstacles.
There is a White Paper on food, giving data on food safety, presented by the European Commission.
http://europa.eu.int/comm/dgs/health_consumer/library/pub/pub06_en.pdf
There has already been extensive consultation and discussion concerning improvements to the EU's food legislation arising from the Green Paper on the general principles of food law.
(http://europa.eu.int/comm/off/green/index_en.htm)
The White paper presents the changes the Commission proposes in this area. However, in addition, the Commission envisages the creation of a European food authority as a further measure. In respect of this proposal, the Commission wishes to elicit public debate, informed comment and broad consultation.
Provided that the food legislation is aimed to decreasing, removing or escaping the health hazard, the systematic methodology of effective appropriate and aimed measures for health prevention is based on risk analysis (risk evaluation, risk management, information exchange).
Protective measures used to be applied in order to provide the community health, though they were an obstacle in the free movement of food. This is why the European Union aspires to united basis in the whole union, in order to apply these principles.

9. Conclusion

Food safety and customer protection are of growing concern to the society, non-governmental organizations, international trade partners and commercial organizations. It is necessary the customers' and the trade partners' trust to be guaranteed since it is of major importance and it is conducted through open and transparent food legislation as well as through undertaking relevant actions by the public institutions on informing the society. It has been proved that diseases caused by contaminated food consumption incur negative effect on business, global economy and life standard. Each year hundreds of people get sick as a result of food intoxication and each year food manufacturing companies refund large sums in order to compensate the customers.

10. Five keys to safer food

WHO introduced the Five Keys to Safer Food poster in 2001, translated in 25 languages, (for Bulgaria adapted by M. Stavreva). The WHO Five Keys to Safer Food are simple rules elaborated to promote safer food handling and preparation practices: keep clean, separate raw and cooked food, cook thoroughly, keep food at safe temperatures, use safe water and raw materials.
(http://www.who.int/foodsafety/consumer/5keys/en/print.html)

KEY 1: KEEP CLEAN (prevent the growth and spread of dangerous microorganisms)

Wash your hands with soap and water (or other means such as wood ashes, aloe extract or dilute bleach) after toilet visits, before and after handling raw food and before eating;
Avoid preparing food directly in surroundings flooded with water;
Wash/sanitize all surfaces and equipment - including hands - used for food preparation;
Protect kitchen areas and food from insects, pests and other animals;
Keep persons with diarrhoea - or other symptoms of disease - away from food preparation areas;
Keep faecal material away from food-preparation areas (separate kitchen and toilet areas);
Avoid eating food raw if it may have been flooded, e.g. vegetables and fruits - see also Key 5.

Why?
Dangerous microorganisms are widely found in the gut of animals and people and therefore also in water and soil in areas with poor sanitation as well as in areas with flooding. These microorganisms can be transferred to food and can, even in low numbers, cause foodborne disease.

KEY 2: SEPARATE RAW AND COOKED FOOD (prevent the transfer of microorganisms)

Separate raw meat, poultry and seafood from ready-to-eat foods;
Separate animal slaughtering and food preparation areas;
Treat utensils and equipment used for raw foods as contaminated - wash and sanitize before other use;
Separately store raw (uncooked) and prepared foods;
Avoid contamination with unsafe water: ensure water used in food preparation is potable or boiled;
Peel fresh fruits before eating.

Why?
Raw food, especially meat, poultry and seafood and their fluids may contain dangerous microorganisms that can be transferred onto other foods during food preparation and storage. Prevent the transfer of microorganisms by keeping raw and prepared food separate. Remember that cooked food can become contaminated through the slightest contact with raw food, unsafe water or even with surfaces where raw food has been kept.

KEY 3: COOK THOROUGHLY (Kill dangerous microorganisms)

Cook food thoroughly, especially meat, poultry, eggs and seafood until it is steaming hot throughout;
For cooked meat and poultry to be safe their juices must run clear and no parts of the meat should be red or pink;
Bring foods like soups and stews to boiling and continue to boil for at least 15 minutes to make sure all parts of the food has reached at least 70°C;
While cooked food should generally be eaten immediately, if necessary thoroughly reheat cooked food until it is steaming hot throughout.

Why?
Proper cooking kills dangerous microorganisms. The most important microorganisms are killed very quickly above 70°C, but some can survive up to 100°C for minutes. Therefore all cooked food should generally reach boiling temperatures and be cooked at such temperatures for extended periods. Remember that big pieces of meat will only heat up slowly. It is also important to remember that in emergency situations with the potential for significant contamination levels in food, the food should be cooked for longer periods.

KEY 4: KEEP FOOD AT SAFE TEMPERATURES (prevent growth of microorganisms)

Eat cooked food immediately and do not leave cooked food at room temperature longer than 2 hours;
Keep cooked food steaming hot (more than 60°C) prior to serving;
Cooked and perishable food that cannot be kept refrigerated (below 5°C) should be discarded.

Why?
Microorganisms multiply quickly if food is stored at ambient temperature - the multiplication is quicker the higher the temperature - and quickest at around 30-40°C. The higher the number of microorganisms in the food the higher the risk for foodborne disease. In general discard food that cannot be eaten within 2 hours - if necessary, food should be kept really hot or really cold. Most microorganisms cannot multiply in food which is too hot or too cold (higher than 60°C or lower than 5°C).

KEY 5: USE SAFE WATER AND RAW MATERIALS (prevent contamination)

Use safe water or treat it to make it safe - e.g. through boiling or treatment with chlorine tablets;
Wash or preferably cook vegetables and peel fruits that are eaten raw;
Use clean containers to collect and store water and clean utensils to dispense stored water;
Select fresh and wholesome foods - discard damaged, spoiled or mouldy food;
Breast-feed infants and young children at least up to the age of 6 months.

Why?
Raw materials, including water, may be contaminated with microorganisms and dangerous chemicals, especially in areas hit by flooding. Likewise the risk of vegetables and fruits being contaminated with water containing sewage is high under a flooding disaster. Toxic chemicals may be formed in spoiled and mouldy foods. Safe water may be seriously contaminated with dangerous microorganisms through direct contact with hands or unclean surfaces. Breast-feeding protects infants against diarrhoea through its anti-infective properties, and minimizes their exposure to dangerous foodborne microorganisms.

References

1. Application of Risk Analysis to Food Standards Issues, Rep[ort of the Joint FAO/WHO Expert Consultation, WHO, Geneva, 1995, 37
2. Codex Alimentarius, Volume I, A General Requirements, FAO/WHO, Rome, 1995,245
3. Codex Alimentarius, Vol. XIV, Supplement 2. Directive pour l'evaluation simplifiee de l'ingestion d'additifs alimentares, OMS, Rome, 1989, 24
4. Conson J.M. Food toxicology (in two parts), Marcel Dekker, INC, New York and Basel, 1988, 1349
5. WHITE PAPER ON FOOD SAFETY, 2000

Chapter 7

Topic 34

Nutrigenomics

Nutrigenomics

Topic 34

Module 34.1

Nutrigenomics - New Research Approaches

Varban Ganev

Learning Objectives

To give students an overview of the development of nutrigenomics as a multidisciplinary field of medical science and practice;
Defining nutrigenomics as an integrative science based on new research approaches;
Introducing basic terms on nutrigenomics;
Introducing basic technologies and research approaches used in nutrigenomics.

Contents

1. Introduction to nutrigenomics
2. Scientific disciplines contributing to nutrigenomics
3. The new technologies
4. Sophisticated bioinformatics approaches
5. Transgenic mouse models and cellular models
6. Detecting the two hits
7. The second strategy - systems biology approach
8. Goals of nutrigenomics research

Key Messages

The basics of genetics, genomics and gene regulation with relation to diet;
The basic approaches: Transcriptomics, Proteomics, Metabolomics, Epigenetics;
New genomics technologies;
General approaches: Hypothesis-driven approach, systems biology approach;
The concepts of nutrigenetics (genetic susceptibility, SNPs, polygenic (complex) diseases, "personalized" diet).

1. Introduction to Nutrigenomics

Nutritional genomics ("nutri-genomics") is a newly emerging multi-disciplinary field of medical science and practice. It is an integrative science based on new research approaches.
The knowledge of nutrigenomics is increasingly becoming a powerful tool for the medical professionals to maintain human health and prevent/control common chronic diseases.
Nutrigenomics emerged and is developing on the basis of the most recent advances in biomedical technologies and our ever increasing knowledge in molecular basis of the fine interactions between the environment and the human genome.

Research in nutrigenomics includes various aspects of:

- transcriptomics - transcription factors and dietary signaling routes;
- genomics - tools and how to apply them;
- bioinformatics and how to make nutrigenomics data useful;
- nutrigenetics and sensitizing genotypes;
- use of models: animal and human studies;
- relationships between nutrigenomics and complex diseases;
- dietary stress and inflammation;
- search for molecular biomarkers with nutritional systems biology;
- nutrigenomics and development of functional foods;
- nutrigenomics and personalized diets;
- regulatory, legal and ethical issues of nutrigenomics, etc.

NUTRIGENOMICS
(NUTRITIONAL GENOMICS)

Integrative science, that seeks to provide a genetic and molecular understanding for how common dietary chemicals affect the balance between health and disease by altering the expression and/or structure of an individual's genetic makeup.

Dietary chemicals include nutrients and bioactive chemicals that do not directly produce energy but exclude man-made chemicals such as pesticides (toxicogenomics).

Fig. 1

WHAT IS NUTRIGENOMICS AND HOW DOES IT RELATE TO ME?

The science of nutrigenomics seeks to provide a molecular understanding for how common dietary chemicals (i.e., nutrition) affect health by altering the expression and/or structure of an individual's genetic makeup.

Fig. 2

Five Tenets of Nutrigenomics:

- **Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases.**
- **Common dietary chemicals can act on the human genome, either directly or indirectly, to alter gene expression or structure.**
- **The degree to which diet influences the balance between healthy and disease states may depend on an individual's genetic makeup.**
- **Some diet-regulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases.**
- **Dietary intervention based on knowledge of nutritional requirement, nutritional status, and genotype (i.e., "personalized nutrition") can be used to prevent, mitigate or cure chronic disease.**

Fig. 3

NUTRIGENOMICS

The sum total of association and laboratory studies show that diet and the chemicals in diet, influence physiological processes. This is achieved by altering the expression (or structure) of a subset of genes in the human genome.

Dietary chemicals have been shown to alter gene expression in a number of ways. For example, they may:

- **act as ligands for transcription factor receptors],**
- **be metabolized by primary or secondary metabolic pathways thereby altering concentrations of substrates or intermediates or**
- **serve as signaling molecules.**

Fig. 4

Nutrigenomics is the field of knowledge produced by the application of high throughput genomics tools in nutrition research.

Applied wisely, it will promote an increased understanding of how nutrition influences metabolic pathways and homeostatic control; how this regulation is disturbed in the early phase of a diet-related disease and to what extent individual sensitizing genotypes contribute to such diseases. Ultimately, nutri-genomics will allow effective dietary-intervention strategies to recover normal homeostasis and to prevent diet-related diseases (1).

Exogenous nutrients can affect gene expression directly (A) or indirectly (B and C): (5)

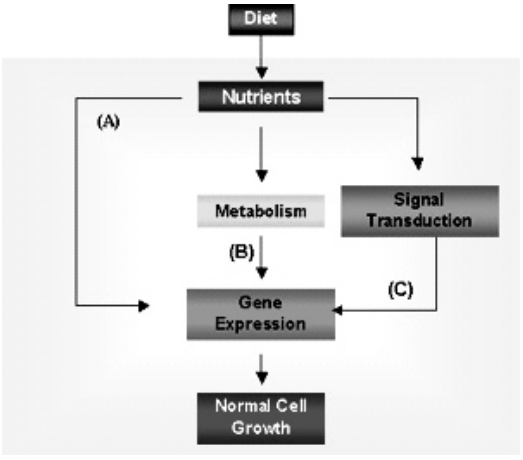


Fig. 5

2. Scientific disciplines contributing to nutrigenomics

Nutrigenomics is an **integrative science** that seeks to provide a genetic and molecular under-standing for how common dietary chemicals affect the balance between health and disease by altering the expression and/or structure of an individual's genetic makeup.

