Chapter 3

EPIDEMILOGY OF “HUNGER IN THE WORLD” THE “HUNGER-OBESITY PARADOX”, THE "FETAL ORIGINS HYPOTHESIS" AND ITS PHYSIOLOGICAL AND ENDOCRINOLOGICAL MECHANISMS

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“Behold, there come seven years of great plenty throughout all the land of Egypt: and there shall arise after them seven years of famine; and all the plenty shall be forgotten in the land Egypt; and the famine shall consume the land; and the plenty shall not be known in the land by reason of that famine following; for it shall be very grievous” (Genesis 41: 29-32).

ABSTRACT

Hunger occurs in sub-Saharan Africa and in South Asia. Every year almost 16,000 children die from hunger-related causes in combination with an impaired immune-system, nearly one child every 5 seconds.

Strange enough in the richest economy of the World, the USA, there is a Hunger-Obesity Paradox. This can be ascribed to coping strategies to food insecurity and food insufficiency in low income households to buy high-fat foods to prevent hunger.

Unraveling the mechanism of starvation, which rely on stores/substrates earlier provided by food components will not be simple but is likely to provide insights into the individual response to starvation and its deteriorating health as a consequence to starvation/ malnutrition.

Several epidemiological studies support the "Fetal origins Hypothesis" followed by the "thrifty phenotype" hypothesis. These state that fetal under-nutrition in middle or late gestation is programmed to exhibit a "thrifty phenotype" resulting in offspring with Metabolic Syndrome (obesity, hypertension, diabetes-2) as a result of in utero environmental alterations and implicate enhanced appetite and adipogenesis as contributory factors. It is suggested via epigenetic inheritance systems starvation of the parents and obesity (=> diabetes 2) of the offspring are related.
Undeniably, to understand the interaction of fuels in the homeostasis of metabolism, there is besides a reductionistic approach for “one-gene” defect mutations, a need for additional research in the -omics of starvation and/or malnourishment and this should be integrated into epidemiological studies.

Systems Biology and its rapid development is based on progress in nutrition, bioinformatics and molecular biology and can give us biomarkers for starvation and ultimately a personalized medical treatment.

1.1. Epidemiology of Starvation and Undernourishment World-Wide

Although 82 countries are listed, the "Hunger Problem" is far away from the Industrialized Western world. To oversee and understand the enormous impact of the problem some facts will be mentioned. 854 million people across the world are hungry (State of Food Insecurity in the World 2006). Nearly one billion individuals are undernourished worldwide (Blackburn 2001). In Figure 1.1 a division of underweight, stunted and chronically undernourished people in the world is given. In Figure 1.2 is depicted an Overview of "Hunger in the World". Colors reflect % of the population undernourished. Black et al. (2003) gave an impressive and shocking review in 'the Lancet' entitled: "where and why are 10 million children dying every year?" Some facts from this review: -Six countries (Sierra Leone, Niger, Angola, Afghanistan Liberia and Mali) account for 50% of worldwide deaths in children younger than 5 year and 42 countries for 90%. -In 2005, about 10.1 million children died before they reached their fifth birthday. About 41% of child deaths occurred in sub-Saharan Africa and another 34% in South Asia (reviewed: Black et al. 2003). -In 2005, about 10.1 million children died before they reached their fifth birthday. About 41% of child deaths occurred in sub-Saharan Africa and another 34% in South Asia (reviewed: Black et al. 2003). Although most child deaths in these countries occur in rural areas, but also urban slum populations can have especially high child mortality rate. To get an impression of these "nothing saying" numbers: every day, almost 16,000 children die from hunger-related causes, nearly one child every 5 seconds (Black et al. 2003). Most of these deaths are attributed, not to outright starvation, but to diseases that move in on vulnerable children whose bodies have been weakened by hunger (State of Food Insecurity in the World 2002). Under conditions of malnutrition or starvation, the immune system becomes impaired, resulting in an immuno-suppression (Chandra 1996, Khovidhunkit et al. 2004, Wellen and Hotamisligil 2005).

1.2. The Hunger-Obesity Paradox

Strange enough when we hear the word “Hunger” we think at the Sub-Saharan Africa and South-Asia (vide Figure 1.2) but in the richest economy of the World, the USA, there is a Hunger-Obesity Paradox. This was first noticed by William Dietz, in 1995 in his case study, “Does Hunger Cause Obesity” (Dietz 1995).

Obesity and hunger exist side by side throughout the United States. What is remarkable and counterintuitive is that the contradictory concepts of hunger and obesity are now known to coexist within the same person and within the same household (Scheier 2005).
Epidemiology of “Hunger in the World” the “Hunger-Obesity Paradox”…


In classes with a low income (poverty) food insecurity and food insufficiency lead to a certain coping strategy. People from these economic classes, “the poor”, buy high-fat foods to prevent hunger. An alternative is that obesity is a physiological response of the body to episodic food insufficiency (van Ginneken 2008). Also environmental factors are related to poverty like the quality what food a family can afford to buy, but also where they can afford to live. This affects their proximity to food stores, fast-food restaurants, social services, public health services, and nutrition assistance programs (Scheier 2005). The local food environment and other environmental factors like physical activity, consumption patterns, and hunger
patterns may paradoxically all contribute to obesity (Morland et al. 2002). So we have to consider that hunger and malnutrition occur both in the underdeveloped “Third-World”-countries as well as in the richest economy of the World, the USA.

1.3. Malnutrition and/or Starvation Impairs the Immune System

The relationship between nutritional status and the immune system started during the decade of the 1950s. Keusch (2003) gave a chronological review of the development of our knowledge and the understanding of the relationship between nutrition and the immune system:

1950s: our knowledge of the immune system was primitive and was focused on the development of antibody humoral immunity. Most of the available information was derived from animal studies which was a bad reflection of the human situation.

1959-1968: the cycle of malnutrition-infection-more nutritional deterioration-more infection was acknowledged and more attention was paid to the reduction of the infection in malnourished children in developing countries.

1970-1980: by improved tools to assess immune function at the areas on the complement system, mucosal immunity and cell-mediated immune responses in humans, the mechanisms underlying the malnutrition-infection cycle were facilitated.

1980-1990: immunologist started to cooperate with nutritionist. The discovery of the decrease in the number of mature, differentiated T-cells during malnutrition was of great importance. Malnutrition was not only confined to children in developing countries but also occurred in up to half of the adult patients hospitalized in the US and also malnutrition was recognized in the elderly.

1990-2000: the role of micronutrient deficiency like vitamin A, iron and zinc as a conditioning factor in host response to infection became widely recognized.

2000 and beyond: The biological revolution initiated by the Human Genome Project, the development of rapid sequencing methods have created the possibility in identifying specific genes during immune responses and production of the relevant proteins that mediate the ultimate responses of the host (reviewed: Keusch 2003).

From Figure 1.3 (derived from: Wellen and Hotamisligil 2005), we can see that starvation and malnutrition can suppress immune functions and increase susceptibility to infections.

The four most common childhood illnesses are diarrhea, pneumonia, malaria and measles (State of Food Insecurity in the World 2002). Black et al. (2003) mention eight causes of child death, in order of proportion: a). Neonatal disorders (cause-specific contribution due to underweight) ≈ 33%, b). Diarrhea ≈ 21%, c). Pneumonia ≈ 20.5%, d). Malaria ≈ 9.0%, e). Other causes ≈ 9%, f). AIDS ≈ 3%, g). Measles ≈ 1.5%, h). Unknown ≈ 1.5%. Epidemiological observations have confirmed that infection and malnutrition aggravate each other (reviewed: Chandra 1996). However, nutrition does not influence all infections equally.

