



# Decreased energy levels can cause and sustain obesity

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## Abstract

Obesity has reached epidemic proportions and has become one of the major health problems in developed countries. Current theories consider obesity a result of overeating and sedentary life style and most efforts to treat or prevent weight gain concentrate on exercise and food intake. This approach does not improve the situation as may be seen from the steep increase in the prevalence of obesity. This encouraged us to reanalyse existing information and look for biochemical basis of obesity. Our approach was to ignore current theories and concentrate on experimental data which are described in scientific journals and are available from several databases. We developed and applied a Knowledge Discovery in Databases procedure to analyse metabolic data. We began with the contradictory information: in obesity, more calories are consumed than used up, suggesting that obese people should have excess energy. On the other side, obese people experience fatigue and decreased physical endurance that indicates diminished energy supply in the body. The result of our work is a chain of metabolic events leading to obesity. The crucial event is the inhibition of the TCA cycle at the step of aconitase. It disturbs energy metabolism and results in ATP deficiency with simultaneous fat accumulation. Further steps in obesity development are the consequences of diminished energy supply: inhibition of beta-oxidation, leptin resistance, increase in appetite and food intake and a decrease in physical activity. Thus, our theory shows that obesity does not have to be caused by overeating and sedentary life-style but may be the result of the “obese” change in metabolism which is forcing people to overeat and save energy to sustain metabolic functions of cells. This “obese” change is caused by environmental factors that activate chronic low-grade inflammatory process in the body linking obesity with the environment of developed countries.

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## 1. Introduction

The prevalence of obesity is increasing at a fast rate and obesity has become one of the major health problems in developed countries affecting over a hundred million people world-wide. Obesity is associated with greater risks of high blood pressure, heart disease, osteoarthritis, type 2 diabetes and several other health problems. To improve prevention and treatment of obesity it is necessary to know the mechanisms that cause it. One of the difficulties in obesity research is that obesity may be a symptom of several different diseases or health conditions. Genetic diseases of fat storage and release may cause severe obesity as well as abnormalities in appetite control enzymes, hormonal imbalances or some psychological or neurological factors. Finally,

obesity may be a result of overeating without any disease. In this paper we will concentrate on obesity that is induced by environmental factors in otherwise healthy people.

Obesity is a result of positive energy balance: more calories are consumed than used up for oxidation, body building and maintenance. Thus, the common approach to reduce obesity is to either decrease the amount of calorie intake by restricting the amount of food consumed, inhibiting nutrient absorption in the intestine, modulating the activity of hypothalamic centers controlling satiety, or to increase energy spending by increasing physical activity or energy dissipation as heat (Keller et al., 1997; Kopecky, 1998). However, obese people very often experience tiredness (Lean, 2000) even without sleep apnea (Vgontzas et al., 1998). During physical exercise they get fatigued faster than lean people (Ardevol et al., 1998; Mattsson et al., 1997) and have decreased exercise capacity (Hulens et al., 2001). Thus, it seems that obese people have at the same time

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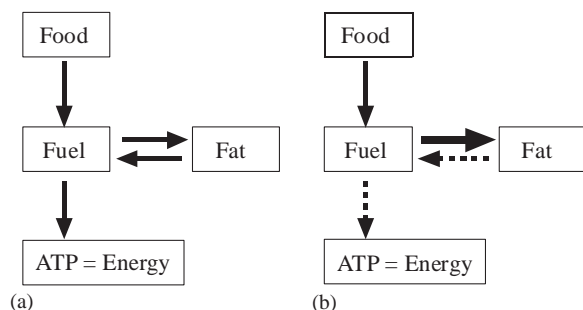


Fig. 1. Fuel distribution between energy production (ATP) and fat synthesis: (a) in normal metabolism; (b) in obesity. Dotted lines indicate decreased efficiency, large arrow—increased efficiency of a process as compared to normal metabolism.

too much and too little energy. The apparent paradox may be explained by considering what metabolic energy is. The currency of free energy in living organisms is adenosine triphosphate (ATP). Nutrients from food are metabolized to fuel and next, either to ATP—and only then they become equivalents of energy—or become precursors of fat or proteins. Fat and proteins may be metabolized to ATP but this is not an automatic conversion and involves several metabolic steps. The schematic distribution of fuel between fat and energy in healthy and obese people is shown in Fig. 1. A common concept in the area of obesity research is an equalization of fat with energy. It is based on a silent and false assumption that steps involved in fat synthesis and in beta-oxidation are never disturbed. Fat is the storage of fuel and only after it is metabolized to ATP does it become energy. Thus, the correct statement is that obese individuals have a deficit of energy in the form of ATP with simultaneous overproduction of fat. Therefore, the logical question is: why in some people, is the energy from food diverted to fat synthesis before all of the energetic needs in the body are satisfied?