In the past decade, nutrition research has undergone an important shift in focus from epidemiology and physiology to **improved knowledge in its molecular and genetic basis**.

This is mainly a result of three factors that have led to a growing realization that the effects of nutrition on health and disease cannot be understood without a profound understanding of how nutrients act at the molecular level (1):

the completion of several large genome projects has markedly altered the research agenda by drawing attention to the importance of genes in human nutrition, and has provided a wealth of new genetic information to be explored;

there has been a growing recognition that micronutrients and macronutrients can be potent dietary signals that influence the metabolic programming of cells and have an important role in the control of homeostasis;

nutrition researchers have increasingly started to recognize that genetic predisposition can be an important contributor to the main causes of mortality that are linked to diet, such as cardiovascular disease, diabetes type II and cancers.

Nutrigenomics covers a wide range of technologies concerned with elucidating how the genetic programme operating in cells and tissues is potentially influenced by diet. John Mathers noted three possible definitions for nutri-genomics (2):

"… the application of high throughput genomics tools in nutrition research"

"… seeks to examine 'dietary signatures' in cells, tissues and organisms and to understand how nutrition influences homeo-stasis"

"… the interface between the nutritional environment and cellular/ genetic processes".

Exogenous nutrients can affect gene expression directly or indirectly:

This was convincingly demonstrated by comprehensive investigation of yeast gene expression using microarrays

A diauxicshift from fermentation respiration resulted in, metabolic reprogramming that identified genes previously unassociated with nutritient utilization.

Although more complex than yeast, we believe the constellation of genes that make up the human genome respond in a similar fashion to the dietary chemicals.

Hence, certain dietary chemicals and/or other environmental factors may alter expression of a given genotype thereby introducing additional phenotypic variation into association analyses.

Fig. 6

All of these draw certain parallels with other "-omics" disciplines, particularly pharmacogenomics and pharmacogenetics, but the comparison cannot be pushed too far.

Nutrigenomics faces complications that the other two areas do not face, notably the length and the complexity of exposures. Integration of the "-omics" disciplines with the science of nutrition and other recently emerged disciplines is leading to identification and understanding of individual and population differences and similarities in gene expression, or **phenotype**, in response to diet (Fig. 7).

Scientific disciplines contributing to nutrigenomics.

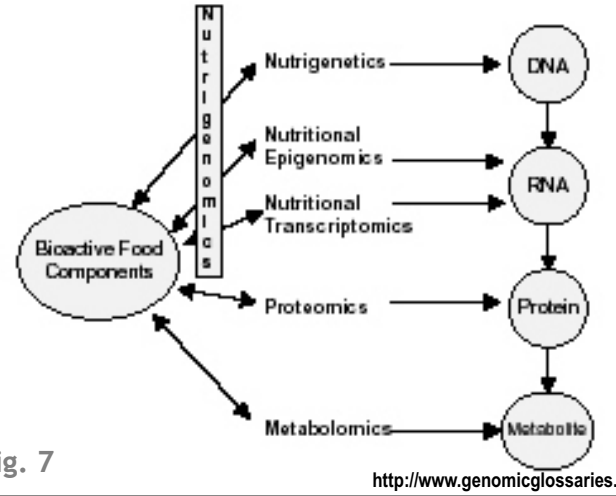


Fig. 7

http://www.genomicglossaries.com

The food components have influences on health that can be explained by investigation of changes in gene expression, in the translation of messages into proteins and then in the metabolites that are manufactured (2). Nutrigenomics has emerged as new technologies, such as transcriptomics, proteomics, metabolomics, and epigenomics have added more complex functional analysis to the basic sequence information provided by the Human Genome Project.

Transcriptomics, for example is a very valuable way of beginning to understand how nutritional exposure influences gene expression on a genomic scale. It is possible to group genes of interest for particular metabolic processes and capture information from all of these at once to see how the cell is functioning at any given time or under certain conditions.

Proteomics investigate differential protein expression again under different conditions or with different underlying pathology. The presence or absence of certain key proteins can give information about the early stages of disease.

Metabolomics examines global patterns of metabolites present in the cell or in body fluids in response to specific dietary exposures.

Epigenetics is the study of modifications to the genome which are copied from one cell generation to the next but which do not involve changes to the primary sequence. These changes, mediated through modification of chromatin proteins such as histones and through the methylation of DNA, contribute to the regulation of transcription and provide a way for the genome to "learn from experience", regulating gene expression in response to dietary and other exposures and leading to altered cellular phenotypes associated, for example, with chronic disease or ageing.
All of these "-omics tools" (for more on "-omics" one can visit www.genomicglossary.com) have been used to study in detail the molecular responses to food substances or the early stages of disease in common diet-related conditions. Who will be susceptible to disease and who will be responsive to dietary modification? At the simplest level, we know, for example, that there are differences in nutrient requirements which for several nutrients follow a normal distribution, with some individuals having very high requirements while others need much lower levels than average. At the heart of individual variation is the 0.1% of variation between the DNA sequences of any two individuals. Much of this variation is accounted for by single nucleotide polymorphisms (SNPs), which are largely responsible for differences in complex characteristics such as the way in which we respond to our environment. These differences are likely to apply throughout life, from the early uterine environment right through to different ageing responses later in life.

3. The new technologies

In the heart of the "-omics", integrated in nutrigenomics are the new molecular **technologies** that have allowed increasingly detailed molecular studies of nutrition.
Subtle changes in gene expression, even at the single-cell level, can now be measured by quantitative techniques such as real-time PCR (Fig. 9) and high-density micro array analysis (Fig. 10). The latter allows the entire nutrition-relevant transcriptome to be studied simultaneously.
Comparable progress in the analysis of the nutrition-relevant metabolome (metabolomics), and the nutrition-relevant proteome (proteomics) should soon allow the analysis of the response of whole systems to nutrients, from genes to organisms.
Indeed, the accurate analyses of the piles of new information coming from the new "-omics" technologies is closely related to the advances in bioinformatics to face the challenges in interpreting the enormous quantities of data generated, with further issues in the areas of data archiving and sharing (Fig. 12). In the future, studying organism responses to particular dietary components at the metabolome, proteome and transcriptome levels will hopefully show valuable organ-specific patterns. An ambitious challenge for the next decade is to translate this type of nutrigenomics data into an accurate prediction of the beneficial or adversary health effects of dietary components.

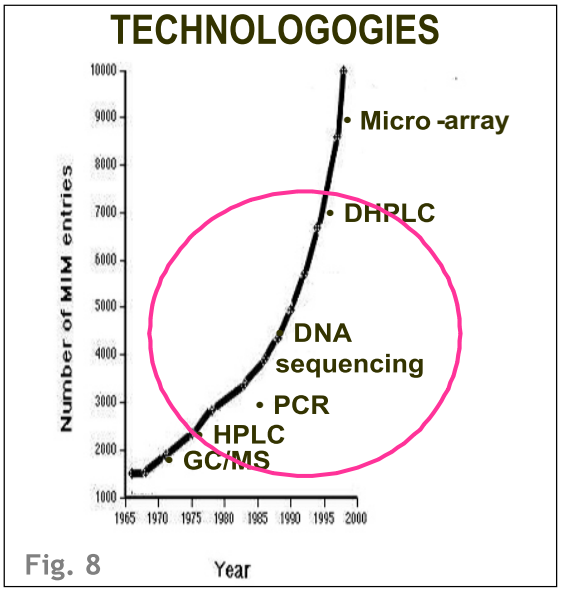


Fig. 8

All of these technologies have so far been applied mostly to cells in culture and to certain model organisms (2). The challenge will come in applying them to people, where problems both of study design and ethics will be encountered. In addition, with human beings, our means of establishing accurate dietary exposures are imperfect and this is compounded by the need for observations over long time periods, by problems of access to the target tissues and of heterogeneity of target tissues (in other words even small samples will contain a large number of different cell types). At the experimental level, standardisation of assays presents further problems.

3.1 Real-time PCR

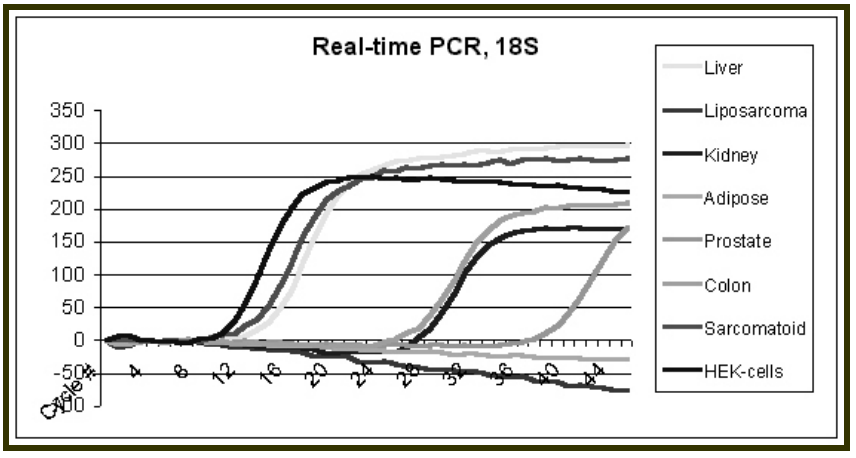


Fig.9 Real-time PCR: allows quantification of specific RNA molecules (analysis of gene expression; transcripts).

3.2 High-density micro array analysis

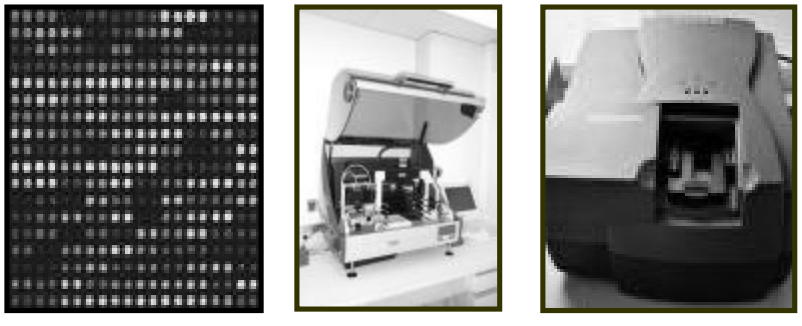


Fig.10 High-density micro array analysis: allows simultaneous detection of thousands of RNA transcripts (transcriptoms) or changes at the level of DNA (genomics).

3.3 Metabolome (A) and proteome (B and C) analyses

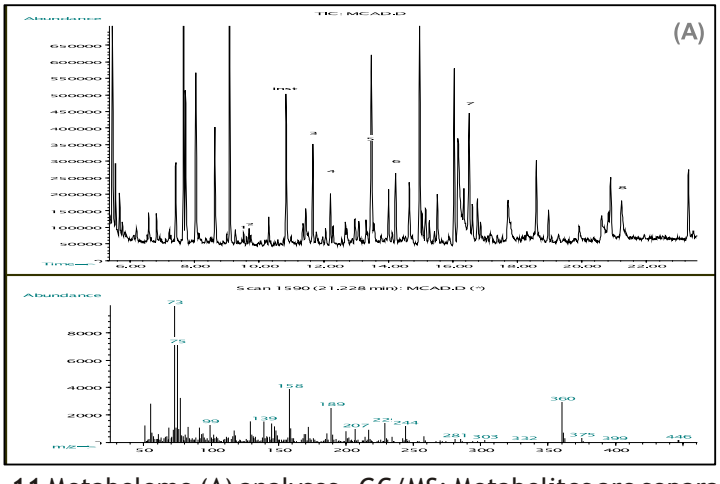


Fig. 11 Metabolome (A) analyses - GC/MS: Metabolites are separated by gas chromatography and analyzed by mass-spectroscopy.