For others (e.g., viral encephalitis, tetanus), the effect of nutritional status is minimal. For still others (e.g., influenza virus, human immuno-deficiency virus), nutrition exerts a moderate influence (reviewed: Chandra 1996).
Figure 1.3. Metabolism and immunity are closely linked. Both over-nutrition and under-nutrition have implications for immune function. Starvation and malnutrition can suppress immune function and increase susceptibility to infections. Obesity is associated with a state of aberrant immune activity and increasing risk for associated inflammatory diseases, including atherosclerosis, diabetes, airway inflammation, and fatty liver disease. Thus, optimal nutritional and metabolic homeostasis is an important part of appropriate immune function and good health. (Figure reprinted with permission of Prof. Dr. G.S. Hotamisligil; Source: Wellen and Hotamisligil 2005).

The absence of complete vital registration systems (at the level of the individual) makes it difficult to predict and develop treatments for malnutrition, starvation and its related diseases especially in the poor densely populated urban areas of Central America or the remote famine-stricken regions of Africa (Blackburn 2001). A credible method, at the level of the individual, for generating valid prediction systems for malnutrition or starvation instead of relying only on epidemiological studies of effects of starvation (Baylin et al. 2005) or causes of death, is a challenge for basic and applied (medical) research (Morris et al. 2003). Therefore at the site of the diagnosis, ‘population screening’ via a “Systems Biology” strategy via Lipidomics (Laaksonen et al. 2008, Meikle et al. 2009) can reveal biological pathways and plasma biomarker candidates, for lipid changes in blood plasma, that can elucidate the mechanism behind malnutrition or starvation (Lusis et al. 2008) (see further §: “Metabolomics and Biomarkers”).

1.4: Psychological Effects of Human Starvation

Continued starvation is the most extreme under-nutrition that can be imposed (Blaxter 1989). In man, metabolic studies to illuminate the consequences of severe food shortages in Europe were undertaken by Benedict and his coworkers in 1919 when the USA entered World War I (Blaxter 1989) and by Keys at the University of Minnesota at the end of World War II (Keys 1950). Especially the, as later considered grueling study of Keys (1950) delivered a standard work: ”The Biology of Human starvation”. Both studies involved giving
young men semi-starvation diets continuously for periods of weeks. The 1385-page book of Keys (1950) presented the first comprehensive record of the physiological and psychological effects of starvation and refeeding. Before I restrict myself to the physiological mechanisms of starvation, I make a small note on the traumatically psychological impact this “Hunger experience” in general had on the volunteers. I quote an interview one of the volunteers gave: food became an obsession.

“I don't know many other things in my life that I looked forward to being over with any more than this experiment. And it wasn't so much …because of the physical discomfort, but because it made food the most important thing in one's life—food became the one central and only thing really in one's life. And life is pretty dull if that's the only thing. I mean, if you went to a movie, you weren't particularly interested in the love scenes, but you noticed every time they ate and what they ate”. (Kalm and Semba, 2005).

Because of the results of the experiment of Keys (1950), it is now generally recognized that starvation alters personality and that nutrition directly and predictably affects mind as well as body (Kalm and Semba, 2005). Disturbances in food pattern like food deprivation, anorexia nervosa, bulimia nervosa, cachexia and obesity are nowadays considered as "diseases" affecting cognitive and social functioning of the patient (reviewed: Kalm and Semba, 2005).

1.5. Epigenesis: Obesity and DM-2 in the Offspring of Parents Exposed to Starvation

Epidemiological studies have largely contributed to our understanding of the influence of disturbed intrauterine nutritional environment of the fetus and the consequences of these events on the health of the offspring. Intrauterine development of the fetus is related to birth size (Gluckman et al. 2005) and in this way can be correlated with pathogenic factors like eg. insulin resistance (IR), glucose intolerance and DM-2 in later life (Ozanne 2001, Reusens et al. 2007), increased incidence of obesity (Ozanne 2001), hypertension and coronary artery diseases (Eriksson et al. 2001, reviewed: Ross and Beall 2008) and mental disorders like schizophrenia (Gottesman 2007). Reusens et al. (2007) reviewed four clear examples of epidemiological studies, which supported the "Fetal Origin Hypothesis" of Barker (1995). This hypothesis stated that fetal undernutrition in middle to late gestation, which leads to disproportionate fetal growth, programs later coronary heart disease (Barker 1995) but also hypertension, and DM-2 in adult life (Godfrey 1998).

Those four clear examples reviewed by Reusens et al (2007) were:

a) In monozygotic twin pairs who were discordant for diabetes, the diabetic twin had a significantly lower birth weight than the normo-glycaemic co-twin.

b) Individuals exposed in utero to famine during the Dutch Hunger Winter have shown directly that poor maternal nutrition, especially during the last trimester of pregnancy, leads to growth restriction of the fetus and is associated with poor glucose tolerance and insulin resistance in 50 year old offspring.
c) In two independent studies on South-African children and Indian children those children born with a low birth weight, but who underweight rapid childhood weight gain, had the worse glucose tolerance, insulin resistance and were thus proposed to be the most susceptible to the development of DM-2 in adulthood.

d) Infants, "small for gestational age" (SGA) in comparison to infants "appropriate for gestational age" (AGA) demonstrated that the SGA infants had a rapid postnatal catch up growth but at an age of three years were more Insulin Resistant than AGA infants.

(Reviewed by: Reusens et al. 2007).

Later this hypothesis was extended to "Fetal Origins of Adult Disease" hypothesis which established a relationship between an adverse intrauterine environment and offspring disease in adult life (Armitage et al. 2005). Later the "thrifty phenotype" hypothesis (Hales and Barker 1992) explained the association between insufficient *in utero* nutrition and the later development of DM-2 (Armitage et al. 2005).

During development of the fetus in an adverse uterine environment due to "fetal programming" (Ross and Beall 2008), the imbalance between the early and postnatal environments may then conflict with the programming that occurred during fetal life, and predispose the offspring to the subsequent development of metabolic diseases in adulthood (Martin-Gronert and Ozanne 2010). This "thrifty phenotype" hypothesis states that during periods of inadequate nutrition, the fetus directs fuel for growth to the most essential organs, for example, the brain, while other organs are deprived of nutrients and are underdeveloped (reviewed: Michels 2003). Intrauterine growth retardation would permanently alter the development and metabolic functions of organs, for example, the pancreas leading to beta cell dysfunction (Meier 2009). These alterations would be beneficial to survival in a poor nutritional environment, but nutritional abundance might lead to metabolic problems, such as obesity susceptibility (Desai et al. 2009) and insufficiency in insulin secretory capacity and insulin resistance (IR) (reviewed: Michels 2003).

As a consequence in the offspring metabolic syndrome (obesity, hypertension, DM-2) will develop as a result of *in utero* environmental alterations (reviewed: Michels 2003, Ross and Beall 2008) and implicate enhanced appetite (Langley-Evans et al. 2010), and adipogenesis as contributory factors (reviewed: Ross and Beall. 2008, Desai et al. 2009). The subsequent offspring obesity in association with an increased appetite is described to the effect of "fetal programming" on leptin concentration, a satiety hormone (Vickers et al. 2000, reviewed: Ross and Beall 2008) but as other hypothalamic neuropeptide appetite regulating factors like neuropeptide Y (NPY) and agouti-related protein (AgRP) (Coupe et al. 2009). The ability of these environmental factors on the fetus to promote a phenotype or disease state mentioned above (e.g. insulin resistance (IR), glucose intolerance, DM-2, obesity, hypertension, coronary artery diseases and mental disorders like schizophrenia), not only in the individual exposed but also in subsequent progeny for successive generations is termed transgenerational inheritance (Skinner et al. 2010). This mechanism of epimutations in the germline that become permanently programmed can allow transmission of transgenerational phenotypes (e.g. DM-2) a process called epigenesis (Godfrey et al. 2007, Bromfield et al. 2008). Epigenesis is defined as heritable changes in gene expression that does not alter DNA sequence but are mitotically and transgenerationally inheritable.
Figure 1.4. Development of complex, multifactorial, polygenic diseases, such as metabolic syndrome as a consequence of e.g. starvation. Nutrition is primarily focused on health and on the earliest phases of disease pathology. In order to effectively apply dietary strategies to prevent disease or to recover homeostasis, validated early biomarkers (vide § 2.5) of the disease state are needed. Nutrition and pharma (pharmacology) are complementary approaches to apply to metabolic stress or metabolic syndrome. (Figure reprinted with permission of Prof. Dr. M. Müller; Source: Afman and Müller 2006).