To address this question we applied a theoretical approach which belongs to a Knowledge Discovery in Databases (KDD) methodology. KDD is a process of identifying relationships between data that exist in databases but are not apparent because of the large number of data (Fayyad et al., 1996). These discovered relationships represent a new knowledge, a theory about the rules governing the data, derived from already existing data. The process involves human decision-making and computer technology. KDD is used in many different areas of science and business. An example of the KDD application to genomic and proteomic databases is a work done by Satou et al. (1997) with the purpose of finding correlations between protein sequence, structure and function. In this application the data mining process was based on the discovery of association rules and the verification of the rules would be a prediction of protein structure or function from the

sequence. The data included information on sequence, secondary structure and EC numbers as indicators of function. Algorithms were used that iteratively perform searches until similar structures were found. KDD was able to identify substructures that were unrelated to the active site but common to proteins which had the same function. We used KDD to discover new relationships in metabolism. This research has allowed us to find out which metabolic disturbances lead to obesity and to formulate a theory for environmentally caused obesity supported by experimental data described already in the scientific literature.

## 2. Method

Growing research produces floods of scientific information. With advancements in computer technology, most of the research data have become readily available in a number of databases. These scientific databases are mostly used as encyclopedias to check detail information. With the development of KDD methods, these databases may be a source of a new knowledge and would be very beneficial in the case of metabolism research. All metabolic processes are connected together into one complex network. The size and complexity of this network exceeds human abilities for searching the entire network or a great part of it. Using KDD there is the potential to speed up metabolic research.

The KDD process consists typically of data mining (DM), incorporation of prior knowledge and finally an interpretation step. Usually DM uses special algorithms or the search for patterns can also be done by queries to database management systems (DBMS) (Fayyad et al., 1996). The KDD procedures used in this work are a prototype. DBMS were used for multiple selections and storage of data from several databases: PubMed at National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), Kyoto Encyclopedia of Genes and Genomes ([www.genome.ad.jp/kegg](http://www.genome.ad.jp/kegg)) and SwissProt ([www.expasy.ch.sprot](http://www.expasy.ch.sprot)). There are two approaches to find relationships between the data, statistical and deterministic (logical). We used the deterministic approach and applied a discovery and verification mode of the KDD process. The discovery mode (presented schematically in Fig. 2) was used to find data and build a new pathway. The “data” was a single experimental result (molecular, biochemical, physiological, etc.). The discovery mode starts with a question to be answered and is iterative. At each step the searches are restricted to the closest neighborhood of the investigated step. Searches started with a single subject. There was a multitude of search terms and each was chosen adequately to the question analysed. This resulted in many abstracts of relevant publications. The knowledge was extracted from some papers that were considered

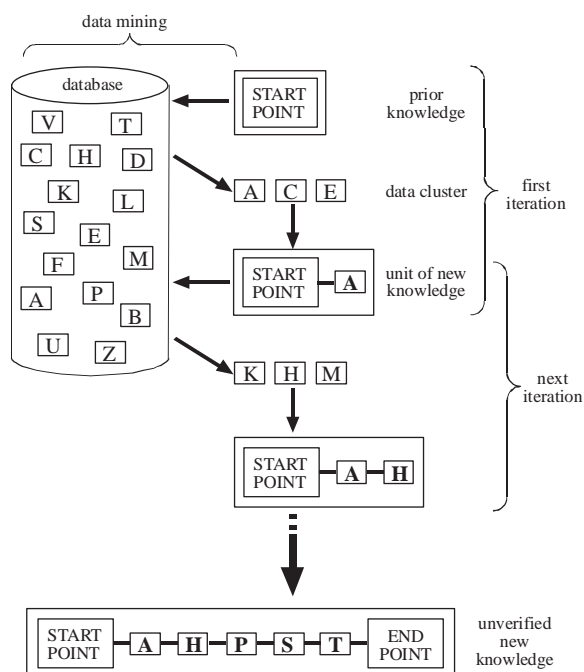


Fig. 2. Schematic representation of the discovery mode of KDD methodology. Individual boxes symbolize data. Connecting boxes represents new knowledge. Horizontal arrows represent searches in databases; slanted arrows—retrieval and analysis of data; vertical arrows—selection and synthesis of new data to the existing stage of knowledge.

interesting and classified for cluster formation. Any of the subjects from a cluster could serve as a keyword for the next search. The human decision at each step involves analysis of the current knowledge, determination of the searches, data clustering, selection and synthesis of some data into a next step of a logical chain of events. Two criteria were used for clustering. First, a decision was made based on the previous knowledge on attributes and on the relevance of each data to the particular step and ultimately to the pathway. Attributes were determined for each data as its closest metabolic neighborhood. The second procedure was frequent term-based clustering that allows to select and cluster data not recognized by present knowledge as interesting for the search. The “interestingness” was defined as a logically possible but not proven connection of the particular data to the step of knowledge under consideration. Only one experimental result supporting a particular step of the discovery process is sufficient to synthesize it into the chain. The constructed chain of events represents a theory discovered from the existing experimental data, a new knowledge. The goal of the verification mode is to find multiple data to either support or disprove the theory. Finding multiple data from different laboratories reduces errors. In the case of contradictory data retrieved, the quantity of the data may determine the

significance. A discrimination level at 25% (1 versus 3) or lower is accepted. In few cases quantitative discrimination was not possible and those results were either not included in the KDD process or presented with the authors comments on the discrepancy (for example the measurements of resting metabolic rate).