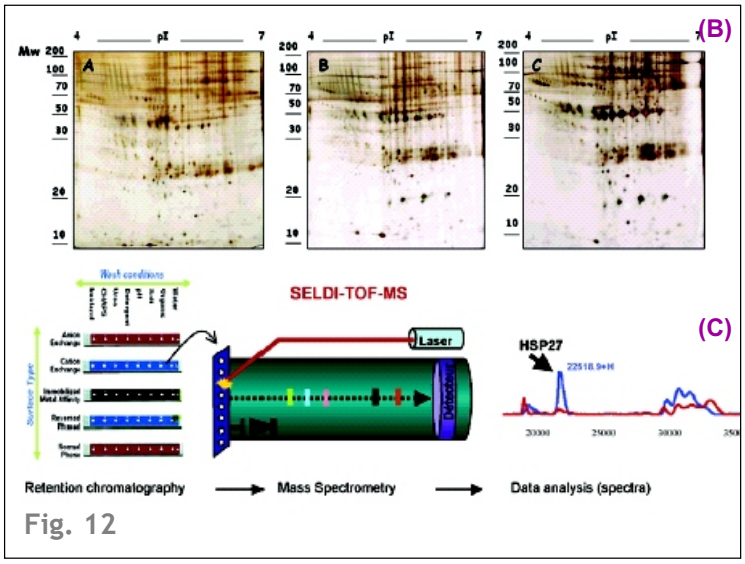


Fig. 12

Fig. 12 Proteome (B) 2-DE analysis of proteins on silver stained gels. The protein spots are recorded as digitalized images using a desktop scanner and imported to a database for matching and detection of specific proteins. (C) SELDI-TOF MS analysis of protein extracts.

Proteins are captured on a solid-phase protein chip (left part). After adding a matrix solution, proteins are ionized with a nitrogen laser and their molecular masses measured by TOF MS (middle). Protein profile is being obtained and analyzed.

4. Sophisticated bioinformatics approaches

Analysis of huge amount of data requires sophisticated bioinformatics approaches. (Fig. 12).
Furthermore, nutrigenomics aims to identify the genes that influence the risk of diet-related diseases on a genome-wide scale, and to understand the mechanisms that underlie these genetic predispositions.
Genomics tools can be used in two different, but complementary, strategies in molecular nutrition research.

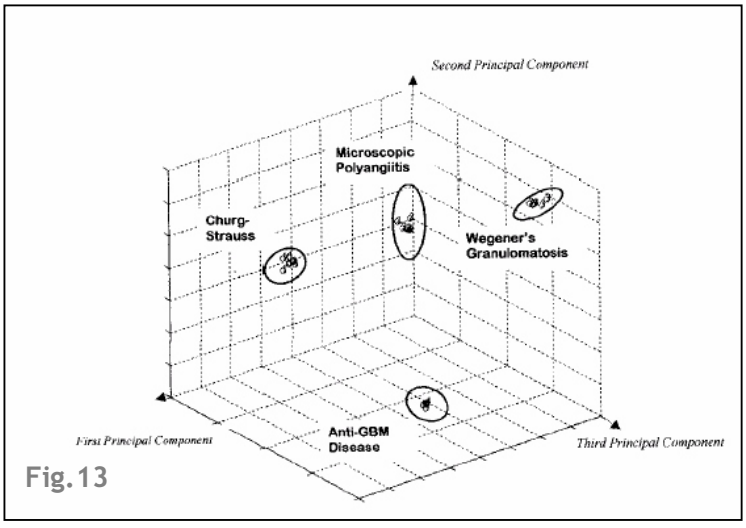


Fig. 13

The first strategy is the traditional **hypothesis-driven approach**: specific genes and proteins, the expression of which is influenced by nutrients (Fig. 13), are identified using above genomics tools - transcriptomics, proteomics and metabolomics - which subsequently allows the regulatory pathways through which diet influences homeostasis to be identified.

5. Transgenic mouse models and cellular models

These models are essential tools in this approach. In future, such models might provide the key to understanding the interactions between metabolic and inflammatory signalling routes (Fig. 14).

One example is the use of **knockout mice for nutrition research**.

To raise nutrigenomics above the level of purely descriptive data, we must understand how food components regulate gene or protein expression. For this purpose, mutant mice (particularly knockout mice) have become an invaluable tool. Using knockout mice, we can unambiguously establish how a particular transcription factor mediates the effect of a specific nutrient: a goal that is impossible to achieve in human studies. In combination with cell-culture studies, the use of knockout mice will greatly contribute to the generation of detailed molecular pathways showing how nutrients regulate gene and protein expression.
Recent studies investigating how polyunsaturated fatty acids (PUFAs) influence lipid metabolism elegantly show the power of knockout models. PUFAs usually stimulate the expression of several genes that are involved in fatty-acid oxidation. However, peroxisome proliferator-activated receptor-α (PPAR-α)-null mice lack this response. In these mice, PUFAs suppress the expression of genes that are involved in lipogenesis. Studies with the same mice showed that PPAR-α is not the nutrient sensor that mediates the lowering of plasma triglyceride levels induced by fish oil.
Similarly, retinoic-acid receptor knockout mice have provided insights into the molecular mechanism of vitamin A action. These mice mimic the symptoms of vitamin A deficiency and are, therefore, important tools for the study of the genomic effects of vitamin A. The increasing number of available knockout (or knockdown) mice should allow us to investigate many more nutrient signalling pathways.

6. Detecting the two hits: pro-inflammatory and metabolic stress (from (1))

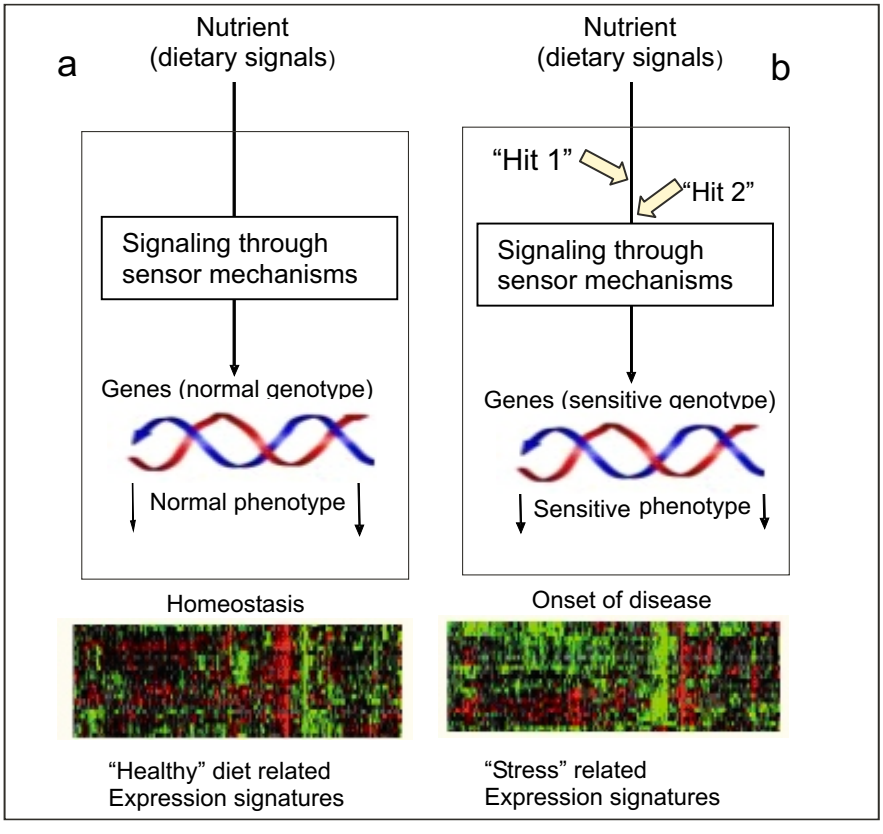


Fig.14 Detecting the two hits: pro-inflammatory and metabolic stress (1)

Cells are regularly exposed to stress, which mainly consists of inflammatory stress and metabolic stress. Inflammatory stress is exerted by cytokines that are released in large quantities by immune cells in response to invading microorganisms.

Cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 induce the hepatic acute-phase response which consists of local and systemic reactions and is accompanied by up regulated or down regulated synthesis and/or activation of liver-enriched transcription factors.
Cytokines promote the synthesis of acute phase proteins, in part by down regulating nuclear receptors, such as peroxisome proliferator activated receptor-α (PPAR-α), which suppress the expression of genes encoding acute-phase proteins such as serum amyloid protein and C-reactive protein.
However, this inflammatory response is a double-edged sword, particularly if it is chronic. Pro-inflammatory cytokines can induce cytotoxicity that, in the worse-case scenario, can lead to liver failure. Pro-inflammatory stress is directly linked to an immune response, whereas metabolic stress describes changes in the plasma and/or cellular concentration of nutrients and metabolites, which might lead to the disruption of cellular function.
One important group of compounds that cause metabolic stress is lipids, or more specifically fatty acids. In healthy individuals, the negative-feedback system that is mediated by PPARs acting as nutrient sensors (see discussion in the main text) can deal with fluctuations in free fatty-acid levels in the plasma (panel **a**). However, in individuals with conditions such as diabetes and obesity that cause permanently elevated plasma levels of free fatty acids (metabolic stress; 'hit one'), who then, as part of an immune response, have cytokine-induced down regulation of PPAR-α and other nuclear receptors (pro-inflammatory stress; 'hit two'), the system is overtaxed (panel **b**). In this case, fatty acids accumulate as triglycerides and spill over into harmful pathways. If triglycerides accumulate in non-adipose tissues, the individual's sensitivity to proinflammatory stress will increase further and might lead to significant organ dysfunction.
For example, a combination of excess fat storage and inflammatory stress in the liver can ultimately result in cirrhosis. In future, nutrigenomics tools should allow the collection of 'healthy' diet-related expression signatures as appropriate baseline data (panel **a**). By comparing these signatures with 'stress' signatures (panel **b**) that are derived from nutrigenomics experiments, we might be able to identify early molecular biomarkers for individuals with sensitive genotypes under sustained metabolic and pro-inflammatory stress that could lead to serious conditions such as cirrhosis or insulin resistance. With enough early warning, dietary intervention might reverse this process, regain homeostatic control and prevent these conditions in at-risk groups.

7. The second strategy - systems biology approach

The second strategy, which is largely theoretical at this stage, is the systems biology approach: gene, protein and metabolite signatures that are associated with specific nutrients, or nutritional regimes, are catalogued, and might provide 'early warning' molecular biomarkers for nutrient-induced changes to homeostasis (Fig. 14). The first strategy will provide us with detailed molecular data on the interaction between nutrition and the genome, whereas the second strategy might be more important for human nutrition, given the difficulty of collecting tissue samples from 'healthy' individuals.
The second strategy is based on *in vitro* experiments using tools such as inducible expression systems, transdominant negative adenoviral constructs and RNA interference (RNAi), and belongs to the main investigative strategies (Fig. 15). The use of laser-capture microdissection for single-cell gene-expression profiling should greatly improve the cell-specific information that is derived from nutrition experiments with intact organisms (*in vivo*). In addition, primary cells and cell lines are wonderful tools for studying the effects of nutrients on gene expression; however, sometimes cell lines display large differences in the expression of important transcription factors compared with primary cells or *in vivo*.