It provides a framework for explaining individual variations and the uniqueness of cells, tissues, or organs despite identical genetic information. The main epigenetic mediators are histone modification, DNA methylation, and non-coding RNAs (reviewed: Tang and Ho 2007). However the exact mechanism of epigenesis during starvation or DM-2 is, as far as we know, until this moment unknown.

1.6. Homeostasis in Metabolism: Fuels and Their Function

a) Glucose and Fatty Acids, Major Metabolic Fuels:

The existence of an organism depends on the continuous provision of energy for metabolic processes. The most important metabolic fuels are glucose and fatty acids (Salway 2004). The maintenance of narrow-controlled blood glucose concentrations (glucose homeostasis) is central for a constant provision of glucose to the brain (Pardridge 1978, Lajtha et al. 1981, Cohen 1987). Glucose homeostasis is a physiologically well-balanced mechanism depending on three coordinated and simultaneously ongoing processes involving insulin secretion by the pancreas, hepatic glucose output and glucose uptake by splanchnic (liver and gut) and peripheral tissues (muscle and fat) (vide Figure 1.5) (Ashcroft and Ashcroft 1992, Baynes and Dominiczak 2004). In normal circumstances, glucose is the only fuel the brain can use (Cohen 1987). Glucose is also preferentially utilized by muscle during the initial stages of exercise (Salway 2004).

b) Long-Chain Fatty Acids, Storage Fuel

Under normal conditions lipids are stored as triacylglycerols (TG’s). These are highly concentrated stores of metabolic energy because they are reduced and anhydrous. The caloric
value of fats is higher than of either carbohydrates or proteins, and therefore long-chain fatty acids are an ideal storage fuel. Lipolysis of TG forms glycerol and FFA. The yield from the complete oxidation of fatty acids is about 9 kcal/g, in contrast with about 4 kcal/g for carbohydrates and proteins. Therefore it is logical that during the course of evolution TG were selected as the major source for energy store and not glycogen. This can be illustrated with an example of a man of 70-kg with fuel reserves of 100,000 kcal in TG, 25,00 kcal in protein (mostly muscle), 600 kcal in glycogen, and 40 kcal in glucose. TG's constitutes about 11 kg of his total body weight. If this amount of energy were stored in glycogen, his total body weight would be 55 kg greater (Stryer 1988). The body has a virtually unlimited capacity for the accumulation of fats (Baynes and Dominiczak 2004).

c) Amino Acids, Fuel During Fasting, Illness, or Injury

Amino acids normally serve as substrates for the synthesis of the body’s own proteins, rather than as a source of energy. However, during a prolonged fast, or after illness or injury, proteins are degraded and the constituent amino acids are converted into glucose in the gluconeogenesis (see further) mainly in the liver. Excess amino acids provided with food are normally converted to carbohydrates either for storage or for energy metabolism (Ashcroft and Ashcroft 1992, Baynes and Dominiczak 2004).

1.7. Glucose Homeostasis: Gluconeogenesis, Glycogenolysis and Glycogenesis And Lipogenesis

As you can see in Figure 1.5, the three major energy stores:

a) glucose and glycogen
b) amino acids and protein
c) Free fatty acids, mainly TG's which can follow different metabolic routes depending on e.g. the tissue/organ (compare liver vs. muscle).

But the ultimate goal is the maintenance of blood glucose levels within a standard range and the coordination of fuel utilization under extreme conditions like starvation to fuel vital organs (brain, heart-, common muscle) with ketone bodies. Several metabolic routes are important in this glucose homeostatis; the catabolic biochemical routes:

a) Gluconeogenesis,
b) Glycogenolysis and
c) Lipid catabolism in β-oxidation

and the anabolic biochemical routes:

d) Glycogenesis
e) Lipogenesis.
A) *Gluconeogenesis (lactate and amino acid catabolism)*: The substrates for gluconeogenesis originate from anaerobic glycosis (lactate) and the breakdown of either muscle protein (alanine) or adipose tissue TG's (glycerol). Muscle handles carbohydrates quite differently in contrast to the liver, as it does not have glucose-6-phosphatase and hence cannot release glucose into the circulation. Instead, it uses glycogen for its own energy needs ("fight or flight"-reaction). However muscle contributes to endogenous glucose production by releasing lactate (mainly by the activity of the enzyme hexokinase), a product of anaerobic glycolysis, which is transported to the liver, where it enters gluconeogenesis. Muscle can use both glucose and fatty acids as energy sources. During starvation, tissue proteins are broken down (tissue wasting) to form amino acids. The liver metabolizes the "glucogenic" amino acids by gluconeogenesis to glucose. The "ketogenic" amino acids are metabolized by the liver to form ketone bodies, while some amino acids are both ketogenic and glucogenic (Salway 2004).

B) *Glycogenolysis (glycogen breakdown)*: Glycogen is stored in muscle and liver. It is mobilized in liver during starvation and in muscle during extreme exercise. Liver is the great provider and during fasting (when glucagon prevails) its reserves of glycogen are broken down to release glucose in the blood where it is transported to the brain for energy metabolism. To achieve this, liver has glucose-6-phosphatase activity. In contrast, muscle (especially white skeletal muscle), uses glycogen entirely for its own benefit as a fuel during vigorous anaerobic exercise, e.g. during adrenaline stimulated "flight
or fight” reactions. It is important to notice that, in contrast to liver tissue, muscle does not have glucose 6-phosphatase activity. It is also important to notice that there is also a biochemical pathway for glucose and lactate between liver- and muscle- tissue. Lactate formed in the muscle in the anaerobic glycolysis is transported to the liver. In the gluconeogenesis in the liver this lactate is converted to glucose and recycled to muscle: the so-called "Cori Cycle".

C) Lipid catabolism: When glucose availability is low during periods of starvation, the TG stored in adipose tissue are hydrolysed to free fatty acids (FFAs) and mobilized into plasma to reach the liver where they play a major role in energy production (Hashimoto et al. 2000). In the liver, the influxed fatty acids are oxidized by the β-oxidation system, leading to the production of acetyl coenzyme A (acetyl-CoA), which then condenses with itself to form ketone bodies (Heijboer et al. 2005). As mentioned earlier these ketone bodies serve as substrate for vital organs like skeletal-, cardiac muscle and brain during starvation.

D) Glycogenesis and lipogenesis: When feeding follows a period of fasting, the first priority of the liver is to replenish its glycogen reserves under the influence of insulin in the Glycogenesis. Once the glycogen reserves are replenished then any surplus glucose will be converted under the influence of insulin to TG's. These are not stored in the liver, which would otherwise lead to hepatic steatosis but are transported from the liver in VLDL to adipose tissue for storage. However when this conditions of overfeeding is prolonged or a "High-Fat" diet is given for an extended period of time this pathogenesis of "fatty liver" will be the consequence (van Ginneken and Poelmann 2010).
Figure 1.6. Cross-talk between tissues in the regulation of glucose metabolism. Insulin is secreted from the \( \beta \)-cells of the pancreas in response to elevations in plasma glucose e.g. after a meal. The hormone decreases glucose production from the liver, and increases glucose uptake, utilization and storage in fat and muscle. The fat cell is important in metabolic regulation, releasing FFAs that reduce glucose uptake in muscle, insulin secretion from the \( \beta \)-cell, and increase glucose production from the liver. The fat cell can also secrete adipokines such as leptin, adiponectin, and TNF (vide: Figure 1.9), which regulate food intake, energy expenditure and insulin sensitivity (Figure modified after: Saltiel and Kahn 2001 and Evans et al. 2004). During starvation the mechanism works in the opposite direction with an antagonist of Insulin \( \rightarrow \) glucagon.