PubMed has abstracts of papers published within the last 30 years. Older information was retrieved either from books or paper copies of journals from the library. This was only necessary for the verification mode. There was enough recent data for the discovery process to build the pathway. An occasional problem was to obtain the whole article from some journals that are not very popular in most libraries. If all journals would have freely available whole articles on the internet, the speed of the KDD process would be significantly faster. The publication language was not a limiting factor in the discovery process. Most articles written in languages other than English have abstracts translated into English. In the verification mode when the whole article was necessary to gather the data, translation was performed. In some cases the abstract had provided the essential data that could be included into the KDD process. The articles that are not abstracted in PubMed were not considered.

An example of one step of KDD procedure is described in detail in the electronic supplement available at [http://www.ncgr.org/staff/dkw/Appendix\\_4.html](http://www.ncgr.org/staff/dkw/Appendix_4.html).

The KDD method of doing research is a new concept for most experimental biologists who are accustomed to formulating hypotheses and then verifying them experimentally. There is no deductive type of hypothesis in the discovery mode of KDD since the direction of search at each iteration is determined by the result of the previous iteration. The thinking process uses both deductive and inductive reasoning. The principle of KDD implies that the discovered theory does not need to be re-verified experimentally because all of the experimental data necessary to formulate and prove it have to be already described in the literature and collected in databases. It is important to stress that experimental data stand for themselves regardless of the purpose of the scientists who have obtained them. However it would be desirable to verify the reliability of the KDD methodology for metabolism research. For this purpose the theory obtained by the KDD process may be considered a hypothesis for experimental research.

### 3. Results and discussion

#### 3.1. Energy metabolism in obesity

From the basic metabolism it is known that the point where fuel metabolism splits between ATP production and fat synthesis is mitochondrial citrate (Stryer, 1988).

Its conversion to isocitrate is catalysed by mitochondrial aconitase and leads to ATP production. If citrate is transported out of mitochondria and into the cytosol, it is converted to acetyl-CoA and malonyl-CoA ultimately leading to fatty acid synthesis. Under normal conditions, citrate distribution is regulated by energy demand and supply. The major result of our findings is the discovery how a perturbation in this regulation leads to obesity. The effect observed in obesity, low energy and overproduction of fat, may be achieved by inhibition of aconitase. If citrate cannot be converted into isocitrate, it will accumulate in the mitochondria and then in the cytosol ultimately activating fat synthesis (Stryer, 1988) in spite of low ATP levels. Furthermore, inhibition of aconitase will additionally decrease the rate of energy production from beta-oxidation because high concentrations of malonyl-CoA and acetyl-CoA carboxylase (ACC) inhibit carnitine palmitoyltransferase I (CPT I) and decrease the supply of fatty acids in mitochondria for oxidation (Ruderman et al., 1999). Thus, this one event, inhibition of mitochondrial aconitase, is able to increase lipogenesis, slow down lipolysis and decrease energy supply for all biochemical processes in affected cells. The changes in energy production and fat metabolism that lead to obesity are shown in Fig. 3.

The inhibition of mitochondrial aconitase is a well-known mechanism of diminishing ATP production. It is used by the immune system as a defense mechanism for killing tumor cells and microorganisms by destroying their energy supply (Drapier and Hibbs, 1986; Hibbs et al., 1988; Drapier et al., 1988). It involves cytokine activation, tumor necrosis factor-alpha (TNF-alpha), interferon gamma, and induction of oxidative stress. The same process of aconitase inhibition also occurs in tissues that do not belong to the immune system including muscle cells, adipocytes and hepatocytes (Andersson et al., 1998; Kapur et al., 1999; Tatsumi et al., 2000; Kurose et al., 1993a). However, aconitase inhibition has never been considered a cause of obesity. Below we provide evidence based on experimental data accumulated during the last 18 years that inhibition of mitochondrial aconitase in tissues capable of fat synthesis may be a major factor causing obesity.

### 3.1.1. Involvement of cytokines

Among the individuals with obesity of unknown etiology there is a large group of people whose obesity is connected with inflammation. Many obese people have elevated levels of C-reactive protein (CRP) that may be a result of bacterial or fungal infection (Visser et al., 1999, 2001). The level of CRP correlates positively with body mass index, waist circumference and low calorie diet invoked weight loss (Heilbronn et al., 2001). Obese people also have higher than normal levels of erythrocyte sedimentation rates (Pasulka et al., 1985), another indication of infection. Since obese people do not have