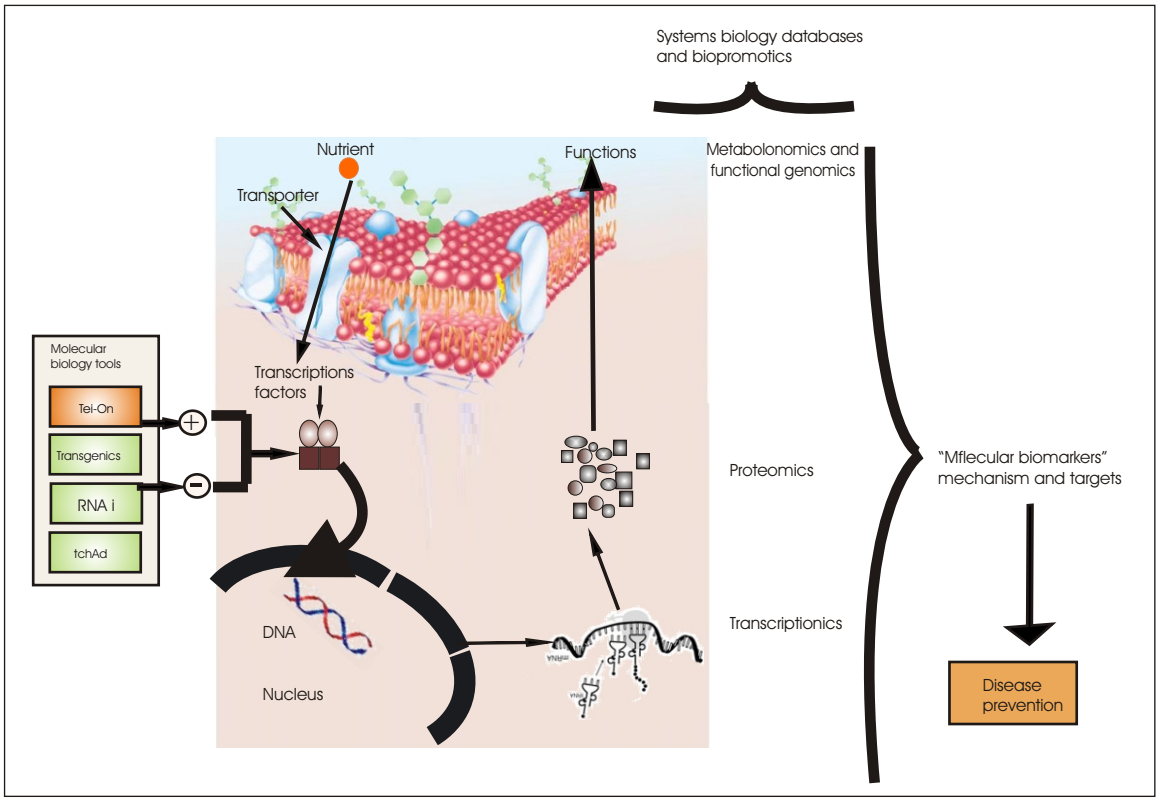


Fig.15 Molecular biology tools, such as transgenic animal or cell models, RNAi, transdominant negative adenoviral constructs (tdnAd) and inducible gene-expression systems will be used to modulate the expression levels and functionality of nutrient sensor systems (1)

This will allow the discovery of dietary target genes and the characterization of the mechanisms that underline dietary sensing. Nutritional systems biology will take advantage of the combination of "-omics", to identify molecular biomarkers. These biomarkers will allow early dietary intervention to reverse the onset of diet-related diseases and to regain homeostasis.
Functional genomics and proteomics approaches, in conjunction with metabolic control analysis are increasingly used to study the metabolic status of cells in an effort to understand the metabolic effects of specific perturbations at the gene and protein level.
Systems biology aims to understand phenotypic variation and build comprehensive models of cellular organization and function. It also seeks to elucidate the interaction and functions of cellular, organ and even organism-wide systems. The optimism for using systems biology in nutrition research relates to the implementation of metabolomics, which allows the extensive, sensitive and rapid measurement of metabolic profiles in blood or organ samples.
We do not know the function of most of the 35,000 - 40,000 genes, >100,000 proteins and several thousand metabolites in humans. We are also dealing with complex genotypes of polygenic diseases. The **integration of all these data will require both intellectual and financial investments** in analytical platforms, dataware housing, laboratory information-management systems, new database structures, algorithms and so on.

8. Goals of nutrigenomics research

Keeping in mind the above described two broad strategies, the **following goals of nutrigenomics research can be defined** (1):

the identification of transcription factors that function as nutrient sensors (See module 34.2) and the genes they target;

the elucidation of the signaling pathways involved, and characterization of the main dietary signals;
the measurement and validation of cell- and organ-specific gene expression signatures of the metabolic consequences of specific micronutrients and macronutrients;
the elucidation of the interactions between nutrient-related regulatory pathways and proinflammatory stress pathways, to understand the process of metabolic disregulation that leads to diet-related diseases;
the identification of genotypes that are risk-factors for the development of diet related human diseases (such as diabetes, hypertension or atherosclerosis) and quantification of their impact;
the use of nutritional systems biology to develop biomarkers of early metabolic deregulation and susceptibility (stress signatures) that are influenced by diet.

References

1. M. Müller and S. Kersten (2003) Nutrigenomics: Goals And Strategies. Nature Reviews - Genetics: 4, 315-322.
2. J. Mathers (2005) The Science Of Nutrigenomics. In: NUTRIGENOMICS - Report of a workshop hosted by The Nuffield Trust and organized by the Public Health Genetics Unit on 5 February 2004. Compiled by H. Burton and A. Stewart. The Nuffield Trust, 6-8.
3. N. Fogg-Johnson and J. Kaput (2003) Nutrigenomics: An Emerging Scientific Discipline. Food Technology: 57, 4, 60-67.
4. R. Jaenisch and A. Bird (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature Genetics Suppl.: 33 245254.
5. J. Kaput, R. L. Rodriguez, Nutritional genomics: the next frontier in the postgenomic era Physiol. Genomics 16: 166-177, 2004; doi:10.1152/physiolgenomics.00107.2003.

Nutrigenomics

Topic 34

Module 34.2

From Nutrients to Genes: Response to Nutrients

Varban Ganev

Learning Objectives

To have understanding of the concepts of molecular nutrition research (signals and signaling pathways, use of animal models);
To have understanding of identification of early biomarkers;
To be able to read and understand literature of the field (molecular nutrition and nutrigenomics);
To have some (practical) knowledge how to apply molecular nutrition and nutrigenomics in the lab;
To be able to extract relevant data/information from internet for molecular nutrition research;
To be able to understand a "nutrigenomics" experiment;
To have understanding on the evolution of genomic versus food patterns. Dietary signaling and sensing.

Contents

Key Messages

Discussion on basic mechanisms of dietary signaling and sensing (Diet x Genome);
Discussion on nutrigenetics (Diet x Genotype);
Introducing some regulatory, legal and ethical issues of nutrigenomics;
Discussion on evolution of genomic versus food patterns;
Nutrigenetics;
Personalized diet;
“Thrifty” genotype;
Regulatory, legal and ethical issues.

1. Dietary signals: from nutrients to genes (Diet x Genes)

In some ways, the nutrigenomics agenda can be seen as analogous to that of pharmacogenomics. However, an important difference is that pharmacogenomics is concerned with the effects of drugs that are pure compounds - administered in precise (usually small) doses) - whereas nutrigenomics must encompass the complexity and variability of nutrition.

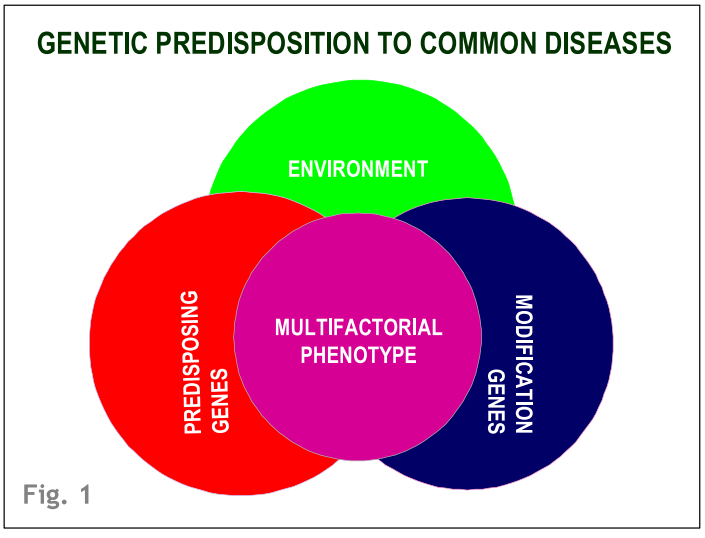


Fig. 1

The body has to process a huge number of different nutrients and other food components. Nutrients can reach high concentrations (μM to mM) without becoming toxic. Each nutrient can also bind to numerous targets with different affinities and specificities. By contrast, drugs are used at low concentrations and act with a relatively high affinity and selectivity for a limited number of biological targets. Despite these differences, nutritional research could benefit greatly, as has pharmacology, from detailed information on the effects of compounds at the molecular level.

It is now evident that, as well as their function as fuel and co-factors, micro- and macronutrients can have important effects on gene and protein expression and, accordingly, on metabolism. The molecular structure of a nutrient determines the specific signaling pathways that it activates. Small changes in structure can have a profound influence on which sensor pathways are activated.

Table 1 Transcription-factor pathways mediating nutrient-gene interactions (1)

Nutrient	Compound	Transcription factor
Macronutrients		
Fats	Fatty acids Cholesterol	PPARs, SREBPs, LXR, HNF4, ChREBP SREBPs, LXRs, FXR
Carbohydrates	Glucose	USFs, SREBPs, ChREBP
Proteins	Amino acids	C/EBPs
Micronutrients		
Vitamins	Vitamin A Vitamin D Vitamin E	RAR, RXR VDR PXR
Minerals	Calcium Iron Zinc	Calcineurin/NF-ATs IRP1, IRP2 MTF1
Other food components		
	Flavonoids Xenobiotics	ER, NFκB, AP1 CAR, PXR

AP1 – activating protein 1; CAR – constitutively active receptor; C/EBP – CAAT/enhancer binding protein; ChREBP – carbohydrate responsive element binding protein; ER - estrogen receptor; FXR - farnesine X receptor; HNF - hepatocyte nuclear factor; IRP – iron regulatory protein; LXR – liver X receptor; MTF1 – metal-responsive transcription factors; NFκB – nuclear factor κB; NF-AT – nuclear factor of activated T cells; PPAR – peroxisome proliterator-activated receptor; SREBP – sterol-responsive-element binding protein; USF – upstream stimulatory factor; VDR – vitamin D receptor.