1.8. Feedback Control of Plasma Glucose by Insulin and Glucagon

Glucose homeostasis is controlled primarily by the anabolic hormone insulin, which is secreted from the \( \beta \)-cells of pancreatic islets of Langerhans (Ashcroft and Ashcroft 1992) and also by several insulin-like growth. Glucose stimulates the secretion of insulin (vide Figure 1.7 (Baynes and Dominiczak 2004). The antagonist of insulin is mainly glucagon, which is synthesized by the \( \alpha \)-cells of the pancreatic islet of Langerhans. In addition there are also several other catabolic hormones like: catecholamines, cortisol and growth hormone, which oppose the action of insulin. They are indicated as anti-insulin or counter-regulatory hormones (Ashcroft and Ashcroft 1992, Mizock 1995). The fine balance between insulin and glucagon action is a key factor in the control of fuel metabolism (Baynes and Dominiczak 2004). As you can see from Figure 1.7 insulin and glucagon both affect Glucose and Lipid Metabolism. Clearly visible from Figure 1.6 is that there is "cross-talk" between various
organs. Evans et al. (2004) described this "cross-talk" between organs, as a control mechanism of metabolism. On one hand we have an increase in blood sugar or fatty acids levels after e.g. a meal and uptake from the small intestine or as a result of stimulation of glucose release from the liver. Important is to remember that changes in glucagon usually oppose alterations in insulin levels. Thus, gluconeogenesis and glycogenesis are often initiated by rising glucagon and falling insulin levels e.g. during starvation.

Figure 1.7. Insulin and glucagon both affect Glucose and Lipid Metabolism. The fine balance between insulin and glucagon action is a key factor in the control of fuel metabolism. Clearly visible from this figure is that there is "cross-talk" between various organs, which is also a basic principle as a control mechanism of metabolism.

1.9. Macronutrient Hormonal Triggers

Anabolism involves the growth, maintenance, and repair of cells and tissues by means of protein synthesis. The opposite of anabolism, catabolism involves the breakdown of cells and tissues especially muscle during starvation. In addition, high levels of catabolic hormones promote fat storage by reducing insulin sensitivity and can devastate the immune system (Blackburn 2001).

Protein: Increased protein intake is positively correlated with and can bolster, growth hormone (somatropin) production, (Isidori et al. 1981), IGF-1 (Clemmons et al. 1985), and glucagon (Schmid et al. 1992). These hormones, collectively, exert an anabolic and lipolytic effect. Especially, somatropin is a powerful lipolytic- (fat mobilizing) (Piatti et al. 1999), anabolic- (muscle-enhancing) (Johannsson et al. 1999), immune system stimulating hormone
(Auernhammer and Strassburger 1995) that also directly influences cholesterol and triacylglycerol levels (Vahl et al. 1999).

Whereas carbohydrates are the primary trigger for insulin, protein is the primary trigger for glucagon (Table 1). Protein also exerts a weak stimulatory effect on insulin, approximately 30% of the effect of carbohydrate on insulin (Westphal et al. 1990).

As mentioned earlier, the fine balance between insulin and glucagon action is a key factor in the control of fuel metabolism (Baynes and Dominiczak 2004).

1.10. Physiological Effects of Human Starvation

A: Control of Metabolic Fuels: When starvation occurs in an animal, there are many physiological changes as the animal attempts to satisfy its energy requirements. At the cellular level, catabolism continues to supply the substances required for anabolism and to continue vital functions.

Reserve stores of nutrients are utilized. Energy is generated from the utilization of proteins, fats, and carbohydrates (Blaxter 1989). If we look only at the primary fuels used, we can distinguish three metabolic phases during food deprivation or starvation in mammals:

a) The post-absorptive phase, which last several hours. During this phase, metabolism is mainly fuelled by glycogenesis. The most readily usable material, the carbohydrate glycogen, is utilized first, which maintains constant blood sugar. However, the energy derived from glycogen is stored in the liver is exhausted within a few hours (Blaxter 1989, MacDonald and Webber 1995). This phase is followed by stored fat from the various adipose deposits (subcutaneous, around the kidney, and in the mesentery and omentum tissue, and finally the marrow of the bones (Keys 1950)).

b) When glucose availability is low during periods of starvation, the TG stored in the adipose tissue are hydrolysed to free fatty acids (FFAs) and mobilized into plasma to reach the liver where they play a major role in energy production (Hashimoto et al. 2000). In the liver, the influxed fatty acids are oxidized by the β-oxidation system, leading to the production of acetyl coenzyme A (acetyl-CoA), which then condenses with itself to form ketone bodies (Heijboer et al. 2005). Ketone bodies generated in the liver are transported out of the liver to serve as fuels for other tissues such as the skeletal-, cardiac-muscle and brain tissue during starvation (Hashimoto et al. 2000).

c) If starvation continues until the adipose stores are depleted, muscle is rapidly degraded for gluconeogenesis. Proteins are used as oxidative substrates and rates of protein synthesis/turnover fall during starvation. The rapid loss in muscle mass cannot be sustained for a long period an eventually kills the animal (Keys 1950, Wang et al. 2006).
Table 1.1. Effect of the macronutrients protein, fat and carbohydrates on the hormonal triggering of Insulin and Glucagon (modified from: Wolever and Bolognesi 1996).

<table>
<thead>
<tr>
<th>Tissue Fuel</th>
<th>ENERGY STORES</th>
<th>IN</th>
<th>MAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Fuel Reserve, Grams</td>
<td>Fat 9000-15000 Starvation 34 days</td>
<td>11 days 3 days</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>350 Muscle Glycogen 14 hours</td>
<td>5 hours 70 minutes</td>
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<tr>
<td>Muscle Glycogen</td>
<td>80 Liver Glycogen 3.5 hours</td>
<td>70 minutes 18 minutes</td>
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<tr>
<td>Liver Glycogen</td>
<td>20 Blood/Extracellular Glucose 40 minutes</td>
<td>15 minutes 4 minutes</td>
<td></td>
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<tr>
<td>Body Protein</td>
<td>6000 Body Protein 15 days</td>
<td>5 days 1.3 days</td>
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*Table 1.2. Energy stores in Human beings during different activities with emphasis on the Starvation Columns (modified from: MacDonald and Webber 1995, Salway 2006a).*

**B: Interaction between the different Organs/Tissues after a meal but also during starvation.** From Figure 1.6 we can see the “cross-talk” between various organs after a meal. Here, the signal initiating “cross-talk” is either an increase in blood sugar or fatty acids levels. This can occur following a meal and uptake from the small intestine or as a result of stimulation of glucose release from the liver.

The opposite happens during starvation. Important is that changes in glucagon usually oppose alteration in insulin level (Figure 1.7). Thus gluconeogenesis and glycogenolysis are often initiated by rising glucagon and falling insulin levels.