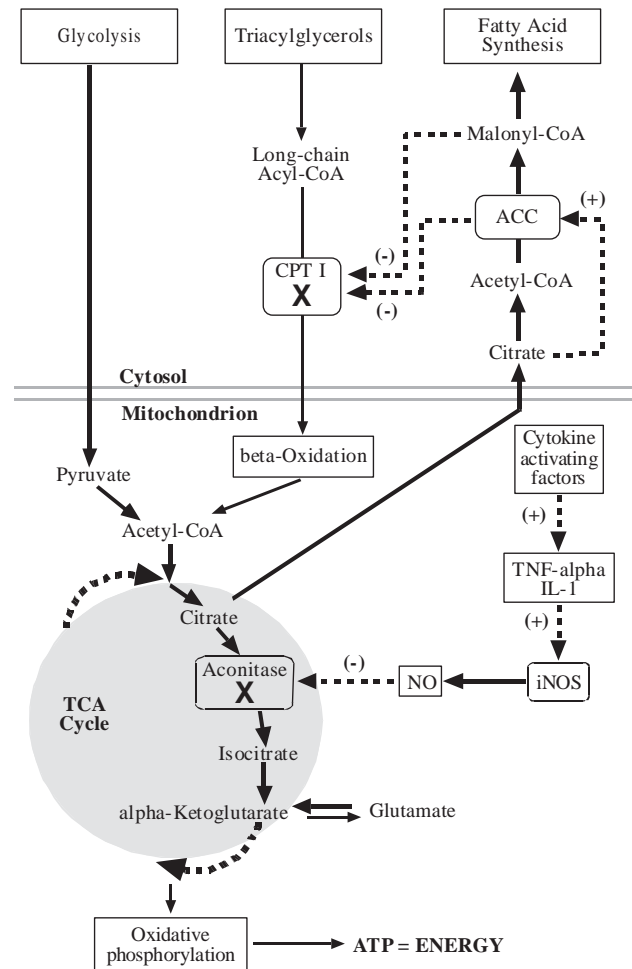


Fig. 3. Energy metabolism in obesity. Bold arrows mark the overactive steps in obese metabolism: cytokines TNF-alpha and IL-1, activate iNOS and NO synthesis; mitochondrial aconitase is inhibited and TCA cycle is disturbed resulting in decreased ATP production and increased mitochondrial citrate levels; citrate is transported to cytosol and activates ACC and fatty acid synthesis; ACC and malonyl-CoA inhibit CPT I and beta-oxidation. Obesity can amplify cytokine expression. (–)—inhibition; (+)—activation, X—inhibited enzyme.

any symptoms of an infection, the inflammation connected with obesity has to be chronic and low grade (Visser et al., 2001).

Inflammation response to infection is mediated by several cytokines. In obese people the levels of three of these cytokines are elevated: TNF-alpha, interleukin 1 (IL-1) and interleukin 6 (IL-6) (Zahorska-Markiewicz et al., 2000; Raymond et al., 2000; Kern et al., 2001; Roytblat et al., 2000). Analysis of the published materials indicates that induction of these cytokines is sufficient for triggering fat synthesis in liver and adipose tissue. For example, in normal rats injection of TNF-alpha or IL-1 stimulates hepatic fatty acid synthesis (Grunfeld et al., 1988, 1990a). The increase in fat synthesis is acute, observed within 30–90 min after a



single dose of cytokine and is sustained for 17 h (Grunfeld et al., 1988). Similarly, fatty acid synthesis is triggered by TNF- $\alpha$  in rat brown adipocytes (Lopez-Soriano et al., 1995). IL-6, which is released by a range of tissue in response to stimulation by TNF- $\alpha$  and IL-1, could mediate the lipogenic effects of the other two cytokines (Grunfeld et al., 1990b). This mechanism of continuous activation of inflammatory cytokines in response to chronic infection may be a primary event in obesity. More evidence for cytokines as a cause of obesity comes from patients whose weight gain was a side effect of depression treatment. A significant increase in the levels of soluble TNF receptor p75 preceded weight gain observed in patients treated for several weeks with antidepressants amitriptyline or nortriptyline (Hinze-Selch et al., 2000). The authors concluded that activation of the TNF- $\alpha$  system may be an early and sensitive marker of weight gain.

Pro-inflammatory cytokines are also activated by factors other than infection. Besides antidepressants described in the previous paragraph, lipopolysaccharides (LPS) from bacterial membranes are well-known potent inducers of TNF- $\alpha$  and IL-1 (Michie et al., 1988). Pro-inflammatory cytokines are also produced in response to exposure to some toxins, for example 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and a water disinfectant by-product dichloroacetonitrile (DCAN). Both of them are wide spread in the environment of industrialized countries and at environmental doses increase TNF- $\alpha$  levels in blood serum and liver of exposed animals or in different cells in culture, adipocytes, monocytes or macrophages (Rier et al., 2001; Kern et al., 2002; Ahmed et al., 2000). Therefore, activation of pro-inflammatory cytokines may depend on the level of environmental pollution and could be the link between the prevalence of obesity and industrialization.

TNF- $\alpha$  and IL-1 inhibit mitochondrial function and energy production in the cells (Tatsumi et al., 2000; Schulze-Osthoff et al., 1992; Zell et al., 1997; Kurose et al., 1996). In the whole organism, decreased energy production may be expressed as lower metabolic rate. Measurements of metabolic rate in obese people give controversial results. This controversy may arise because it is difficult to select whose obesity has the same cause and thus, the same metabolism. Also, since obesity can arise as a result of small energy imbalance, it is possible that existing techniques are not sensitive enough to measure such small differences (Goran, 2000). However, in the case where a difference in resting metabolic rates was observed between obese and lean people after a meal, a treatment with aspirin and ephedrine, which decrease the inflammation, increased metabolic rate mostly in obese people and alleviated the difference caused by obesity (Horton and Geissler, 1991). Since at least two cytokines, TNF- $\alpha$  and

IL-1 can induce fat synthesis, inhibition of only one of them may not be sufficient to prevent this induction. That may be why some of the attempts to reverse the abnormalities in lipid metabolism of the obese Zucker rat by TNF antibody were not successful (Lopez-Soriano et al., 1997).