This fine-tuned molecular specificity explains why closely related nutrients can have different effects on cellular function.
One example is how the nutritional effects of fatty acids vary depending on their level of saturation. The ω-3 polyunsaturated fatty acids have a positive effect on cardiac arrhythmia, whereas saturated C16-18 fatty acids (stearic acid and palmitic acid) do not. Furthermore, ω-6 unsaturated C18 fatty acids (oleic acid and linoleic acid) decrease plasma levels of low-density lipoprotein (LDL) cholesterol. The challenge for the next decade is to identify nutrient-influenced molecular pathways and determine the down-stream effects of specific nutrients. Nutrigenomics can assist in this identification because it allows the genome-wide characterization of genes, the expression of which is influenced by nutrients.
It is only with a complete understanding of the biochemical links between nutrition and the genome that we will be able to comprehend fully the influence of nutrition on human health.
Transcription factors are the main agents through which nutrients influence gene expression. The nuclear hormone receptor superfamily of transcription factors, with 48 members in the human genome, is the most important group of nutrient sensors (Table 1). Numerous receptors in this superfamily bind nutrients and their metabolites. These include retinoic acid (retinoic acid receptor (RAR) and retinoid X receptor (RXR)), fatty acids (peroxisome proliferatoractivated receptors (PPARs) and liver X receptor (LXR)), vitamin D (vitamin D receptor (VDR)), oxysterols (LXR), bile salts (farnesoid X receptor (FXR), also known as bile salt receptor) or other hydrophobic food ingredients (constitutively active receptor (CAR) and pregnane X receptor (PXR)).
Nuclear receptors bind with RXR to specific nucleotide sequences (response elements) in the promoter regions of a large number of genes. During ligand binding, nuclear receptors undergo a conformational change that results in the coordinated dissociation of co-repressors and the recruitment of co-activator proteins to enable transcriptional activation. In metabolically active organs, such as the liver, intestine and adipose tissue, these transcription factors act as nutrient sensors by changing the level of DNA transcription of specific genes in response to nutrient changes. Nuclear hormone receptors have important roles in the regulation of numerous processes, including nutrient metabolism, embryonic development, cell proliferation and differentiation. So, it is easy to envision how nutrients, by activating these receptors, are able to influence a wide array of cellular functions.
To briefly illustrate the strategy that cells use to adapt to changes in nutrient and metabolite concentrations through these nutrient-sensing transcription factors, we discuss two examples: bile-salt sensing and fatty-acid sensing during feeding and fasting.

1.1 Bile-salt sensing

Bile salts are metabolites of cholesterol that are formed in hepatocytes and secreted across the canalicular membrane by the ATP-binding cassette transporter (ABC) ABCB. Bile salts are important components of bile, and are necessary for lipid digestion in the intestinal tract. However, at elevated concentrations, these potent detergents are cytotoxic. An ingenious sensor mechanism protects cells from these cytotoxic effects, allowing them to rapidly reduce the free intracellular concentration of bile salts. The nuclear hormone receptor FXR is the nutrient sensor that mediates this response to elevated levels of bile acids. Through this receptor, bile acids increase the expression of numerous gene products that are involved in lipid metabolism, including ileal bile-acid binding protein, PPARα, short heterodimeric partner, phospholipid transfer protein, apolipoprotein E (APOE), APOCII and the bile-salt export pump (ABCB11).Overall, the increased expression of these genes inhibits the synthesis of bile acids and stimulates the transport of bile acids out of the cell, through ABCB11, into the bile canaliculi.

1.2 Fatty-acid sensing during feeding and fasting.

Fatty acids influence human health in numerous ways. Epidemiological studies show that certain fatty acids are linked to the increased occurrence of certain diseases. Nutritional trials, in which the fats are enriched in specific fatty acids, show that fatty acids influence several indicators of health status. Unfortunately, until recently, our understanding of the molecular mechanisms that

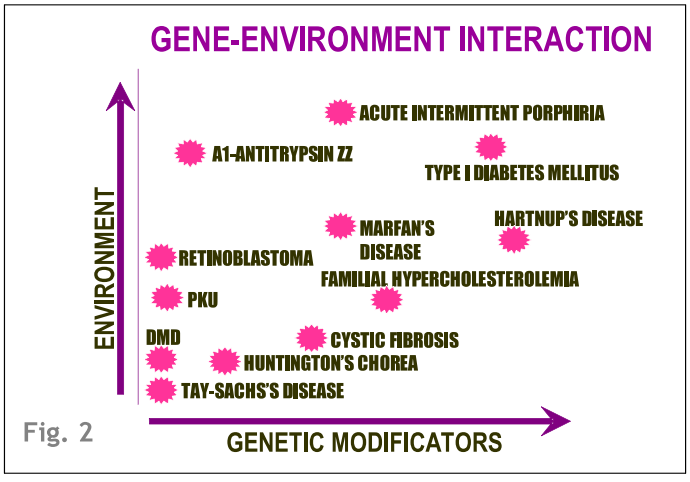


Fig. 2

underlie these results was patchy. Early studies indicated that dietary poly-unsaturated fatty acids potently repress the hepatic expression of several genes involved in fatty acid synthesis. However, it was not until several nuclear hormone receptors were discovered and characterized that some details of the manner in which fatty acids induce changes in gene expression emerged.

We now know that PPARs - another group of nuclear hormone receptors - act as nutrient sensors for fatty acids and influence the expression of specific genes.

One of the three PPAR isotypes - PPAR-α - is present mostly in the liver and is important during food deprivation and fasting. During fasting, free fatty acids are released from the adipose tissue.

These fatty acids then travel to the liver, where they undergo partial or complete oxidation. However, these fatty acids also bind PPAR-α, which then increases the expression of a suite of genes through binding to specific sequences in their promoter regions. Further, genes can also have their expression increased indirectly, through the genes that are directly affected by PPAR-α. The target genes of PPAR-α are involved in numerous metabolic processes in the liver, including fatty acid oxidation and ketogenesis, apolipoprotein synthesis, amino acid metabolism, cellular proliferation and the acute-phase response. This is an elegant pathway in which the signal that initiates adaptive changes in liver metabolism during fasting originates from the adipose tissue and acts through a receptor, the expression of which is upregulated by fatty acids during fasting.

2. Nutrigenetics and personalized diets (Diet x Genotypes)

Nutrigenomics is focused on the effect of nutrients on the genome, proteome and metabolome, whereas **nutrigenetics examines the effect of genetic variation on the interaction between diet and disease or on nutrient requirements**. Genetics has a pivotal role in determining an individual's risk of developing a certain disease. Population differences in SNPs can have an important effect on disease risk. Inter-individual genetic variation is also likely to be a crucial determinant of differences in nutrient requirements.

For example, one study indicates that individuals with a C→T substitution in the gene for methylenetetrahydrofolate reductase (MTHFR) might require more folate than those with the wild-type allele. Conversely, several studies indicate that diet has an important influence on the risk of developing certain diseases in which genetic predisposition has a role. One interesting example of the complicated interaction between genetics, diet and disease comes from a study of the occurrence of hepatocellular carcinoma in Sudan; there was a stronger relationship between the risk of developing the disease and the consumption of peanut butter contaminated with aflatoxins in Sudanese people with the glutathione S-transferase M1 (GSTM1) null genotype than there was in those lacking this genotype. The availability of the sequence of the human genome, coupled with the ongoing cataloguing of human genetic variation, provides nutrigenetics with an enormous resource with which to work. The goal of the Single Nucleotide Polymorphisms Consortium is to map all the important polymorphic sites in the human genome. The challenge for molecular epidemiology is to identify specific polymorphisms that are linked to altered risk of disease or sensitivity to diet.

Gene expression patterns produce phenotype, which represents the physical characteristics or observable traits an organism, e.g., hair color, weight, the presence or absence of a disease. Phenotypic traits are not necessarily produced by genes alone. Phenotypic expression is influenced by nutrition. For example, diets alter cholesterol levels and types (LDL, HDL, and their ratios), homocysteine levels, and obesity (nutritional component), and these responses differ among individuals (genetic component).

At the molecular level, variations in one DNA building block result in variations in gene structure. That variation, or SNP, can lead to variations in the protein structure after the gene or its variant is expressed.

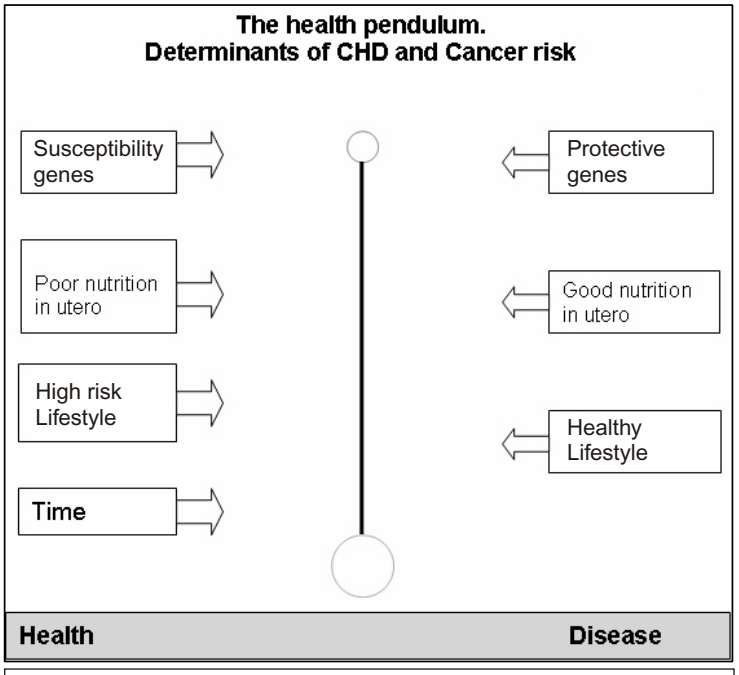


Fig. 3 The balance between genetic and epigenetic factors. R. Jaenisch, A. Bird, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals, Nat. Genet. Suppl. 33 (2003) 245254.

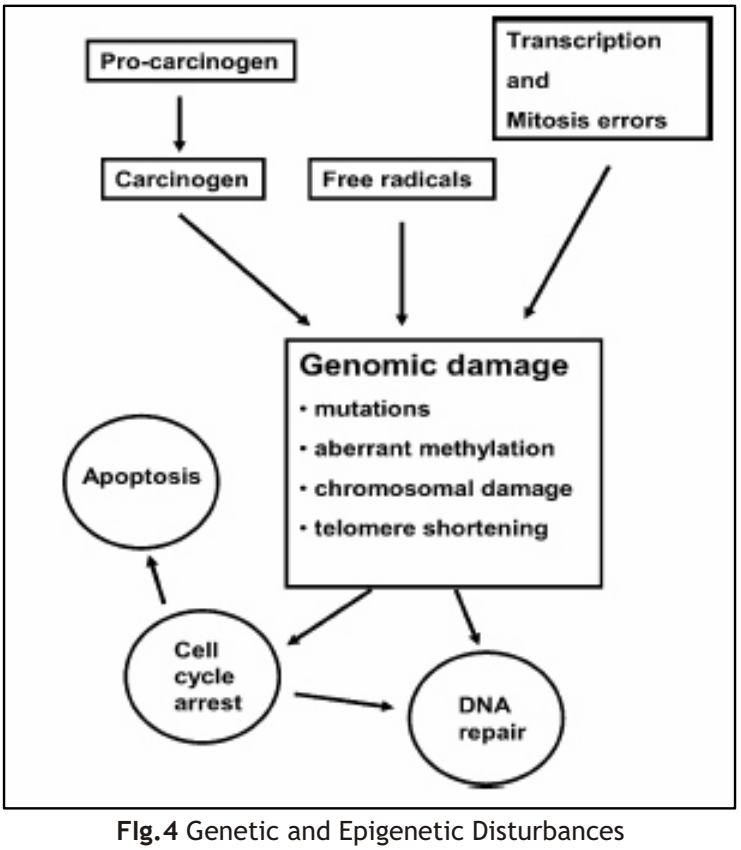


Fig.4 Genetic and Epigenetic Disturbances

Some structural variations in a protein may have an impact on its function, while some may not. Multiple genes may contain one or more SNPs, in distinctive patterns, associated with phenotypic patterns of nutritionally related health states, such as homocystenemia, high (or low) cholesterol, or variations in HDL and LDL cholesterol and their subunits. Patterns of multiple SNPs are called haplotypes.