In Figure 1.8 is the “cross-talk” between various organs given during starvation (source Figure modified from Cahill 2006).
Figure 1.8. Overall scheme of starvation fuel metabolism. Liver derives its major energy by partial oxidation of FFA to β-hydroxybutyrate and acetoacetate; muscle and kidney by complete oxidation of FFA to CO2 and H2O. Brain utilizes both β-hydroxybutyrate and acetoacetate and glucose. FFA, free fatty acids; RBCs, red blood cells (Figure modified after: Cahill 2006).
a) **Body mass Index.** Henry (2001), has examined the literature on starvation, using Body Mass Index (BMI) to define the limits of human survival to starvation. Data included is based on normal weight subjects who died from starvation, famine or anorexia nervosa. Despite the diversity of sources, the data shows a remarkable consistency. In particular, the review illustrates a sex difference in the limits of survival when based on BMI classification. In males, a BMI of around 13 appears to be fatal. The coefficient of variation (CV) of the BMI is 8.7%. In contrast, females survive to a lower BMI of around 11, although with greater index variability (CV 14%). Several females had BMI's as low as 9 and 10. Based on these figures a mean BMI of 12 as the lower limit for human survival emerges - value first proposed by James et al. (1988). The ability of females to withstand a greater degree of food deprivation may be due to the following three reasons: a) Females have greater body stores of fat than males, b) The contribution of fat energy to total energy expenditure is greater in the female, resulting in a greater conservation of protein, c) Females appear better able to mobilize adipose tissue from most sites in the body (vide review: Henry 2001).

b) **Muscle:** In the “Minnesota Starvation Experiment” of Keys (1950), the volunteers received first 3 months 3200 cal of food per day. This period was followed by a 6 months semi starvation period in which they received 1800 cal of food per day (mimicking the war-torn areas of Europe). During this semi-starvation period most of the participants lost >25% of their body weight. The final three months were a nutritional rehabilitation period (Kalm and Sebena 2005). During food restriction, FFA from adipose- and muscle tissue are used as energy and are transported via the blood plasma to the liver. During prolonged fasting brain, heart and muscle shifts to using ketone bodies instead of FFA as energy and glucose as energy source of the brain. FFA is channeled to ketogenesis through \( \beta \)-oxidation when the supply of carbohydrate is short (e.g., during fasting) or under conditions of high circulating glucagons or low circulating insulin (e.g., diabetes mellitus) (Muurling 2004). On the other hand FFA are used for synthesis of TG that are packaged into lipoproteins when the supply of carbohydrate is abundant (e.g., during feeding) or under conditions of low circulating levels of glucagons or high circulating insulin concentrations (Muurling 2004).

c) **Liver:** In the post-absorptive (fasting state), Triacylglycerols (TGs) that are contained in adipose tissue are continuously hydrolysed by an enzyme called hormone sensitive lipase (HSL). HSL in the fed state is inhibited by insulin. Most of the generated free fatty acids are released into the blood and transported to other organs where they can be used as energy substrate. FFA release generally exceeds demand, especially in resting conditions. When not used for \( \beta \)-oxidation in mitochondria, FFAs can undergo re-esterification into TG, that can subsequently be deposited in the cytoplasm of the hepatocyte (hepatic steatosis). During starvation the liver represents the major sink of fatty acids in the form of TG (Heijboer et al. 2005). The fat constitution and composition of the liver after a period of starvation is largely unknown. Earlier we demonstrated with LC-MS- and localized \(^1\)H-MRS-techniques that the concentration of lipid compounds in the liver has been changed, quantitatively and qualitatively, due to the rearrangement and repartitioning of the adipose stores (van Ginneken et al. 2010).
Brain: The response of an organ to a condition of starvation or a Fat-Diet is tissue-dependent and most tissues can use a variety of substrates for energy metabolism, i.e., they are capable of utilizing lipids and proteins as well as carbohydrates. An exception is the brain, which is mostly exclusively dependent on glucose and ketone bodies (Pardridge 1978, Lajtha et al. 1981). Under normal nutritional conditions systemic glucose is the only significant source of energy for the brain, while under conditions of starvation, body carbohydrate reservoir diminishes first, blood glucose level drops and lipid catabolism is enhanced (Pardridge 1978). The liver produces ketone bodies as by-products of excess lipid catabolism which is under these conditions an important “fuel” for the brain in combination of a minimal amount of glucose levels produced by gluconeogenesis (Cohen 1987). Lipids cover about 60% of the brain’s dry weight making brain tissue the second most lipid-dense tissue after adipose tissue (McIlwain and Bachelard 1985). It is suggested that the brain’s inability to use fatty acids and amino acids for energy, constitutes a mechanism of protecting it from self destruction in order to maintain its integrity, at the expense of the rest of the body during starvation. This protection is based upon the brain’s inability to catabolize substances of which it is built, for energy production (Cohen 1987).

Blood plasma and lipoproteins: Because blood is a transport medium that carries nutrients, lipids and waste products between cells, we made in this review a comparison of the blood composition in two different situations, starvation versus obesitas, in a mouse model. Starvation is a situation of catabolism when an animal casu quo human uses reserve stores of nutrients to supply the substances required for anabolism and to sustain vital functions. The liver takes up free fatty acids (FFA) from plasma, which are transported by lipoproteins as triacylglycerols (TG). Lipoproteins are water-soluble protein complexes, which consist of a hydrophobic core, containing triacylglycerols (TG) and cholesterol esters (CE), and a hydrophilic monolayered shell, composed of phospholipids (PL), free cholesterol (FC), and specific proteins (apolipoproteins) (Salway 2006b). Five major classes of lipoproteins can be distinguished in blood plasma, including chylomicrons, very-low-density lipoproteins (VLDL), intermediate- density lipoproteins (IDL), low-density lipoproteins (LDL) and high density lipoproteins (HDL) (Muurling 2004). The function of LDL and VLDL is to transport CE from and to the liver and other organs, while HDL removes cholesterol (Ch) from peripheral tissues. The Free Fatty Acids (FFA) can be metabolized in mitochondria of the hepatocytes by β-oxidation to provide energy. FFA is broken down to acyl-CoA, which can be used in the tricarboxylic acid cycle for ATP production, FFA and TG synthesis or formation of ketone bodies. TG and CE can be used to form VLDL, which can be transported through the blood. TG from VLDL are subsequently hydrolysed into FFA and glycerol by lipoprotein lipase (LPL), which is located on the wall of blood capillaries. TG are also transported in chylomicrons in which dietary lipids are packaged and this is processed in a similar way. The LDL, VLDL and chylomicron remnants enter the liver again where cholesterol can be repackaged into VLDL or it can be removed from the body as bile acids (Rhoades et al. 1995). So FFA and TG are transported by the blood plasma where they can be stored or used as energy by the underlying tissue, e.g. liver-, adipose- or muscle tissue. For an overview of the
different lipoproteins and their function see Table 1.3 (Salway 2006b). Finally, a switch in lipoproteins can be expected during different nutritional stages like starvation or obesity. As a consequence this may have an effect on the hepatic delivery of substrates like Cholesterol, Triacylglycerols, and precursors-lipids like Sphingomyelin (resulting in the toxic product: ceramide). Finally, changes in lipoprotein concentrations due to nutritional intervention may result in inflammation reactions mainly during conditions like obesity and diabetes-2 (vide Figure 1.3) which if this effect of inflammation is extended may result in atherogenesis (Khovidhunkit et al. 2004).

f) Adipose tissue: Adipocytes are known to secrete hormones (vide Figure 1.9) (reviewed: Kershaw and Flier 2004). These adipocytokines exert different functions in fatty acid oxidation and insulin sensitivity. Alterations in the pattern of hormone secretion, which occurs in enlarged fat cells, can lead to impaired fat cell oxidation and insulin resistance (Heilbronn et al. 2004). The fat cells can become “dysfunctional”.
Table 1.3. Plasma lipoproteins are spherical structures with a hydrophilic exterior and a hydrophobic (lipid-containing) core. Their function is to transport lipids in the hydrophilic environment of the blood. The outer surface of lipoproteins is rich in phospholipids and apolipoproteins (like: ApoA1, ApoB48, ApoB100, ApoC2, ApoE), which confer upon the lipoproteins many of their specific properties. Schedule modified from Salway (2006b), modified. Abbreviations: VLDL=Very low density lipoprotein, IDL=Intermediate density lipoprotein, LDL=Low density lipoprotein, HDL=High density lipoprotein.

