THE BACKGROUND OF β-OXIDATION DISORDERS IN HUMANS. II. LABORATOY STUDIES AND CLINICAL DATA

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SUMMARY

Laboratory method for studies of clinical disorders of β-oxidation is described. 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 β-oxidation and cholesterol and triacylglyceroles levels lowering are reported.

Key words: energy production, β-oxidation, chromium ions, human, rats, broilers, laboratory method

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Energy delivery in organisms is done by balanced processes of glycolysis and β-oxidation connected with Krebs' cycle. High carbohydrate diet as well as high lipid one can substitute, in part, each other. As long as processes of degradation of sugars and lipids and its content in diet are balanced the catabolism of these compounds deliver proper amount of ATP and is stored in the form of glycogen and lipids to overcome their eventual short supply. Processes of glycolysis, glycogenolysis, gluconeogenesis, oxidation of fatty acids, ketogenesis and ketolysis are controlled by hormonal and enzymatic regulation mechanisms. In clinical practice disturbances in energy delivery to tissues manifest as hypoketotic hypoglycaemia and loss of consciousness and irreversible central nervous system damage (this is not constant and does not appear in the short-chain fatty acids degradation disorders) (1), Reye's syndrome and myoglobinuria. Cardiac or skeletal muscles myopathy combined and/or hepatic involvement at periods of metabolic decompensation are typical, since these tissues depend on fatty acid oxidation.

Glycolysis is quite simple process of degradation of glucose after its uptake to the cell. The main steps are: phosphorylation of glucose, its transformation into fructose, hydrolysis of phosphofructose into two C-3 units and after decarboxylation complete decomposition in Krebs' cycle. Beta-oxidation is more complicated. After transmembrane transport into the cell fatty acid molecule must be transferred through mitochondrial membranes (as esters of carnitine) by two acyl-carnitine transferases , 1 and 2 (2-5). After hydrolysis of the ester bond fatty acid is transferred on CoA and degraded by β-oxidation delivering acetyl-CoA for Krebs' cycle. Factors affecting the activity of β-oxidation have not been well learned yet.

Disorders of glycolysis could be assayed by simple measurements of glucose lever after overnight fasting and/or glucose and fructose phosphorylation using "ready for use" commercially available kits. Studies of β-oxidation disorders require more complicated methods. These methods are based on decomposition of radiolabelled fatty acid (generally palmitic, oleic or myristic acid). In mitochondrial β-oxidation process tritiated water is liberated and its amount is the indicator of the activity of fatty acid degradation (6, 7). Such assay answers the question if activity of β-oxidation is normal or lowered and what is the degree of this metabolic block. This method could not answer the question on the place responsible for lowered decomposition of fatty acid. It could be through-membrane transportation of substrate (which is carnitine-dependent process), as well as the activity of acyl-carnitine transferases.

The second problem of improper β-oxidation is the storage of lipids which leads to increased risk of atherosclerosis and cardiovascular diseases (8). This is the reason for studies of factors affecting the level of lipids (triacylglyceroles, phospholipids and cholesterol) by its decrease and/or by the activation of lipids degradation. Among others the most interesting role is played by chromium ions (Cr^{3+}) and non-digestible oligosugars. It is known, that chromium ions exhibit insulin-like effect on glucose uptake by cells (9, 10). However it is impossible to answer the question if chromium affects the activity of glycolysis. It is known (11, 12) that chromium affects the glucose concentration in blood in *diabetes mellitus type 2* as glucose level lowering factor.

In humans, determination of the total activity of fatty acids is important in medical diagnoses (6, 7, 13, 14). It can improve the therapy of patients with β-oxidation disorders. On the other hand, the assay of the activity of fatty acids degradation can answer some questions in obese patients with elevated risk of atherosclerosis and cardiovascular diseases. Another problem of interest are factors stimulating the activity of β-oxidation not only for the purpose of medical therapy but also for industrial breeding of animals.

LABORATORY ASSAY OF THE ACTIVITY OF β-OXIDATION

The method is based on the ability of cells to utilize fatty acids as an energy source. Firstly Manning et al. (7) used fibroblasts as cells for studies of β-oxidation activity. These cells were also used by other laboratories (13, 15). This material requires previous isolation of fibroblasts and their culturing to obtain proper amount of cells for studies. In our experiments we chose lymphocytes isolated from the whole venous blood by simple centrifugation. The cells were incubated for 1 hour at 37 °C in Hank's Balanced Salt Solution (HBSS) in the presence of palmitic acid at a concentration of 26.2 pmol/sample supplemented with 1 μCi of [9,10-^{3}H]-palmitic acid. After four rounds of β-oxidation cycle tritiated water liberated from radiolabelled substrate was separaded from remaining radioactive palmitic acid on the column (Bio-Rad AG 1X-8) (6). Its amount was the indicator of the activity of β-oxidation. The activity was calculated as pmoles of decomposed fatty acid per mg of lymphocyte protein per 1 min [see (6)]. This allowed to study the problems concerning inherited β-oxidation disorders in Clinic of Metabolic Diseases of Children Health Center, Warsaw, Poland in the period of 1994-2000 (data not published). In the material comprising 1600 samples, we found heavy disorders only in 2.64% of the samples. A physiological level of β-oxidation in human lymphocytes was estimated by us for healthy adults as 26 – 28 pmol/min/mg of lymphocyte protein. Acceptable level must be higher than 18 pmol of palmitic acid decomposed by 1 mg of lymphocyte protein per 1 min at the concentration of substrate of 26.2 pmol, whereas in case of the heavy disorder – below 10 pmol/min/mg of protein. The frequencies of inherited disorders of β-oxidation of fatty acid differ by population/ethnical group. The highest prevalence has been found in closed populations such as Jewish (religious rules). Giros et al. (16) reported 2% of β-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 β-oxidation, but also α-oxidation, ether phospholipid and isoprenoid biosynthesis (14, 17). In our study (data not published) 1,600 samples were tested. We found 6.21% of lowered β-oxidation level and 2.64% of very low activity of β-oxidation of palmitic acid.