The key element that distinguishes nutrigenetics from nutrition research is that the observable response to diet, or phenotype, is analyzed or compared in different individuals (or genotypes). Classical nutrition research essentially treats everyone as genetically identical, even while realizing that some individuals require more or less of specific nutrients.

Molecular biology and biochemical approaches often assume the same: that an enzyme or flux through a metabolic or signal transduction pathway is the same in each individual. Similarly, the key element that distinguishes nutrigenetics from both molecular and genomics research is that nutrigenetics analyzes genetic expression in response to variations in diet.

Molecular and genomic research often assumes that the environment does not influence genetic expression. Nutrigenetics combines these concepts. **An individual enzyme, pathway, or collection of pathways will be unique to groups or each individual, depending on the variation (defined by SNPs, haplotypes, and other polymorphisms) inherited**. The expression or activity of these variant forms of normal genes differs depending on the amount and type of food ingested and the interactions between the food and the specific genotype.

Understanding these interactions has significant implications: turning genes on or off or changing the abundance of certain proteins in response to different dietary chemicals may affect the balance between health and illness. Causes of chronic diseases, such as cardiovascular disease, cancer, or cognitive decline in aging, are not well understood because they are multifactorial in nature. While we have clues to some dietary and other environmental or lifestyle factors that appear to contribute to occurrence of those and other chronic diseases, effects are not consistent among individuals. Thus, clear cause/effect relationships are still emerging.

Occurrences of chronic diseases are “encoded” by a combination of factors, all acting on the body over time to create the disease phenotype of variable severity. These factors may include a number of genes, common genetic variants (i.e., SNPs, haplotypes), environmental factors, risk-conferring behaviors, and socioeconomic status. The genetic factors contributing to complex disease are difficult to identify because they typically exert small effects over long periods of time. Moreover, other unrelated genes and environmental factors, such as diet and lifestyle, can modify the magnitude of their effect. This is nicely depicted by the health pendulum shown on Fig. 3.

The most commonly used approach for analysis of the contribution of genetic variations in response to diet or food components is the **case-control approach**, where the frequencies of genotypes are compared between groups of exposed individuals with different responses.

More than 100 studies are published to date. However, their conclusions should be considered cautiously, having in mind the study of Hirschhorn et al. (5) on more than 600 association studies on multifactorial (chronic) diseases, showing that only six of the associations have been confirmed in more than three studies.

The "failure" to identify single genes responsible for chronic diseases led to the common variant/common disease (CV/CD) hypothesis, which is largely responsible for the current genome-centric approach to the study of chronic diseases.

This hypothesis, in its simplest form, is that combinations of naturally occurring gene variants (i.e., alleles of unlinked genes) rather than mutations produce any given chronic disease.

This is exemplified by the number of chromosomal regions associating with obesity (Fig. 7), illustrating that susceptibility to this disease is multigenic. The link to nutrigenetics is that some of these naturally occurring gene variants will alter metabolism of nutrients, which in turn will alter the regulation genes involved in maintaining health or promoting disease.

Fig. 5 **CHRONIC DISEASES MAY RESULT FROM DIFFERENT MOLECULAR PATHWAYS**

- **Almost 1700 genes fit the description of oncogene**
- **Different tumors may be initiated and/or promoted by one or more different oncogenes, each activating its own cascade of altered regulatory processes. Cancers of the same organ or cell type that appear to be morphologically and histologically similar may have unique molecular expression profiles. Glioblastoma provides one example. Data from high-density oligonucleotide arrays showed that EGFR positive tumors express 90 genes differently from EGFR negative tumors.**
- **Mutations in many different genes such as leptin, leptin receptor, agouti signaling peptide, attractin, insulin signaling protein, and carboxypeptidase-E cause obesity in mice and humans.**

Fig. 6 **NUTRITION x GENOME: ASSOCIATION APPROACH**

- **Case/control studies – variations in candidate genes are associated with certain response to diet**
- **More than 100 studies**
- **Limitations: sample sizes that lack appropriate statistical power, control groups that are not appropriately matched to cases, population stratification that occurs because of genetic admixtures among the study participants, and overinterpreting data (among others)**
- **Examples: Lipid, diet, smoking, sex, activity, alcohol vs. APOA1, APOA4, APOB, APOC3, APOE, APOH, LPL, CETP, LCAT, LDLR, HL, Cholesterol 7a-hydroxylase, Intestinal FABP, Neuropeptidase Y, M/N blood group, Alcohol dehydrogenase-3, Paraoxonase, Microsomal transfer protein**

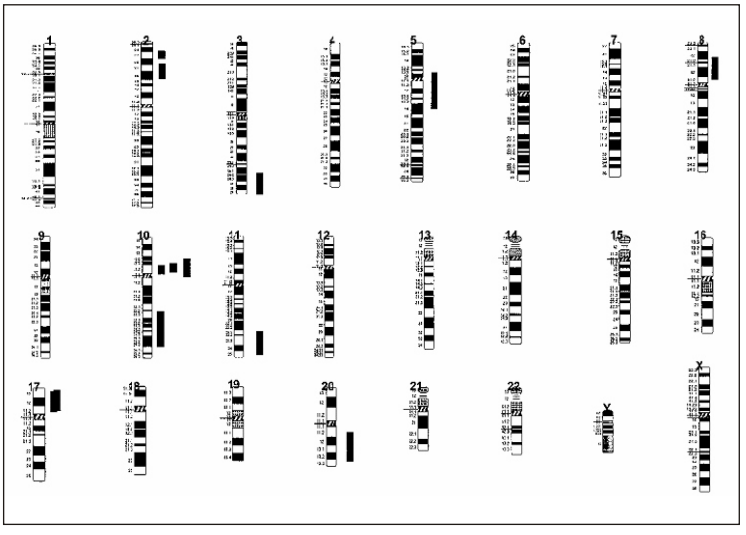
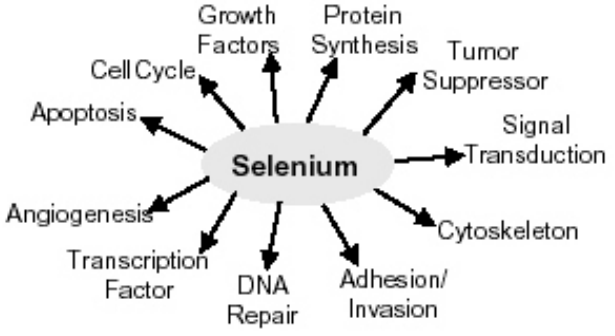


Fig.7 More than 10 chromosomal loci associate with obesity.

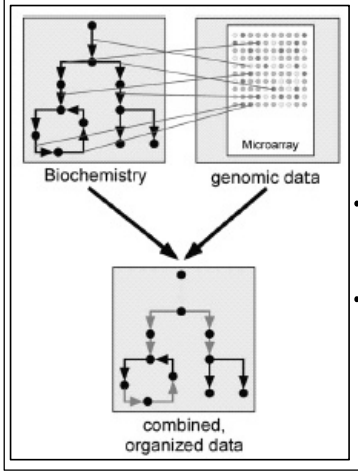
Multiple effects of gene-modifiers

Fig. 8 **MULTIPLE EFFECTS OF ENVIRONMENTAL FACTORS (NUTRIENTS)**



Categories of genes that are mediated by dietary selenium in human prostate cancer cells as determined by microarray analysis – Dong et al. [2002].

Fig. 9 **MULTIPLE EFFECTS OF GENE-MODIFIERS**



Global changes in gene expression in mouse liver in response to fasting and sugar-fed conditions using high density microarrays (Bauer et al., 2004):

- **starvation response correlated with processes promoting anti-aging and longevity;**
- **most of these potentially beneficial changes were suppressed by sugar feeding.**

On the other hand, a **single environmental factor (e.g. food component) can influence the activity of several genes simultaneously** (Fig. 8), i.e. diet or environment will also affect the expression and, in some cases the abundance, of the enzymes and proteins.

Altering the concentrations of enzymes in the pathways will alter flux through pathways and ultimately the physiology of the organism. For example, in response to fasting and sugar-fed conditions mice liver reacts with global changes in gene expression as shown by high density microarrays:

starvation response correlated with processes promoting anti-aging and longevity;

most of these potentially beneficial changes were suppressed by sugar feeding;

down-regulation of fatty acid synthesis and upregulation of fat breakdown to provide energy. Fat catabolism also entails activation of lipid signaling cascade, which provides protection from genotoxic side products. This group includes CYP450 species and lipid-activated nuclear receptors.

Mutations in many different genes such as leptin, leptin receptor, agouti signaling peptide, attractin, insulin signaling protein, and carboxypeptidase-E cause obesity in mice and humans:

Up-regulation of amino acid catabolism and urea cycle, since endogenous proteins are broken down during starvation to provide fuel and essential metabolites. The urea cycle regulates the nitrogen level and also helps prevent conditions where excess nitrogen and ammonia become toxic. In this regard, the up-regulation of the urea cycle can be seen as a protective reaction that functions as an antioxidant lipid signaling cascade. Since excess amino acids and proteins can not be stored, the increase in the urea cycle can be brought about by two opposite physiological conditions, i.e. the absence and the excess, of dietary proteins, as for example in the high protein 'Atkins' diet.

Significant transcriptional regulation of IGFs (insulin-like growth factors) and IGFBPs (IGF binding proteins). IGF1 is a major growth signaling molecule that is transcriptionally activated by insulin and GH under good nutrient conditions, thereby allowing cell growth and proliferation. Under starvation, these signals are absent so that IGF1 expression is strongly reduced, while its deactivating binding proteins IGFBP1 and IGFBP2 are up-regulated.

Unexpected response to starvation in steroid metabolism. Because of the shortage of acetylCoA, expression of enzymes that catalyze de novo synthesis of cholesterol is strongly reduced. However, due to the breakdown of fat and cell membranes cholesterol is still available as a starting metabolite for steroid synthesis. Strikingly, most of the enzymes for synthesis of DHEA are up-regulated, whereas those catabolizing DHEA or converting it to other hormones are down-regulated.

Fig. 10 MULTIPLE EFFECTS OF GENE-MODIFIERS

List of lifespan-prolonging reactions identified at the transcriptional level in mouse liver upon starvation.

Lifespan-prolonging responses to starvation	Underlying metabolic process
Anti-oxidative and anti-xenobiotic reactions	fat breakdown
Elevated urea cycle	breakdown of endogenous AA
High DHEA level	steroid metabolism
IGF1 repression	insulin and GH signaling
Up-regulation of genome stabilizing factors	stress response

The common study design errors in the case-control studies include small sample size, poorly matched control groups, population stratification, overinterpretation of data, and others. These methods and approaches are being improved to eliminate such errors and to reliably identify genes associated with complex phenotypes.
Several recent association studies on multifactorial diseases have included dietary variables in studies testing whether a single gene variant is associated with a complex phenotype:

Hypertension
A variant (designated AA) of the angiotensinogen (ANG) gene is linked with circulating ANG protein, which in turn, is associated with increased blood pressure. The Dietary Approaches to Stop Hypertension (DASH) diet positively affects individuals with the AA genotype, but the same diet was less effective in reducing blood pressure in individuals with a GG genotype. A large percentage (~60%) of African-Americans have the AA variant, with the remainder heterozygotic (AG) at this position (6).