### 3.1.2. Role of nitric oxide

The effect of TNF- $\alpha$  and IL-1 on energy production is mediated mostly by nitric oxide (NO) (Tatsumi et al., 2000; Kurose et al., 1993b; Geng et al., 1992). TNF- $\alpha$  and IL-1 activate inducible nitric oxide synthase (iNOS) and increase NO concentration in different types of cells including hepatocytes (Kapur et al., 2000; Binder et al., 1999; Tang et al., 2001; Duval et al., 1996). The levels of iNOS in obese rats were found to be 4 times greater than normal (Zhou et al., 2000). On the other hand, treatment of mice with NO synthase inhibitor, NG-nitro-L-arginine methyl ester, caused a decrease in body weight in obese animals but did not affect lean mice (Morley and Flood, 1992, 1994). This indicates a relationship between obesity and NO concentration. The connection of obesity and NO was also observed in people where NO concentration correlated with body fat mass (Choi et al., 2001). Inhibition of NO release by NG-monomethyl-L-arginine acetate in subcutaneous adipose tissue of healthy people increased lipolysis (Andersson et al., 1999). There is direct evidence that the increase in NO level affects energy production. It was observed that addition of nitroglycerin, a widely used NO donor, to rat reticulocytes in vitro significantly decreased ATP levels in the cells (Maletic and Kostic, 1999).

NO and its derivatives cause oxidative stress to mitochondria and inhibit several enzymes the same way as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Beltran et al., 2000; Brown and Borutaite, 1999; Tretter and Adam-Vizi, 2000). The most sensitive enzyme to oxidative stress is aconitase (Tretter and Adam-Vizi, 2000; Stadler et al., 1991; Gardner et al., 1994; Yan et al., 1997) thus it will be the main target of low NO concentrations. Aconitase requires  $(\text{Fe-S})_4$  clusters to be active (Drapier and Hibbs, 1986). The interaction of NO or reactive oxygen species (ROS) with  $(\text{Fe-S})_4$  clusters causes redox changes connected with iron removal and reversible inactivation of this enzyme (Drapier and Hibbs, 1986; Janero and Hreniuk, 1996; Drapier and Hibbs, 1996). High NO concentrations inhibit aconitase completely as well as other enzymes of the TCA cycle and oxidative phosphorylation (Tretter and Adam-Vizi, 2000; Stadler et al., 1991). In this case the disturbance in energy production is much more severe and may lead to apoptotic cell death, which is linked to heart failure (Muller-Werdan et al., 1998; Sabbah, 2000), neurodegenerative diseases (Sheu and Blass, 1999; Schapira,

1999; Mastrogiacoma et al., 1996) and may be involved in many of the complications of obesity.

### 3.1.3. Effects of aconitase inhibition on fuel metabolism

As mentioned above, inhibition of mitochondrial aconitase has tremendous effects on energy metabolism. It decreases ATP production in TCA cycle by preventing citrate to isocitrate conversion. In consequence, accumulating citrate activates ACC, the committing step in fatty acid synthesis. Thus, inhibition of aconitase diverts metabolism from energy production to energy storage. It is well documented that stimulation of fatty acid synthesis by TNF-alpha is induced by citrate accumulation (Grunfeld et al., 1988, 1990a; Lopez-Soriano et al., 1995). An increase in citrate concentration in the cytosol of rat hepatocytes was seen as early as 15 min after an intravenous administration of a single dose of TNF-alpha and an acute increase in fatty acid synthesis followed within next 15 min (Grunfeld et al., 1990a). The citrate concentration and ACC activity were elevated by more than 50% (Grunfeld et al., 1988) and there was no change in the activity of several other enzymes involved in fatty acid synthesis or glucose metabolism (Lopez-Soriano et al., 1995).

The same effects as caused by cytokines and NO on the TCA cycle can be reproduced in cells treated with fluorocitrate, an inhibitor of aconitase. In cultured astrocytes, both a significant accumulation of citrate and inhibition of ATP production were observed (Hassel et al., 1994). Similar results were observed in dogs treated with fluorocitrate where depletion of ATP and accumulation of citrate in the liver accompanied inhibition of TCA cycle (Bosakowski and Levin, 1986, 1987). After the inhibition of aconitase the continuation of the TCA cycle is sustained by alpha-ketoglutarate synthesis from glutamate and glutamine which is substituting for glucose in ATP synthesis (Hassel et al., 1994). The TCA cycle is fueled by amino acids which explains an increased muscle protein turnover in obese rats, variations in amino acid metabolism as compared with lean animals (Domenech et al., 1993; Rodriguez et al., 1997) or changes in amino acid metabolism in hepatocytes of normal rats treated with TNF-alpha (Yasmineh et al., 1995). These results strongly support the theory that fuel metabolism disturbances observed in obesity are caused by aconitase inhibition. They also indicate the importance of protein for energy production in obesity.

