

# THE BACKGROUND OF $\beta$ -OXIDATION DISORDERS IN HUMANS. I. THEORY

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## SUMMARY

*Disorders in energetical processes are described from the point of view of energy production and the possibility of substitution of substrates for ATP synthesis. The effects of other diet components such as microelements ( $Cr^{3+}$  ions) and non-digestible oligofructans (inulin and oligofructose) as stimulatory factors on the activity of  $\beta$ -oxidation and cholesterol and triacylglyceroles levels lowering are reported.*

**Key words:** energy production,  $\beta$ -oxidation, chromium ions, human, rats, broilers

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Energy delivery between meals in organism is controlled by hormonal and enzymatic regulation of processes of glycolysis, glycogenolysis, gluconeogenesis, oxidation of fatty acids, ketogenesis and ketolysis. Sudden interruption of energy delivery for metabolic processes should result in loss of consciousness and irreversible damages of central nervous system. Protracted shortage of energy affects mostly central nervous system, muscles and heart. In extreme chances alarming signal is pronouncement of hypoketotic hypoglycaemia (this is not constant and does not appear in the short-chain fatty acids degradation disorders) (1), Reye's syndrome and myoglobinuria. Reye's syndrome consists of a number of severe disorders such as acute encephalopathy accompanied by hepatomegaly and lipid storage in the liver. Glycogen level in the liver falls down while concentration of amino acids in blood increases. This is the effect of disorders in amino acids and ammonia metabolism. Decomposition of fatty acids in  $\beta$ -oxidation process is strongly affected by the structure of mitochondria. In hepatocytes from patients with Reye's syndrome membranes of mitochondria exhibits improper structure which leads to disorders in  $\beta$ -oxidation of fatty acids. Cardiac or skeletal muscles myopathy combined and/or hepatic involvement at periods of metabolic decompensation are typical, since these tissues depends on fatty acid oxidation. A number of genetic disorders may result in energy deficit due to improper glycolysis and/or  $\beta$ -oxidation of fatty acids.

Mitochondrial  $\beta$ -oxidation of fatty acids is the process assuring inflow of energy between meals. Process becomes activated after longer lasting break in feeding and after exhaustion of liver and muscular glycogen only. Activation of  $\beta$ -oxidation of fatty acids launches hormonal impulse stimulating lipolysis caused by lowering of the level of insulin and remission of hyperglycaemic hormones. Fatty acids transported to cells in the form of complexes with albumins can pass the biological lipid bilayer membrane of the cell and/or membranes of the subcellular structures after binding with carnitine (3-hydroxy-butyric acid betaine) and can undergo  $\beta$ -oxidation process in mitochondria. Acetyl-CoA, di-

carbon fragment, is gradually released from a side of activated carboxyl group of chain of fatty acid across enzymatic splintering off in position  $\beta$ . Then acetyl-CoA becomes metabolized in Krebs cycle. It is the substrate for synthesis of ketone bodies (in ketogenesis), and for synthesis of glucose in the process of glukoneogenesis. During a prolonged starvation, under correct process of  $\beta$ -oxidation organism can work in this new energetic arrangement without a greater disturbances for a long enough time.

Derivatives of fatty acids -  $\alpha$ ,  $\Omega$  - dicarboxylates - can also undergo  $\beta$ -oxidation. Additionally these compounds exhibit an antibacterial properties and have been used in the treatment of hyperpigmentary disorders. Dicarboxylic fatty acids from oxalate (DC 2) to sebacate (DC 10) are transported by the inner membrane anion channel (IMAC). Additional possibility of the transport of DC 6 - DC 10 is electroneutral mechanism that appears to reflect the transport of the fatty acid through the lipid bilayer. This transport mechanism is probably the major pathway by which the longer dicarboxylates enter cells, bacteria and mitochondria. DC 2 and DC 4 are the only dicarboxylates that can be transported by the classical dicarboxylate carrier (2).

Other problem is metabolism of  $\beta$ -methyl-fatty acids (i.e. phytanoic acid). In normal, health subjects decomposition of this acid starts from  $\alpha$ -oxidation followed by regular  $\beta$ -oxidation of formed acyl-CoA after shortening of the fatty acid chain by one carbon atom. In the patients with Refsum's syndrome the metabolism of this compound is impossible because of block of initial  $\alpha$ -oxidation of the fatty acid and manifests in the storage of phytanoic acid (lipomatosis).