Cardiovascular health
Apo-A1 plays a central role in lipid metabolism and coronary heart disease. G to A transition in the promoter of APOA1 gene is associated with increased HDL-cholesterol concentrations, but the results across studies are not consistent. Ordovas et al. (7) found that the A allele (or variant) was associated with decreased serum HDL levels. The genetic effect was reversed, however, in women who ate more polyunsaturated fatty acids (PUFA) relative to saturated fats (SF) and monounsaturated fats (MUFA). In men, this type-of-fat effect was significant when alcohol consumption and tobacco smoking were considered in the analyses. If confirmed by other studies, the APOA1 gene shows a classical gene-environment interaction. Such interactions may help explain why candidate gene studies show inconsistencies. Food intake therefore may alter susceptibility to diseases mediated by increased HDL-cholesterol levels.

Cancer
Methylenetetrahydrofolate reductase (MTHFR) is a key gene in one-carbon metabolism and indirectly in all methylation reactions. Several laboratories have noted that the C667T polymorphism (ala to val), which reduces enzymatic activity, is inversely associated with occurrence of colorectal cancer. Dietary recalls were used to assess intake of folate, vitamin B-12, vitamin B-6, or methionine (and in one study, alcohol) in individuals with the CC or TT phenotypes. Low intakes of these vitamins were associated with increased risk for cancer among those with the MTHFR TT genotype. MTHFR variants are also implicated in cardiovascular disease.

Another application of nutrigenetics information is expected to be in treatment of enzyme deficiencies. Some genetic diseases in humans are caused by defective enzymes. A subset of these enzymes is altered by naturally occurring SNPs which increase the Michaelis constant, Km, of coenzyme for enzyme. Km is a biochemical measure of the affinity of coenzyme or substrate for enzyme - an increased Km results in decreased affinity. In certain cases, increasing the coenzyme concentration may ameliorate the decreased enzymatic activity.
The medical applications for such cases would be that if genetic tests were available for the variant gene and if that variant was shown to be the only cause of a disease process, a physician or nutritional expert could recommend increasing or decreasing intake of a specific vitamin or food.
For example, increased dietary intake of nicotinic acid or nicotinamide might increase NADPH coenzyme concentrations enough to alter the equilibrium of GPDH/GPDH-NADPH (Table 2). The same approach will not work for ALDH because the NAD substrate concentration could not be increased enough to overcome the increased Km caused by the substitution of lysine for glutamic acid at position 487. Elson-Schwab and Ames (8) have established a Web site (www.kmmutants.org) that summarizes nutritional information for a large number of coenzyme-containing enzymes.

Table 2 Examples of enzyme sensitive to cofactors

Enzyme	Cofactor	Δbp	ΔAA	%f	Km[b]
MTHFR	FAD	C609T	P187S	~15	Increase
ALDH	NAD	-	E487K	~50[c]	150-fold increase
GPDH	NADP	C131G	A44G	11	5-fold increase[d]

[a]MTHFR – methylene tetrahydrofolate reductase; ALDH – aldehyde dehydrogenase; GPDH – glucose 6-phosphate dehydrogenase; Δbp – change in base pair ; ΔAA – change in AA; %f – percent of reference activity; Km – Michaelis constant

[b]Increased Km for cofactor – decreased affinity

[c]Heterozygote + homozygote

[d]May be aided by increased intake

3. Specific nutrients and foods for specific individuals or groups

Significant contributions from genomics, molecular biology, and nutritional disciplines have been made toward understanding the different components of complex, chronic disease phenotypes. However, a comprehensive, integrated picture still eludes us. This is specifically true for the effect of food on health and disease process.
Epidemiological studies repeatedly have demonstrated associations between diet and cardiovascular disease, cancer, and other chronic diseases. However, the specific cause/effect linkages between nutrient type and amount and health or disease phenotype are only beginning to emerge. The promise of nutrigenetics is that scientific research can deliver scientific evidence of health benefits of **specific nutrients and foods for specific individuals or groups**.
A recent high-resolution recombination map of the human genome has greatly improved our knowledge of the genetic order of polymorphic markers, the precision of estimates of genetic distances, and the SNP map of the human genome. SNPs should provide powerful molecular tools for investigating the role of nutrition in human health and disease. Incorporating studies of SNPs into metabolic and epidemiological studies might also help to define optimal diets. Although the implementation of this type of personalized diet is still in its infancy, progress in the next few years is likely to be rapid. Indeed, several small biotechnology firms have been founded that focus on nutrigenomics/nutrigenetics and the commercialization of personalized diets. However, if the use of genotypes in the dietary prevention of disease is to be established, the field of molecular nutrition must first be successful in identifying the mechanisms driving the connection between diet and phenotype according to specific genetic variations. Understanding how nutrient sensing transcription factors mediate the effects of dietary components on gene expression will be crucial if this endeavour is to succeed. So, although personalized diets would be an interesting application of nutrigenomics the implementation of such an approach lies far ahead of us and over the next 10

years the focus should be on understanding how nutrients interact with the genome at the molecular level.
The **analogy of pharmacogenetics to nutrigenetics** is readily evident. The goals of these areas are similar: customization of therapy, prevention and management of disease, and market segmentation based on personalized criteria. Through analysis of gene expression, SNPs, haplotypes, and biochemical and physiological results, scientists are verifying individual and group differential responses to diet.
When asked, 75-90% of consumers state that they make food choices with the intent of benefiting their or their family's health. **Health management and market segmentation through diet** are possible, well established, and continue to grow. Consumers seeking cholesterol management solutions have an array of foods available, including oatmeal, fat type and amount, carbohydrate type and amount, and stanols/sterols. Dairy products and soy for bone health, cancer, weight management, and cardiovascular health represent additional well established and emerging health segments. Dietary choices based on genetics are not new. Phenylketonuria and alcohol dehydrogenase deficiency are well-known conditions and can be avoided by avoiding consumption of phenylalanine and alcohol, respectively. Currently, food selection for health is based largely on generalized information, and, in some cases, more specific information derived from effects of diet on biomarkers such as bone density, cholesterol, serum triglycerides, or blood glucose, among others.
Epidemiological studies support the role of diet in health but have not revealed causeeffect linkages that are emerging through the combination of the previously discussed scientific disciplines. Each of those disciplines contributes to unique, yet interrelated understanding of chronic disease and the role of diet in phenotypic expression of wellness or disease. The role of fatty acid intake and metabolism in depression (DHA/EPA); obesity, colon cancer, heart disease (PPARs); and partitioning of energy into adipose or muscle tissue (CLA) are but a few examples of gene/diet interactions for which phenotype variability is being unraveled. Food clearly represents a nearly ideal channel through which to realize the benefits that nutrigenetics promises.

4. Regulatory, legal and ethical considerations

Knowledge resulting from the scientific combination that underpins nutrigenomics leads to a potential **change in the borderline between medicine and foods**. The distinction between those current definitions will be challenged with growing evidence of nutrient effects on disease processes at the cellular level and a role for nutrients in disease prevention and management. Modern pharmaceuticals evolved from thousands of years of traditional lore concerning the uses of plants and herbs as medicines.
Research efforts over the past 100 years have led to the widespread adoption of Paul Ehrlich's Principles of (Chemo)therapy:

Drugs need not be of natural origin and could be developed by planned chemical synthesis;
Systematic exploration of structure/function relationship distinguishing therapeutic activity from toxicity is needed;
Maximization of ratio of dose required to cure disease to that producing toxicity (broad therapeutic index) is needed;
The importance of developing animal models of diseases for quantitative measurements of both therapeutic potency and toxicity is needed.

Highly sophisticated methods are now used to identify, characterize, and test potential drugs for effectiveness in humans to meet the criteria of these principles. However, the growing interest, acceptance, and use by the public of dietary supplements, not to mention herbal medicines, has outpaced the scientific, medical, and food industry's ability and capacity to carefully analyze the chemicals, their combined and independent activities, and their effectiveness and safety.
Nutrigenomics, by definition, will require clinical validation of effects, including safety, in the target market segment. Clearly there is an opportunity and a **growing need for consideration of a regulatory framework** that will accommodate emerging science as well as deliver consumer benefits and afford consumer protection. While scientists suspected that food, like drugs, had cellular-level effects, the extent of that truth could not be supported with scientific evidence.

Today, the proof is here and is growing. It is not suggested that food be regulated as drugs. The suggestion is that thoughtful consideration be given to heretofore unexpected effects of nutrients and foods on health. Such consideration, if managed with foresight, ideally would support research to identify genes, adjunct diagnostic tests, and foods that would afford opportunity for optimal consumer health and wellness.
At the consumer level, nutrigenomics will first be encountered as diagnostic testing for genetic patterns of SNPs, coupled with food products or supplements, and diagnostic monitoring of biomarkers that will track genetic response to diet. **Consumer counseling** will be essential to translate the meaning and recommended actions suggested by one's genetic profile. Successful incorporation of any food into an individual's diet will depend completely on whether the food fits an existing dietary pattern and has excellent sensory properties. In other words, like many other health trends in the food industry, nutrigenomics will thrive and deliver the benefits inherent in the concept only if the products deliver consumer benefits and satisfy consumer preferences.
Questions of ethics, privacy, compliance, insurance reimbursement, value creation and capture, and economic return, and, as noted above, the need for additional physiological and biochemical studies to identify and validate effects of dietary modification on phenotypic expression must be resolved.
The science from various disciplines that constitute nutrigenomics continues to emerge and is being integrated into useful information on which the food industry can act. Clearly, this is not the food industry as is has been in the past. Alliances, partnerships, or consortia among varied commercial partners will be essential to deliver on the scientific and commercial potential of nutrigenomics.

5. Evolution of genomics versus food patterns

Our evolution as human beings took millions of years, whereas the human civilization exists from thousands of years. The evolution of human genome has been shaped in favor of survival under limited food/calories intake, which resulted in formation of **"thrifty" genotype**. The selective advantage of this genotype is in putting together metabolic network aiming at survival with minimal food/calories intake and storage of the chemical energy exceeding this minimal requirement.
However, with the radical changes in our diet and physical activity patterns in industrial societies (the major hallmarks being excess intake of food and restricted energy expenditure) during the last "seconds" of our human history, the "thrifty" genotype is turning more and more into "susceptibility" genotype. Recently, the WHO (2002) predicted significant growth during the next two decades in the prevalence of diseases resulting from this bio-social conflict, such as type 2 diabetes, obesity, atherosclerosis, etc.

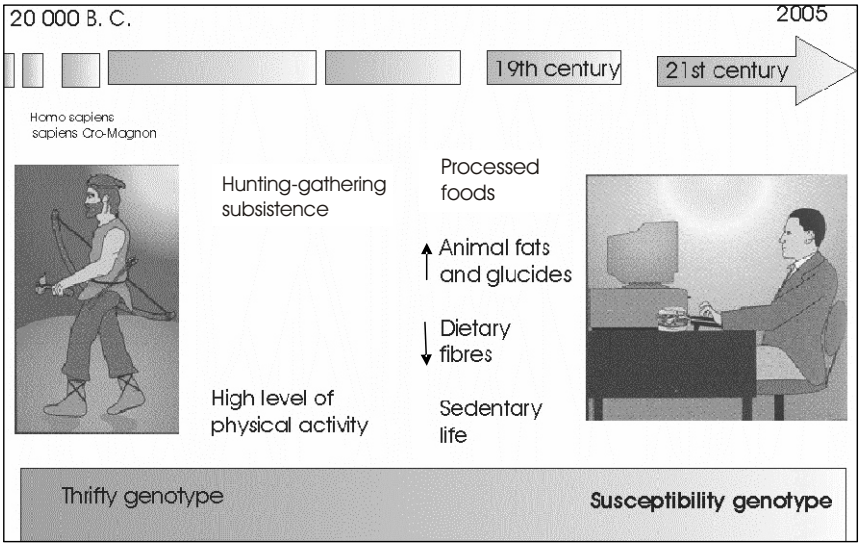


Fig. 11

Exposing our “thrifty” genes to a new diet, characterized by much higher callory intake is very well demonstrated with the story of the Aboriginal Canadians Ojie-Cree (Fig. 12). Population of Ojie-Cree migrated from their natural habitat at the Sandy Lake to Toronto area, Ontario, about 1980's. This population showed a high rate of coronary heart disease some two decades later, which occurred to be about three times higher than the one of the All Ontario population, as well as to the rate among Ojie-Crees inhabiting their original settlements. When one of the genes belonging to the “thrifty” genotype was studied (HNFIA) it has been found that the Ojie-Crees living in both Sandy Lake area and Toronto area are frequent carriers of a mutation (S 319), which is exotic among the non-Aboriginal Canadians. Apparently, the high frequency of this mutation among the Aboriginal Canadians is a result from selection in favor of survival advantages.
However, the exposure of carriers to drastically changed environment (sedentary life style, high saturated fatty acids diet, etc.) in their new settlement is converting the “thrifty” into “susceptibility” genotype.