Plasma lipoproteins

<table>
<thead>
<tr>
<th></th>
<th>Chylomicron</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Intestine</td>
<td>Liver</td>
<td>Derived from VLDLs</td>
<td>Derived from VLDLs and IDLs</td>
<td>Intestine and liver</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Transport dietary TAG and cholesterol from the intestines to the periphery</td>
<td>Forward transport of endogenous TAG and cholesterol from liver to periphery</td>
<td>Precursor of LDLs</td>
<td>Cholesterol transport</td>
<td></td>
</tr>
<tr>
<td><strong>Density (g/mL)</strong></td>
<td>&lt;0.95</td>
<td>0.95-1.006</td>
<td>1.006-1.019</td>
<td>1.019-1.063</td>
<td>1.063-1.21</td>
</tr>
<tr>
<td><strong>Diameter (nm)</strong></td>
<td>100-500</td>
<td>30-80</td>
<td>25-50</td>
<td>18-28</td>
<td>5-15</td>
</tr>
</tbody>
</table>

Components of lipoproteins (%)

<table>
<thead>
<tr>
<th></th>
<th>Chylomicron</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TAG</strong></td>
<td>90</td>
<td>65</td>
<td>30</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cholesterol/Ester</strong></td>
<td>5</td>
<td>13</td>
<td>40</td>
<td>45</td>
<td>18</td>
</tr>
<tr>
<td><strong>Phospholipids</strong></td>
<td>4</td>
<td>12</td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>
Hormones secreted by fat cells play important roles in glucose and lipid metabolism. Adiponectin, for example, is secreted only by adipocytes and increases tissue sensitivity to insulin. ASP, another adipocyte-secreted hormone, increases lipid deposition in adipocytes by enhancing glucose uptake and deposition into triglycerides. Other hormones are resistin, angiotensin, plasminogen activator inhibitor-1, tumour necrosis factor-alpha, interleukin-6, leptin, and adipin (reviewed: Bays et al. 2002; Kershaw and Flier 2004). Most of the hormones secreted by fat cells are known to play a role in immune and/or inflammatory response (Heilbronn et al. 2004) and play a role in organ cross talk (Giorgino 2009). Based upon the preceding discussion, Mora and Pessin (2002) have proposed in a review an adipocentric view of insulin signalling and intracellular trafficking. They propose to consider adipocytes not only as primary site for whole-body energy storage but also as a critical endocrine organ, since it has become increasingly apparent that loss of adipose tissue in both animal models and humans also leads to metabolic disorders that result in severe states of insulin resistance and potential diabetes. However, despite the large number of molecules secreted by adipocytes, our understanding of the pathways and mechanisms controlling intracellular trafficking and exocytosis in adipocytes is poorly understood (Mora and Pessin, 2002). Finally, it is even possible that enlarged fat cells not have a pathophysiological significance by themselves, but are instead only a manifestation of other unknown pathogenic factors. In this hypothesis these factors may lead independently to enlarged adipocytes, insulin resistance and other traits (Heilbronn et al. 2004).

1.11. Metabolic Flexibility during Starvation

Tissues/Organs: The size and functional capacity of most visceral organs and muscle change in response to the physiological demands (like starvation or obesity) that are placed upon them. The gastrointestinal organs are very metabolically active and have been estimated to account for as much as 40% of the basal metabolic rate (Blaxter 1989). Thus, a reduction in organ size during fasting may confer a significant energetic savings, which may contribute to
a marked reduction in the basal metabolic rate of fasting animals (Wang et al. 2006).

*Partitioning between protein and fat:* It is hypothesized in the review article of Dulloo and Jacquet (1999) that the size of the initial protein compartment (which can ultimately serve during starvation as an energy reserve), can be as important as the initial percentage of fat at the start of starvation of the individual.

There is an inter-individual variation between individuals in protein sparing during the early phase of starvation, probably related to age-associated susceptibility to muscle wasting (Dulloo and Jacquet 1999), but also related to gender (Henry 2001).

In nutritional related diseases like obesitas (resulting in DM-2), the latter is often associated with a reduced oxidative capacity and impaired metabolic flexibility, i.e. an impaired switching from fatty acids to glucose in response to insulin. Thus, a reduced fat oxidative capacity and metabolic inflexibility are important components of muscle Insulin Resistance (Phielix and Mensink 2008). At this moment it is not known if the metabolic flexibility (switching between the substrates glucose and fatty acids) during starvation, can be impaired.

2. **METABOLOMICS AND BIOMARKERS FOR STARVATION**

2.1. **Reductionistic Approach vs. Systems Biology Approach**

Reductionism can either mean (a) an approach to understanding the nature of complex things by reducing them to the interaction of their parts, or to simpler or more fundamental things or (b) a philosophical position that a complex system is nothing but the sum of its parts, and that the account of it can be reduced to accounts of individual constituents. In contrast, Systems Biology has a holistic view and focuses on complex interactions in biological systems, claiming that it uses a new perspective.

The *Reductionistic vs. Systems Biology* approach is related to a discipline eg. reductionism is propagated in sciences like Chemical Engineering (Sorger 2005), Biochemistry and Biophysics (Fontecave 2010), while Systems Biology is propagated in Sciences like Biology, Pathobiology (Loscalzo et al. 2007). The major “drive” and interest for a Systems Biology approach is linked to the progress in collecting tremendous experimental datasets and at the system-level understanding of native biological and pathological systems to provide potential therapeutic targets (Friboulet and Thomas 2005, Ahn et al. 2006).

Medicine is at the edge, clinical medicine focuses on the parts and the Systems Biology on the systems (reviewed: Ahn et al. 2006, Loscalzo et al. 2007). While the “traditional” approach in medicine is based on four principles:

a) the focus on a singular, dominant factor which is the deeply rooted belief that each disease has a potential singular target for medical treatment. For example, an infection has to be treated with antibiotics but the underlying mechanism eg. an impaired immune system is not treated.

b) emphasis on homeostasis. This is based on two principles: i) homeostasis needs to be maintained by placing a deviating physiological parameter (eg. high blood pressure) within its physiological range, ii) because reductionism often disregards the dynamic
interaction between parts, the system is often depicted as a collection of static components.

- c) Inexact risk modification. The often in medical epidemiology "one risk-factor to one-disease" approach has certain limitations.
- d) Additive treatments. This is characterized by a reductionistic approach: one risk to one-disease analysis and the inability eg. for diseases with multiple risk factors like DM-2 and calculate their collective influences (reviewed: Ahn et al. 2006).

The need to make sense of complex genetic interactions has led some researchers from a component-level to system-level perspective. In understanding the difficulty of complex biological systems research has to be directed to the components of a biological system, their interactions and the behaviors and properties of the whole system.

This is the basis for a Systems Biology approach. The progress made in this new research area is also related to the progress made the last decade in other research area's like: Molecular Biology, Computational Science, Statistics, Chemistry, and Mathematics (reviewed: Ahn et al. 2006).

Figure 2.1. The complexity of the Human metabolism with its complex of the bodily function of living organisms (physiology) and the interaction between the different organs and tissues in healthy organisms or during disease (vide standard work: Salway 2006a).
2.2. Reductionistic Approach via Transgenic/Knock-Out Mouse Models

Via a reductionistic approach animal mouse mainly with genetically engineered mice were used to get insight which genes are actually mutated in human disease like starvation or DM-2 (Rees and Alcolado 2005, Davey and MacLean 2006). By eliminating these genes
(knock-out) or inserting mutations into specific genes, creating transgenic animals, (knock-in) some mechanisms of the starvation process can be elucidated and possibly via this approach biomarkers for starvation or DM-2 can be found. The advantages are clear: a). The transgenic approach is relatively straightforward and inexpensive (Davey and Maclean 2006), b). The different mouse strains gave for single gene defects valuable information eg. for the maturity-onset diabetes of the young (MODY) syndrome (Stride and Hattersley 2002), syndromes of severe insulin resistance (Krook and O’Rahilly 1996), mitochondrial diabetes (Maassen et al. 2004), the different obese mouse strains like ob/ob, db/db and fa/fa (Leibel et al. 1997, Chagnon and Bouchard 1996, Zhang et al. 1994, Lee et al. 1996: reviewed in Rees and Alcolado 2005). However there are also major disadvantages using only a reductionistic approach with rodent models. Jang and Remmen (2009) and other researchers (see below) critically noted that several questions still need to be addressed:

a) Most of the studies using the knockout and transgenic approach do not take into account the method effect, especially using the knock-out method which can be lethal or stressful during development (Davey and Maclean 2006).