Increased activity of fat synthesis in obese people is seen in the process of de-novo liver lipogenesis (Diraison et al., 2002). In healthy lean people de-novo liver lipogenesis is observed only when they stay on a low-fat high-carbohydrate diet (Marques-Lopes et al., 2001). On a high-fat diet, the body's need for fat is supplied by food. On the contrary, in obese people, a high-fat diet does not stop liver de-novo lipogenesis (Rafecas et al.,

1992). On high-carbohydrate diets the level of de-novo lipogenesis is higher in obese than in lean people and the extent of lipogenesis is positively associated with body mass (Marques-Lopes et al., 2001). These observations indicate that in obese people regulation of fat synthesis is disturbed and is in agreement with our theory that inhibited aconitase is forcing fat synthesis regardless of fat supply to the cells. Increased de-novo lipogenesis and impaired beta-oxidation in liver may be responsible for fatty liver observed in a type of obesity connected with diabetes (McCullough, 2002).

Inhibition of aconitase is affecting not only energy production when nutrients are abundant, but also at the time of nutrient deficiency at starvation or low-calorie dieting. After exhausting glycogen stores, energy is produced from fat and proteins. Metabolites of protein catabolism and fatty acid oxidation enter the TCA cycle and experience the same disturbance in the transition from citrate to isocitrate. High citrate concentration induces the synthesis of malonyl-CoA which inhibits CPT I and significantly slows down fatty acid oxidation (Ruderman et al., 1999; Saha et al., 1999; Kim et al., 2000). This mechanism was also described in starved rats after TNF-alpha treatment where increased levels of citrate in liver were observed with impaired ketogenesis and increased availability of free fatty acids (Beylot et al., 1992). There is evidence that obese people respond to starvation differently than lean people. The hyperketonemia is twice lower in obese people and after prolonged starvation, protein oxidation is 2–3 times less efficient than in lean people (Elia et al., 1999). This indicates that obese people loose weight slower and at the same time have less energy available for metabolic processes.

The analysis of fuel metabolism shows that aconitase is the only enzyme that when inhibited can cause increased fat synthesis with simultaneous inhibition of ATP production. To induce fat synthesis citrate has to accumulate. Thus, inhibition of any step before citrate synthesis may affect ATP production but will not activate fat synthesis. On the other side, any step beyond alpha-ketoglutarate may push the TCA cycle metabolites to produce glutarate without citrate accumulation. Between the steps of citrate and alpha-ketoglutarate synthesis, there is only one enzyme beside aconitase. It is isocitrate dehydrogenase and is much more resistant to NO and oxidative stress than aconitase. Inhibition of this enzyme will not make much difference, since it will be achieved at higher concentrations of NO when aconitase is already inhibited (Tretter and Adam-Vizi, 2000).

Obesity may be developed only if the individual is exposed to cytokine inducing factors for a long period of time or continuously. Intermittent changes in ATP supply would cause normal temporary switching of metabolism between energy storage and usage and body

weight would be periodically balanced. This effect was observed in people treated with nitroglycerin where only continuous therapy caused significant weight gain. This weight gain, as predicted by our theory, was not observed in the intermittent therapy (Parker et al., 1991). Another necessary factor in obesity development is a relatively low level of oxidative stress and aconitase inhibition. High levels of oxidative stress would also inhibit pyruvate dehydrogenase and citrate synthesis (Tretter and Adam-Vizi, 2000; Stadler et al., 1991) ultimately inhibiting TCA cycle without induction of fat synthesis. Both of these requirements are fulfilled by the exposure to environmental levels of toxins or LPS. Thus, when the TCA cycle is inhibited by oxidative stress at the aconitase step over a long period of time or continuously, the regulation of energy metabolism is seriously disturbed. It creates a sick cell that is deprived of energy and simultaneously produces fat.

### 3.2. The consequences of energy deprivation

#### 3.2.1. Appetite control and involvement of leptins

The supply of energy is the most basic requirement of every cell and every organism. Inadequate energy supply in the body will be transformed into increased appetite. Although the mechanism of such signaling is not yet known, there is evidence that it exists (Koch et al., 1998; Friedman et al., 1999; Ji et al., 2000). In the experiments on rats, treating animals with metabolic inhibitors, 2,5-anhydro-D-mannitol or/and methyl palmoixirate, controlled the ATP levels in liver cells. The researchers observed the effect of decreasing ATP concentrations on rats eating habits including the frequency of eating, the amount of food consumed at one eating session and the total amount of eaten food. They discovered a direct correlation between eating habits and the level of ATP, ATP/ADP ratio and the phosphorylation potential in liver cells only. These experiments indicate that there is an integrated metabolic control of food intake with liver ATP levels acting as a major sensor of energy status in the body. In obese people the levels of hepatic ATP are decreased. Nair et al. (2003) have shown that the hepatic ATP content is inversely related to BMI, decreasing steadily with increasing BMI.