Lowering of the proper level of palmitic acid oxidation should result from a number of factors as fatty acid transport to the tissues, formation of carnitine-acyl complexes, their transport through the mitochondrial membrane into the mitochondria and decomposition of fatty acid chain in the mitochondria. In addition an attention should be payed to extracellular factors [L-carnitine (5) and Cr^{3+} ions (18)] which (among others) play a role in β-oxidation. L-carnitine is necessary for the transport of fatty acids across the membrane in the form of acyl-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, neurohormones, synaptic morphology and synaptic transmission of multiple neurotransmitters (19). These functions should explain neurological effects of β-oxidation disorders on central nervous system. The deficit of L-carnitine (if necessary) could be supplemented with delivery of the 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 cases of β-oxidation disorders caused by its low level intravenously. Final activity of β-oxidation is the effect of all of these processes, proper or disturbed in its activity.

In the case of β-oxidation of mirystic acid among 112 samples 5.36% of mid- and 2.67% of heavy disturbances of β-oxidation were found. These disorders were accompanied by lowering of serum carnitine level: by 34.8% for free and 24.11% for a total. This indicated, that physiological level of L-carnitine is higher than that required for proper β-oxidation. In 3 cases, the activity of fatty acid β-oxidation was not affected at the level of free- and total L-carnitine as low as 12–18 μmol/l and 14–23 μmol/l respectively, while the normal range is 35–45 μmol/l and 42–80 μmol/l, respectively (data not published).

Chromium ions activate process of β-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-time more effective than those in lymphocytes of chickens fed with standard fodder (without addition of chromium yeast). Activity of β-oxidation of [9,10-^{3}H] palmitic acid increased from 40.151±5.96 pmoles/min/mg of protein for controls to 85.88±5.81 pmoles/min/mg of protein in samples from broilers fed with the diet supplemented with 0.5 ppm of chromium yeast. The stimulatory effect of the chromium ions was observed as long as 4 weeks after removing chromium supplementation from the diet. Additional effect of feeding of the animals with the diet supplemented with chromium were lower concentrations of fat and cholesterol in muscles (21).

In humans addition of inulin to the experimental diet resulted in lowering of blood cholesterol and triacylglyceroles. Letexier et al. (22) reported that the addition of 10% of inulin to the diet of healthy volunteers has a beneficial effect on plasma lipids by decreasing hepatic lipogenesis and plasma triacylglycerol concentrations compared to that after placebo ingestion ($p<0.05$), but cholesterol synthesis and plasma cholesterol concentrations were not significantly different between the 2 groups. Experiments by Brighenti et al. (23) were done with the addition of 18% of inulin to the breakfast. They found no changes in body weight whereas plasma total cholesterol and triacylglycerol significantly decreased by 7.9±5.4% ($p<0.05$) and 21.2±7.8% ($p<0.05$), respectively. Inulin seems to have a lipid lowering potential in normolipidemic men. Additional beneficial effect of oligofructans is increased calcium ions absorption (24) and no effect on minerals excretion (26).

The latest results of our experiments (26) 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).

Lymphocytes from control group utilized 5.991 ± 0.348 pmol of palmitic acid per 1 min per 1 mg of lymphocyte protein. Low chromium supplementation (0.3 ppm) had no stimulatory effect on the activity of β-oxidation of palmitic acid by rat lymphocytes. Slight inhibition of fatty acid degradation was found in group of

animals fed with diet with addition of 10% of polyfructans (4.329 ± 0.188 pmol/min/mg of protein). In groups of animals fed with diet supplemented with 5 ppm of chromium the activity of β-oxidation was higher as compared to that one in groups receiving diet with 0.3 ppm supplementation and in control group. Higher content of sugars at chromium concentration of 5 ppm resulted in higher fatty acid utilization (7.347 ± 0.323 pmol/min/mg for 5% inulin, 7.207 ± 0.284 pmol/min/mg for 5% oligofructans, 11.877 ± 0.664 pmol/min/mg for 10% inulin and 12.356 ± 725 pmol/min/mg for 10% oligofructans, respectively). Multifactorial ANOVA and Tuckey test at $p<0.05$ showed that β-oxidation of fatty acids was significantly affected by experimental factors as well as their interactions. Independently on other factors higher level of dietary fibre oligosugars – FOS (10%) and higher level of supplementary Cr (5 ppm) markedly increased fatty acid utilization by 37.5% and 76.9%, respectively. Also significantly higher stimulatory effect of Cr was detected in the case of diets enriched with oligofructose alone and generally higher level of FOS (10%).

It looks that the problems of energetically processes in animals are affected by a number of factors including hormones, enzymes and dietary components. Excluding genetically inborn errors the metabolism of sugars and lipids should be modified by addition to the diet of such factors as nondigestible oligofructans and mineral ions (mainly Cr^{3+}) in the form of chromium yeast. These factors play a role in energy production by activation of lipolysis lowering simultaneously the level of triacylglyceroles and cholesterol. This have beneficial effect on cardiovascular diseases. The effect of intake of L-carnitine as commercially available "fat eater" looks to be important only in the case of very low level of this compound in the blood, a very rare disease.

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