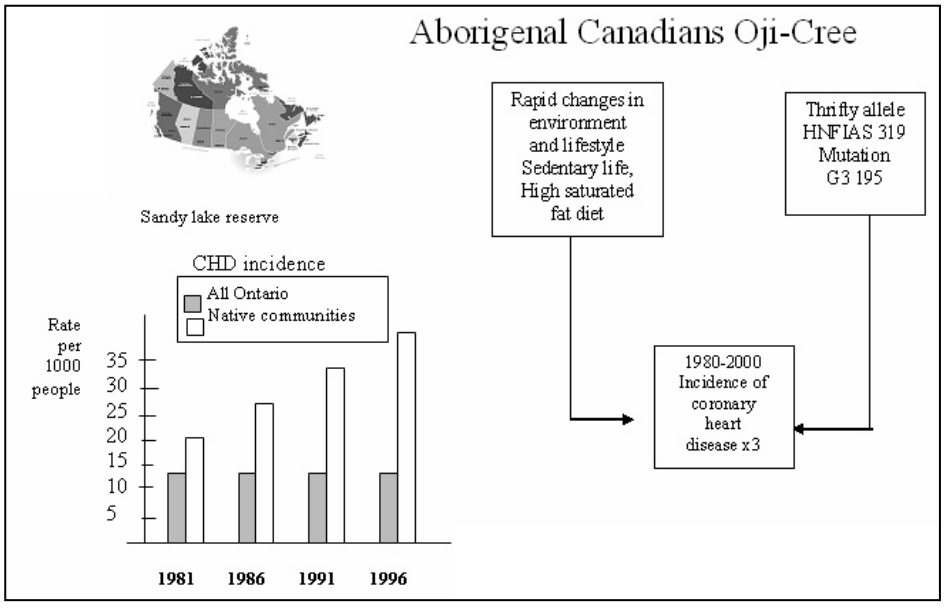


Fig.12 The concept of “thrifty” genotype. Increased incidence of CHD among carriers of a “thrifty” gene exposed to rapid changes in their environment.

Similarly, Native Americans moving from Mexico to Texas, US, have several times higher risk to develop obesity and type 2 diabetes, when compared with their relatives inhabiting Mexican mountains.

6. Concluding remarks

Nutrigenomics is an **integrative science**, which seeks to provide a genetic and molecular understanding for how common dietary chemicals affect the balance between health and disease by altering the expression and/or structure of an individual's genetic makeup. The conceptual basis for this new branch of genomic research can best be summarized by the following

Five tenets of nutrigenomics:

Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases;

Common dietary chemicals can act on the human genome, either directly or indirectly, to alter gene expression or structure;

The degree to which diet influences the balance between healthy and disease states may depend on an individual's genetic makeup;

Some diet-regulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases;

Dietary intervention based on knowledge of nutritional requirement, nutritional status, and genotype (i.e., "personalized nutrition") can be used to prevent, mitigate or cure chronic disease.

The sum total of molecular studies shows that diet and the chemicals in diet, influence physiological processes. **This is achieved by altering the expression (or structure) of a subset of genes in the human genome**. Dietary chemicals have been shown to alter gene expression in a number of ways. For example, they may:

act as ligands for transcription factor receptors;

be metabolized by primary or secondary metabolic pathways thereby altering concentrations of substrates or intermediates or

serve as signaling molecules.

The ability of cells to adapt to environmental change by regulation of gene expression is essential for organism survival. Organisms vary their gene expression in the absence or presence of nutrients by increasing and decreasing production of cellular proteins necessary for life sustaining function.

Ultimately, the science of nutrigenomics **promises to offer the health practitioner greater knowledge, enabling them to predict potential genetic responses to nutritional intake and to target and modify associated behavior**. A major step will be to establish biomarkers needed to quantify a positive biological response to nutrient intake. This will be a valuable step that differentiates nutrigenomics from the general public health messages of the early 21st century, which led to punitive dietary restrictions unrelated to individual health outcomes. Once verifiable protocols, based on genomic biomarkers are established, nutrigenomics will revolutionize health care leading to the reduction of individual health risk.

7. Glossary

Acute-phase response
The early and immediate set of homeostatic control reactions that are induced during inflammation.

Allele
One of the variant forms of a gene at a particular location on a chromosome. Different alleles produce variation in inherited characteristics such as hair color or blood type. In an individual, one form of the allele (the dominant one) may be expressed more than another form (the recessive one).

Canalicular membrane
The apical membrane of liver epithelial cells (hepatocytes) that lines the bile canaliculus. Members of the ABC-transporter super family that are localized in this membrane are responsible for bile secretion.

Comparative genomics
A new field of biological research in which the genome sequences of different species: human, mouse and a wide variety of other organisms from yeast to chimpanzees are compared. By comparing the finished reference sequence of the human genome with genomes of other organisms, researchers can identify regions of similarity and difference. This information can help scientists better understand the structure and function of human genes and thereby develop new strategies to combat human disease. Comparative genomics also provides a powerful tool for studying evolutionary changes among organisms, helping to identify genes that are conserved among species, as well as genes that give each organism its unique characteristics.

Gene expression
Process by which genes are activated to make proteins that in turn carry out a range of functions within the body.

Genotype
The genetic identity of an individual that does not show as outward characteristics.

Inducible expression systems
Expression systems that regulate mammalian gene expression with, for example, tetracycline or its derivatives (Tet-On/Tet-Off gene expression systems).

Inflammation
The complex series of reactions that occur in the host as a response to injury, trauma or infection of a tissue, which prevent ongoing tissue damage, isolate and destroy the infective organism and activate the repair processes that are necessary to return the organism to normal function.

Ketogenesis
The production of ketone bodies such as acetoacetate and □-hydroxybutyrate which are the intermediate products of fatty acid catabolism and can be used to provide energy.

Laser capture microdissection
A method in which cells are cut out from a tissue sample using a laser beam, allowing single cell expression analysis.

Ligands
Atom, molecule, group or ion that is bound to a central atom of a molecule, forming a complex.

Macronutrients
Organic compounds, including proteins, amino acids, carbohydrates and lipids, that are required in large amounts in the diet.

Metabolomics
The study of the metabolome, which is the entire metabolic content of a cell or organism, at a given time.

Microarray technology
A new way of studying how large numbers of genes interact with each other and how a cell's regulatory networks control vast batteries of genes simultaneously. The method uses a robot to precisely apply tiny droplets containing functional DNA to glass slides. Researchers then attach fluorescent labels to DNA from the cell they are studying. The labeled probes are allowed to bind to complementary DNA strands on the slides. The slides are put into a scanning microscope that can measure the brightness of each fluorescent dot; brightness reveals how much of a specific DNA fragment is present, an indicator of how active it is.

Micronutrients
Dietary compounds, including vitamins and minerals that are required in small amounts in the diet.

Nutrigenetics
The relationship between genotype and the risk of developing diet-related diseases, such as cancer, diabetes type II and cardio-vascular diseases.

Nutrigenomics
The study of the genome-wide influences of nutrition or dietary components on the transcriptome, proteome and metabolome, of cells, tissues or organisms, at a given time.

Pharmacogenomics
A term often used to mean the influence of DNA sequence variation in drug targets, Phase I or Phase II drug-metabolizing enzymes, and transporters on the effect of a drug, which ultimately allows physicians to design individualized therapy.

Phenotype
The observable traits or characteristics of an organism, for example hair color, weight, or the presence or absence of a disease. Phenotypic traits are not necessarily genetic.

Polymorphism
A common variation in the sequence of DNA among individuals. Single nucleotide polymorphism (SNP) is common, but minute, variation that occurs in human DNA at a frequency of one every 1,000 bases. These variations can be used to track inheritance in families. SNP is pronounced "snip".

Proteomics
The study of proteomes (the complete collection of proteins in a cell or tissue at a given time), which attempts to determine their role inside cells and the molecules with which they interact.

RNA interference
RNAi - The process by which double-stranded RNA silences homologous genes.

Saturation
The binding state of a CC bond in a fatty acid molecule.

Systems biology
The study of whole biological systems (cells, tissues and organisms) using holistic methods.

Transcription factors
Bind to specific DNA sequences in the promoter region of specific genes, thereby either enhancing or suppressing gene expression.

Transcriptome
The complete collection of gene transcripts in a cell or a tissue at a given time.

Transdominant negative adenoviral construct
A recombinant adenovirus that infects cells, resulting in the high-level expression of a mutant protein that, for example, specifically blocks a given signaling pathway (superrepressor) by competing with the endogenous protein.

More at http://www.genomicglossaries.com/content/Basic_Genetic_Glossaries.asp

References

1. M. Müller and S. Kersten (2003) Nutrigenomics: Goals And Strategies. Nature Reviews - Genetics: 4, 315-322.
2. J. Mathers (2005) The Science Of Nutrigenomics. In: NUTRIGENOMICS - Report of a workshop hosted by The Nuffield Trust and organized by the Public Health Genetics Unit on 5 February 2004. Compiled by H. Burton and A. Stewart. The Nuffield Trust, 6-8.
3. N. Fogg-Johnson and J. Kaput (2003) Nutrigenomics: An Emerging Scientific Discipline. Food Technology: 57, 4, 60-67.
4. R. Jaenisch and A. Bird (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature Genetics Suppl.: 33 245254.
5. Hirschorn J N, Lohmueller K, Byrne E, Hirschorn K A comprehensive review of genetic association studies. Genetics in Medicine, 4 (2), 45-61, 2002.
6. Svetkey LP, Moore TJ, Simons-Morton DG, Appel LJ, Bray GA, Sacks FM, Ard JD, Mortensen RM, Mitchell SR, Conlin PR, Kesari M; DASH collaborative research groupAngiotensinogen genotype and blood pressure response in the Dietary Approaches to Stop Hypertension (DASH) study. J Hypertens. Nov;19(11):1949-56. 2001
7. Ordovas, I.M., D. Corella, L.A. Cupples et alPolyunsaturated fatty acids modulate the effects of the APOAl G-A polymorphism on HDL-cholesterol concentrations in a sex-specific manner: The Framingham Study. Am. J. Clin. Nutr. 75(l):38-44, 2002.
8. Ames B, Elson-Schwab I, Silver E. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased Km): relevance to genetic disease and polymorphisms. Am J Clin Nutr 2002;75(4):616-58

LIFE LONG LEARNING SYSTEM FOR TRAINING MEDICAL DOCTORS AND STUDENTS IN NUTRITION

STUDY MANUAL
Educational Modules

Volume 2

Edited by:

Regina Komsa-Penkova
Remy Meier
Lubos Sobotka

www.lll-nutrition.com
e-mail: edunutrimed@gmail.com; rkomsa@gmail.com
Remy.Meier@ksli.ch; pustik@lfhk.cuni.cz

Printed by EA AD, Pleven, Bulgaria, 2005