There is another appetite signaling mechanism, which is better known than the one described above. This signaling involves leptins (Friedman and Halaas, 1998). These proteins are produced by adipocytes and decrease appetite and food intake. Thus, the more adipose tissue is in the body, the stronger the signal decreasing appetite would be. However, most obese people have elevated levels of leptins as compared to lean people (Szymczak and Laskowska-Klita, 2001; Considine et al., 1996). In spite of that, their appetite is not decreased (Widdowson et al., 1997). This effect is called leptin resistance and it is one of the puzzles about obesity. The signaling leading

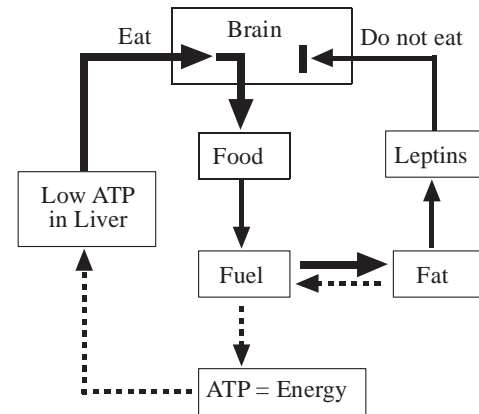


Fig. 4. Possible mechanism of leptin resistance. Signals triggered by low ATP levels in liver cells have higher priority and override “do not eat” signal coming from leptins. The final result is an increase in appetite. Dotted arrows indicate less efficient processes, large arrows—more efficient processes than in normal metabolism.

to leptin resistance is presented schematically in Fig. 4. Considering the interaction of the two signaling mechanisms: leptins and liver-ATP, gives an explanation of the puzzle. In obesity both of these mechanisms send signals to the brain but the signals are opposite: leptins to decrease the appetite and low ATP levels to increase the appetite. Since the energy supply is the most basic and important need of every living organism, supplying energy would be the priority signal and would override any others. Thus the final outcome would be an increase in appetite. Analysis of both types of signaling, leptins and liver-ATP signaling, is an example of the disturbed appetite control in obesity. Accepting liver signaling as the most important regulator of appetite helps to understand why it is so difficult for obese individuals to comply with low calorie diets. Liver is an energy distributor in the body and low levels of ATP in liver indicate that all of the energy sources of the body are used up. Thus, the message coming to the brain from liver cells is an “emergency” signal difficult to ignore.

In most people the level of aconitase inhibition may be very small and still the additional amount of calories to satisfy energetic needs in spite of aconitase inhibition may lead to significant obesity. Let us consider an example of an individual with a daily need of 8000 kJ. Assuming that the energy is produced with an efficiency of 95%, that individual will have only 7600 kJ of energy available in the form of ATP. The remaining 5% will be metabolized to fat. Thus, that individual would have an appetite for and consume an extra 421 kJ a day to produce the needed 8000 kJ (95% of 8421 = 8000). This 421 kJ might only be an additional cup of fruit juice a day, yet the calories will accumulate to some 150,000 kJ within a year and will be converted to few kg of new fat tissue.

### 3.2.2. Decreased energy spending

Another effect of energy deficiency is the need to restrict energy spending. Obese people have less muscle ATP available to support their energetic needs (Lennmarken et al., 1986). It has been shown that low ATP levels are connected with the feeling of fatigue (Park et al., 1998; Green, 1997; McFarlane and McDonald, 2002). Fatigue has been connected with obesity (Lean, 2000; Vgontzas et al., 1998). In school children the level of fatigue was significantly related to the degree of obesity (Okamoto et al., 2000). A normal reaction to fatigue is a need to rest and an obese person would usually choose the activities requiring the least energy and thus, favor sedentary life-style.

Exercise changes metabolism to increase the efficiency of ATP production. Energy production will also be changed in obese individuals. The inhibition of beta-oxidation may be attenuated because decreasing ATP levels activate ATP kinase that inhibits ACC and in consequence prevents inhibition of CPTI (Winder and Hardie, 1996). This effect prevails at high exercise intensity (Rasmussen and Winder, 1997) where beta-oxidation levels were higher in obese than non-obese people (Kanaley et al., 2001). However, most obese people restrain from exercise as may be evidenced by low rate of compliance with long-term exercise regimens (Borg et al., 2002; Wing, 1999) and may indicate that some energy production pathways are still disturbed in exercise. Comparison of obese people with non-obese indicated that obese people get fatigued at lower workloads and energy expenditure levels and required a longer recovery time after exercise (Ardevol et al., 1998; Mattsson et al., 1997). Also, in addition to exhaustion at peak intensity obese people experienced musculoskeletal pain, which was not present in non-obese people (Hulens et al., 2001), certainly making exercise unpleasant. Moreover, physical exercise elicits a temporary inflammatory response of a subclinical nature with elevated levels of TNF-alpha, IL-1, IL-6 and interferon gamma (Shek and Shepard, 1998; Rhind et al., 2001; Shepard and Shek, 1998; Kimura et al., 2001; Moldoveanu et al., 2000). In obese people it would be an aggravation of the inflammatory process and may further deteriorate the regulation of fuel distribution. Besides, substantial weight loss achieved by exercise and/or dieting in spite of lowering total muscle fat did not decrease the accumulation of intramyocellular fat deposits observed in obesity (Malenfant et al., 2001). This suggests that the reason for intra-muscle fat accumulation is not a total calorie balance but a change in fuel metabolism. Since exercise and/or calorie restricted diets do not ameliorate the metabolic change observed in obesity, their primary role in weight regulation for individuals with environmental etiology is questionable. Thus, although overeating and sedentary life-style are the factors which allow accumulation