b) Mice are mammals but besides large (genetically caused?) differences between strains the question can be posed if the results of mouse studies can be extrapolated to the human situation. To give two examples from an endocrinological point of view: i). adrenals of mice produce corticosterone rather than cortisol, which is produced by the adrenals in humans (Davey and Maclean 2006), ii). differences also exist in substrate preference of the steroidogenic enzymes between species (Payne and Hales 2004). An example is 17β-hydroxysteroid dehydrogenase (17HSD1) which uses estrogens as substrate in humans, whereas in rodents the enzyme catalyzes the conversion of both estrogens and androgens (Perkins and Payne 1988). An example from a genetic point of view is the study of Maegawa et al. (2010). They studied DNA methylation in normal colon tissues in young and old mice and found evidence of changes affecting over 20 percent of genes in the process of starvation. DNA methylation is an epigenetic event that affects cell function by altering gene expression. This age-related methylation instability is tissue-specific but the researchers questioned if this age-related DNA methylation phenomenon is specific to human tissues. So the question arises if a mouse model is suitable for the human situation (Maegawa et al. 2010).

c) There are several examples in genetically modified mice, which gave unexpected results, probably because metabolic/physiological/ endocrinological processes have a pleiotropic origin and more genes are involved. An example is the study of Malek et al. (2001) were a knock-in mouse model for the protein p27Kip1, an inhibitor of cell division was developed. This was an important goal because abnormally low amounts of p27 are associated with pathological states of excessive cell proliferation, especially cancers. Unexpected the researchers found that cells expressing p27Kip1 were unable to down-regulate p27 during the S and G2 phases of the cell cycle, but that this had a surprisingly modest effect on cell proliferation both in vitro and in vivo.

d) The genes encoding thyroid hormone receptor α and β (TRα and TRβ) encode four thyroid hormone receptors and four variant isoforms with antagonistic properties. So there have now 13 mutant knockout and knock-in mouse strains developed to
understand the specific functions of specific receptors. But the question remains making this mouse models: "which isoform is deleted and which is expressed?" More specific, the pleiotropic effects of T3 make it difficult to separate cell autonomous from indirect effects because of the unknown functions of the different isoforms (Flamant and Samarut 2003).

So the reductionistic approach with transgenic/knock-out strategies has in most cases failed to unravel candidate genes (=potential biomarkers), in the "garden variety" of the genetics of starvation and/or obesity (assuming there is such an entity). Therefore a "systems Biology" approach is clearly warranted. The "systems Biology" approach aims to understand phenotypic variation to assemble comprehensive data and models of cellular organization and biochemical function, and to elucidate interactions and pathways (Kitano, 2002). The term – omics represents the rigorous study of various collections of molecules, biological processes, or physiological functions and structures as systems (Keusch 2006). In principle metabolomics can provide certain advantages relative to other -omics technologies (genomics, transcriptomics, proteomics) in human starvation like is reviewed for diabetes-2 research by Bain et al. (2009).

1. Estimates vary, but e.g. in the Human Metabolome Database (HMDB)-Canada (Human Metabolome Project, Wishart et al. 2009), currently lists ~ 6,500 discrete small molecule metabolites, significantly less than the estimate of 25,000 genes, 100,000 transcripts, and 1,000,000 proteins. Trujillo et al. (2006) estimated the human genome to encode over 30,000 genes, and to be responsible for generating more than 100,000 functionally distinct proteins.

2. Metabolomics measures chemical phenotypes that are the net result of genomic, transcriptomic, and proteomic variability, therefore providing the most integrated profile of biological status.

3. Metabolomics is in theory a precise tool for discerning mechanisms of action (see further "personalized medical treatment " (van der Greef et al. 2006) and possible toxicological effects of drug therapies or in case of starvation of malnutrition the identification of novel and safety biomarkers that can be used in the assessment of new intensive treatments e.g. the development of new "functional foods" (Blackburn 2001).

4. An important area in which metabolomics has great potential is the discovery of biomarkers related to metabolic processes or age related diseases.

2.3. Lipidomics

A biomarker is defined as a substance used as an indicator of a biological state. It is characteristic that it is objectively measured and evaluated as an indicator of normal biological processes, nutritional intervention, pathogenic processes, or pharmacological responses to a therapeutic intervention (Laaksonen et al. 2008).

In this section we will in an extensive way discuss the principles of Metabolomics and the requirements for a potential candidate biomarker for therapeutic purposes. Here we restrict ourselves to a biomarker for human starvation.
Figure 2.3. Overview of the "omics" technologies (genomics, transcriptomics, proteomics, metabolomics and lipidomics) at several levels in the organism to elucidate the mechanism in a certain disease, or elucidate a biochemical pathway or mechanism or developing predictive tools like "biomarkers" which can be applied in a personalized medical treatment.

As mentioned earlier (Wang et al. 2006), based on the primary fuels three phases can be distinguished during starvation: a). postabsorptive phase with major fuel glycogen from the liver (stores for 3.5 hr) and muscle (stores for 14 hr). b). TG-stores from adipose tissue transported as FFAs in blood plasma and in the β-oxidation of mainly the liver transformed to ketone bodies for vital organs like skeletal-, heart-muscle and brain (fat stores: 34 days). c). Degradation of muscle proteins in the gluconeogenesis mainly in the liver (protein stores: 15 days) (reviewed: MacDonald and Webber 1995, Wang et al. 2006 and Salway 2006 a,b).

A potential biomarker from the proteomics fraction may be the protein Albumin, which is one of the major products of hepatic protein synthesis. It has a diagnostic value for nutritional status and catabolism (Doweiko 1991). A disadvantage of taking a protein as biomarkers is that we are already in the third phase of starvation (Wang et al. 2006) which lasts for humans, based on the human protein stores, only 15 days (MacDonald and Webber 1995, Salway et al. 2006 b,c). For development of an early valid prediction system for human malnutrition or starvation, which is applicable under "field-conditions" e.g. in the densely populated urban area's in South-East Asia or remote famine-stricken regions of Africa) (Blackburn 2001), a biomarker from the lipid fraction (Lipidomics) is a safer approach for three reasons.

a) In phase-2 in the diagnosis process of the starvation (Wang et al. 2006) we have more time because lipid stores are in principle for healthy patients sufficient for 34 days (reviewed: MacDonald and Webber 1995, Salway 2006 b,c).
b) Fatty acids measured as components of cholesterol esters or phospholipids present in plasma or serum reflect intake of dietary fat over the last few weeks (Katan et al. 1997).

c) Fat and fat-soluble substances have the advantage over other nutrients of a long half-life and readily accessible storage depots. In case no storage depots are found we are already in the situation of starvation, under-nutrition or eating disorders (Arab 2003).