Physical exercises require increased amount of high-energy compounds (such as ATP and phosphocreatinine) for work of muscles. On the other hand, the brain is almost completely dependent on glucose and/or ketotic bodies delivery. As long as the requirement of ATP is balanced by the activity of glycolysis and by the intake of glucose, delivery of energy is high enough. When the sugar delivery is lower than the requirements of the

organism, the deficit may be covered by  $\beta$ -oxidation (acetyl-CoA production). Problems begin at the moment of short supply of glucose delivery at lowered activity of  $\beta$ -oxidation.

While hypoglycaemia could be assayed by a simple estimation of glucose level in the blood, the examination of proper activity of  $\beta$ -oxidation is much more complicated. Investigations of the entire process of  $\beta$ -oxidation of fatty acids was for the first time described by Manning et al. (3) and Olpin et al. (4). They used cellular cultures of isolated fibroblasts and tritium labeled palmitic acid as a substrate. This procedure required to obtain fragments of skin, isolation and culturing of cells in order to obtain sufficient quantities of material for investigations. Use of lymphocytes isolated from venous blood (5) permits considerably to shorten the time of the assay and answer the question, whether a process of  $\beta$ -oxidation of fatty acids proceeds correctly. When results of investigation point out disturbances of this process a possibility exists to investigate individual stages of this metabolic pathway, i.e. acyl-carnitine transferases (6, 7), 3-oxothiolase (8) or connected irregularities concerning a level and transport of L-carnitine (9).

The enzymatic reactions of mammalian  $\beta$ -oxidation in mitochondria were elucidated some 40 years ago. In the pathway leading to this process a great number of enzymes i.e. membrane-associated acyl-CoA dehydrogenase and carnitine-acyl transferases are engaged (10). Heavy inherited  $\beta$ -oxidation disorders should manifest clinically as usual by recurrent Reye-like episodes (acute hepatopathy and encephalopathy). The findings at the tissue level are dominated by steatosis of organs with a high  $\beta$ -oxidation level (liver, kidney, heart and skeletal muscles) and a toxic effect of metabolites of  $\beta$ -oxidation process (11). An additional effect of  $\beta$ -oxidation disorders is obesity, associated with metabolic disorders related to alterations in lipid mobilization and oxidation (12). Lipogenesis is stimulated by a high carbohydrate diet, whereas it is inhibited by polyunsaturated fatty acids and by fasting (13). In humans, determination of the total activity of  $\beta$ -oxidation of fatty acids is important in medical diagnostics (3, 4, 5, 14).

Genetically inherited disorders of  $\beta$ -oxidation are dangerous for organism when they are serious. They can result from genetically conditioned defects of oxidation of long chain (LCHAD) or mid-chain fatty acids (MCHAD), a deficit of acyl-carnitine transferases (CPT-1 and CPT-2) (16, 18), acyl-CoA dehydrogenases (M-MAD), keto-thiolase and transport of carnitine.

Lowering of the proper level of palmitic acid oxidation could result from a number of factors such as fatty acid transport to the tissues, formation of carnitine-acyl complexes and their transport through the mitochondrial membrane into the mitochondria and decomposition of fatty acid chain in the mitochondria. In addition, it is necessary to pay an attention to extracellular factors as L-carnitine (17) and  $\text{Cr}^{3+}$  ions (18) which (among others) play a role in  $\beta$ -oxidation as well. L-carnitine is necessary for transport of fatty acids across the membrane in the form of acyl-L-carnitine and acetyl-L-carnitine. Acetyl-L-carnitine (ALCAR) contains acetyl and carnitine moieties, both of which have neurobiological properties. Other reported neurobiological effects of ALCAR include modulation of brain energy and phospholipid metabolism, cellular macromolecules including neurotrophic factors and neurohormones, synaptic morphology and synaptic transmission of multiple neurotransmitters (19). These functions should explain neurological effects of  $\beta$ -oxidation disorders on central nervous

system. The deficit of L-carnitine (if necessary) could therefore be solved by supplementation of this compound (20). L-carnitine provides a natural pathway for removal of the toxic metabolites in these disorders and is life saving therapy but, with poor oral absorption (25%). This makes it necessary to administer this compound in case of  $\beta$ -oxidation disorders caused by its low level intravenously. Final activity of  $\beta$ -oxidation is the effect of all of these processes, proper or disturbed in its activity.