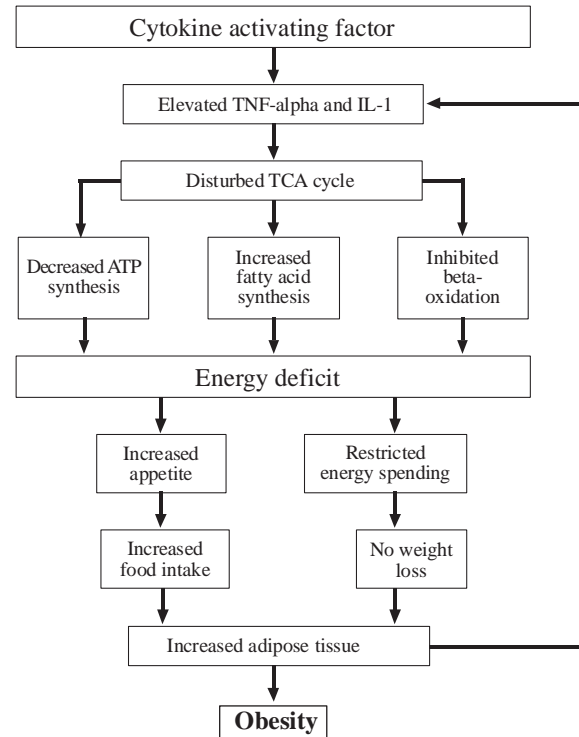


Fig. 5. Vicious circle of events leading to obesity. Closing of the circle is caused by the ability of adipose tissue to produce TNF-alpha and IL-1.

of fat, they do not have to be the reason for obesity; they may be a result of an already disturbed “obese metabolism” which is forcing people to overeat and preserve energy.

The “obese metabolism” is a self-perpetuating vicious circle, as shown in Fig. 5. It starts with an inflammation-inducing factor, toxin or low-grade infection, which activates TNF-alpha and IL-1. The TCA cycle is then inhibited causing lower energy production and more food intake. Overeating and limited energy spending increase the amount of fat tissue of an individual. It is further worsening the metabolism because adipocytes have the ability to amplify TNF-alpha and IL-1 (Kern et al., 1995; Hotamisligil et al., 1995). Therefore, the more adipose tissue one has, the higher level of these cytokines would be present in the body further increasing oxidative stress and fueling the damaging process. The deteriorating system of fuel distribution in the body may ultimately lead to type 2 diabetes which develops in many obese people. How soon the symptoms of diabetes appear may depend on the rate of this deterioration.

It is noteworthy that overeating and sedentary life-style without an inflammatory process can lead to obesity but cannot start the “vicious circle” of events that perpetuate obesity. Although adipocytes have the ability to produce inflammatory cytokines, they require stimulation for cytokine synthesis similarly as



macrophages (Sewter et al., 1999). Thus, the adipocytes may amplify the response to LPS but they do not activate the inflammatory state without reason. Over-eating may cause weight gain but without inflammation there will be no elevation in TNF- $\alpha$  and IL-1 levels. Furthermore, energy production, beta-oxidation or fat synthesis will not be disturbed. Thus, there will be no circle that would perpetuate obesity.

The presented mechanism of obesity development does not have to be exclusive, further research may discover other possible connections between obesity and environment. To test the usefulness and reliability of the KDD process for metabolic research several experiments may be performed. The most direct evidence could be obtained from the treatment of obese people to achieve normal levels of pro-inflammatory cytokines by removing any type of infection, contamination of food and environment, and then observing weight change without any restriction of food intake or exercise. The same experiment, if performed on sufficiently large number of people, may indicate the fraction of people whose obesity was connected with inflammation. If obesity was caused by reasons other than inflammation, no weight loss will be observed. To show the connection between obesity and aconitase inhibition, it is possible to observe weight changes in animals treated with low doses of fluorocitrate for an extended period of time.

In conclusion, the presented metabolic mechanism of environmentally caused obesity indicates energy deficiency as an engine working toward development of obesity. It is obvious that all efforts to stop this engine by further decreasing the energy cannot be successful. Thus, the low calorie diets and exercise regimens seem to be a redundant burden of already exhausted people. It also explains the low rate of success in long-term adherence with those regimens and ability to permanently keep normal weight. According to the presented theory, obesity is caused by the metabolic mechanisms of defense. Activation of cytokines and iNOS is an important immune function in the protection of the organism from infections, toxins and tumor cells. Inhibition of these mechanisms for the purpose of obesity amelioration may have dangerous consequences since it would compromise the efficiency of the immune system. Although temporary help may come from studies of special nutrient balancing, in the long run, considering the number of people affected by obesity, the best solution may be finding and eliminating the source of continuous cytokine activation.

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