2.4. Personalized Medical Treatment and Biomarkers For Starvation

*Personalized medical treatment:* Over the past century, traditionally medical care has centered on standards of care based on epidemiological studies of large cohorts. However, large cohort studies do not take into account the genetic variability of individuals within a population. E.g. at the level of genomics, according to the National Human Genome Research (NHGRI) about 99.9 percent of the DNA sequence is identical in all people, but the 0.1 percent difference is critical because it represents the genetic variations in the susceptibility of a person for getting a disease, the severity of the disease, and what is the impact of the medical/therapeutic treatment (Wishart et al. 2009).
Personalized medical treatment seeks to provide an objective basis for the consideration of such individual differences. It is often defined as “the right treatment for the right person at the right time” (Bennet 2005). “Traditionally, personalized medicine has been limited to the consideration of a patient’s family history, social circumstances, environment and behaviors in tailoring individual care. Nowadays all the information generated by genomics (studying variations in genes that cause disease), proteomics (seeking for abnormal protein patterns) and metabolomics (mining for abnormal metabolite patterns) collected by the application of new measurement tools (GC-MS: Gas chromatography mass spectrometry; LC-MS: liquid chromatography mass spectrometry; MD-LCMS: multidimensional liquid chromatography mass spectrometry; NMR: Nuclear magnetic resonance; PTM: Post-translational modification
(van der Greef et al. 2006) was one of the preconditions for the strategy of a personalized medical approach. The other precondition was the development of techniques in the processing of data by Bioinformatic Science, computation and modeling in combination with the development of new statistical techniques for application in biomedical research to evaluate the tremendous amount of datasets (van der Greef et al. 2003, 2004). These trends during the last decade in Systems-Biology gave an enormous boost to this research area and we are close to a personalized diagnostic “Industry” (Wishart et al. 2007).

Examining biomarkers for starvation in personalized medicine would provide a scientific baseline for clinical trials of anti-starvation medicines and the effects of the recently development of “functional foods” after/during starvation (Blackburn 2001).

**Biomarkers for starvation:** A particular area of interest of Metabolomics-based System Biology, will be identification of novel safety biomarkers that can be used in the assessment of new intensive treatments both during their discovery phase and in clinical use e.g. to study age-related diseases in combining the diagnostics with the patient therapy (van der Greef et al. 2006). Recent technological advances in metabolomics (van der Greef et al. 2003, 2004) and lipidomics (Meikle et al. 2009) can potentially make a real contribution in increasing efficacy of drug development pipelines (Laaksonen et al. 2010).

![Figure 2.5. Nutritional genomics. Research and discovery in nutritional genomics elucidate the reciprocal interactions among nutrients, metabolic intermediates, and the mammalian genome. Understanding the interrelationships among human genetic diversity, genome function, and dietary components will enable precise manipulation of genome function and stability throughout the life cycle for optimal human health and disease prevention. (Figure reprinted with permission of Prof. Dr. J. Stover; Source: Stover 2004).](image-url)
Metabolomics is a discipline dedicated to the systematic study of small molecules (i.e. metabolites) in cells, tissues, and different biofluids. Metabolite levels can be regarded as amplified responses of biological systems to genetic or environmental changes Laaksonen et al. 2010). From Figure 2.7 we can see that the balance between "disease" or "healthy" is determined by factors like on one hand: environment, behavior and diet and for a diseased person by genetics and medical treatment.
In this respect molecular mediators in blood plasma (Baylin et al. 2005) are important to study because they can serve as biomarkers to trace people vulnerable and susceptible for the pathogenesis or starvation. The process to find a proper biomarker is underestimated and has to follow a long route:

A) The Discovery phase,
B) Qualification phase one, which encompasses:
   1. Identification of the biomarker,
   2. Validation analysis,
   3. Proof of the biomarker in a new study,
C) Qualification phase two, which encompasses:
   1. Study precision,
   2. Study robustness,
   3. Study limitation,
   4. Test biomarker in wider population,
D) Finally the application phase in the population is reached (Koulman et al. 2009).

2.5. Biomarkers from Lipidomics

A prerequisite is that homeostatic conditions are needed for the biomarker to reflect accurately long-term intake and not to be biased by lipolysis (Arab 2003). Fatty acids can be measured as free fatty acids in serum, components of circulating triacylglycerols, components of erythrocyte membranes, phospholipids or cholesterol esters, or adipose tissue from various sites.

Lipid compounds of serum or plasma: reflect the dietary fat intakes of the past few hours (triacylglycerols) or the past few days (cholesterol ester and phospholipid fatty acids). Fatty acids measured as components of cholesterol esters or phospholipids present in plasma or serum reflect intake of dietary fat over the last few weeks (Katan et al. 1997).

Also Zock et al. (1997) found the fatty acid composition of serum cholesterol esters suitable qualitative biomarkers.

Lipid compounds in erythrocytes: red blood cells provide a marker reflecting the last month and offer a more aggregated time period than does serum (Arab 2003). This estimation is based on the lifespan of erythrocytes (~120 d, the half-life of erythrocytes) (Arab 2003).

Lipid compounds from adipose tissue: this is a preferred medium for the measurement of fatty acids as a reflection of long-term dietary intake and has been considered the best indicator of long-term essential fatty acid intake (Baylin et al. 2005), under the restriction when no severe weight loss has occurred (Arab 2003). Adipose tissue, be it gluteal, abdominal, subcapular, pectoral or from another site, reflects long-term storage of fats under homeostatic conditions. This is because of the oxidative, low fuel requirements of adipocytes and the large energy content. It has to be remarked that white adipose tissue is metabolically active (reviewed: Arab 2003).
Fasting whole Blood: Baylin et al. (2005) compared, for epidemiologic studies fasting whole blood as biomarker of essential Fatty Acid intake in comparison with adipose tissue and plasma. Adipose tissue is considered the best indicator of long-term essential fatty acid intake but sampling has more complications. It the comparison whole blood vs. plasma he concluded that fasting whole blood is a suitable biomarker of long-term essential Fatty Acid intake, and its performance in comparison to that of fasting plasma. Furthermore in epidemiological studies it is the sample of choice because of its ability to predict intake, its accessibility, and minimum sampling processing (Baylin et al. 2005).

**CONCLUSIONS**

a) Nearly one billion individuals are undernourished worldwide.

b) Every day, almost 16,000 children die from hunger-related causes, nearly one child every 5 seconds.

c) Under conditions of malnutrition or starvation, the immune system becomes impaired (mainly due to a decrease of mature differentiated T-cells), resulting in an immuno-suppression.

d) The “Hunger-Obesity Paradox”, in the richest economy of the World, the USA, is that the contradictory concepts of hunger and obesity are now known to coexist within the same person and within the same household due to food insecurity and food insufficiency leading to “coping-strategies” in the low income classes to buy High-Fat food to prevent Hunger.

e) Epidemiological observations have confirmed that infection and malnutrition aggravate each other resulting for children in the four most common illnesses: diarrhea, pneumonia, malaria and measles.

f) It is now generally recognized that starvation alters personality and that nutrition directly and predictably affects mind as well as body.

g) The “Fetal Origin Hypothesis”, “The Fetal Origins of Adult Disease” and the "Thrifty phenotype" hypothesis states that due to “fetal programming”, during periods of inadequate nutrition, the fetus directs fuel for growth to the most essential organs, for example, the brain, while other organs are deprived of nutrients and are underdeveloped. As a consequence in the offspring metabolic syndrome (insulin Resistance, obesity, hypertension, DM-2 and coronary diseases) will develop as a result of in utero environmental alterations and implicate enhanced appetite, and adipogenesis as contributory factors.

h) This mechanism of epimutations in the germline that become permanently programmed can allow transmission of transgenerational phenotypes (e.g. DM-2) a process called epigenesis and is defined as heritable changes in gene expression that does not alter DNA sequence but are mitotically and transgenerationally inheritable.

i) The fine balance between insulin and glucagon action is a key factor in the control of fuel metabolism. Clearly visible from this figure is that there is "cross-talk" between various organs, which is also a basic principle as a control mechanism of metabolism.
The energy stores in human beings during starvation are: fat (34 days), muscle
glycogen (14 hours), liver glycogen (3.5 hours), blood/extracellular glucose (40
minutes), body protein (15 days) and a Body Mass Index of 12 as the lower limit for
human survival.

A valid prediction systems at the level of the individual for malnutrition or starvation
is a challenge for basic and applied (medical) research.

This can be applied via a “Systems Biology” strategy with predictive “Biomarkers”,
which can make ‘population screening’ and ‘Personalized medical treatment
possible.

Lipid compounds from “whole blood” is a suitable biomarker for epidemiological
studies because of its ability to predict intake, its accessibility, and minimum
sampling processing.

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