The frequencies of inherited disorders of  $\beta$ -oxidation of fatty acid differ in human populations. They are the highest in closed populations such as Jewish (religious rules). Giros et al. (21) reported 2% of  $\beta$ -oxidation disorders among 116 cases of peroxisomal diseases in Spain in the period of 10 years (1987-1997). Disorders of peroxisomes metabolism should affect not only  $\beta$ -oxidation, but also  $\alpha$ -oxidation, ether phospholipid and isoprenoid biosynthesis (14, 22). In our studies from the years 1994-2000 (data not published) we found 6.21% of lowered  $\beta$ -oxidation level and 2.64% of very low activity of  $\beta$ -oxidation of palmitic acid. In the case of  $\beta$ -oxidation of myristic acid we found 5.36% of mid- and 2.67% of serious disturbances of  $\beta$ -oxidation. These disorders were accompanied by lowering of serum carnitine level. Physiological level of L-carnitine turned to be higher than that one required for proper  $\beta$ -oxidation (data not published).

Limited disturbances of activity of  $\beta$ -oxidation not always result in pathological disorder. However, in connection with a low level of glucose they can lead to losses of consciousness. In the case of large requirements of organism for the high-energy compounds (physical effort, exercises etc.) at the limited delivery of sugar and simultaneous lowering of production of energy as result of lowered  $\beta$ -oxidation, the quantity of ATP produced in respiratory chain united with Krebs cycle does not cover energetic requirements of organism.

Delivering to the organism the greater than required quantities of energy in the form of carbohydrates causes transformation of their excess into glycogen and fatty acids.

In cows fed at restricted energy intake and overfed during the dry period the mode of feeding affected only concentration of fatty acids but not their composition (23).

An important component of diet, affecting metabolism of sugars and lipids, are ions, among them chromium. Chromium in the form of  $\text{Cr}^{3+}$  is a factor enhancing the uptake of glucose by cells activated by insulin and its influence on membrane insulin receptor and activation of receptor-dependent phosphorylases and phosphatases. Simultaneously, chromium ions activate process of  $\beta$ -oxidation in lymphocytes (18). Oxidation of palmitic acid in lymphocytes from broilers fed with fodder with addition of 0.5 ppm of chromium in the form of chromium yeast was 2-times more effective than those in lymphocytes of chickens fed with standard fodder (without addition of chromium yeast). The stimulatory effect of the chromium ions was observed as long as 4 weeks after removing chromium supplementation from the diet.

The latest results of our experiments (24) performed on rats fed with the diet supplemented with chromium ions in the form of chromium yeast to the final concentration of 5 ppm and with 10% of non-digestible oligofructans led us to conclusion that these components increased the rate of fatty acids decomposition as compared to those with lower concentration of these additives (0.5 ppm for chromium and 5% for oligofructans, respectively). In humans addition of inulin to the experimental diet resulted in

lowering of blood cholesterol and triacylglycerols (25, 26). It is possible, that the same effect is accompanying the observed changes in rats.

The need to cover energetic requirements of organism originates from two processes dependent each on another transformation of glucose and transformation of fatty acids. Under normal circumstances, shortages of sugar are balanced by utilization of products of fatty acids degradation for gluconeogenesis. Excess of delivered sugars is stored in the form of glycogen and transformed into fat. Especially essential is equilibrium of these processes for people executing strenuous physical work. An energetic deficit caused by limited availability of sugar, which cannot be covered by energy originating from decay of fatty acids can lead to serious pathological condition (loss of consciousness, neurological disorders and dysfunction of skeletal muscles and cardiac myopathies). Morning hypoglycaemia after overnight fasting is a signal of existing irregularities in energetic metabolism of organism. It should suggest the necessity of examination of activity of metabolic transformations of glucose and fatty acids and (in the case of lowered activity of  $\beta$ -oxidation) of the level of L-carnitine. Beneficial to health could be delivery of L-carnitine to organism (preferable intravenously) and possibly of ions, among them of ions of chromium (in the form of  $\text{Cr}^{3+}$ .)

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Received September 23, 2003

Received in revised form and accepted February 4, 2004