CALORIC RESTRICTION AND ANOREXIA NERVOSA WITH REGARD TO THE OXIDANT - ANTIOXIDANT BALANCE

DAS OXIDATIVE/ANTIOXIDATIVE GLEICHGEWICHT BEI REDUZIERTER KALORIENZUFUHR UND PATIENTINNEN MIT ANOREXIA NERVOSA

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Abstract

It is known, that caloric restriction reduces the production of reactive oxygen species (ROS), lowers the incidence of ROS associated diseases and increases the life-span of experimental animals. On the other hand, anorexia nervosa on humans, which is also associated with low caloric intake, could jeopardize health. In the present review, we emphasize the effects of caloric restriction and anorexia nervosa on antioxidant systems, and oxidative damage of macromolecules. The findings of the available literature suggest that anorexia nervosa can alter the endogenous antioxidant systems including a reduction of small molecular antioxidants and antioxidant enzymes. This phenomenon may lead to enhanced sensitivity towards oxidative stress. One of the consequences could be increased concentrations of inflammatory cytokines (e.g. interleukin-6) and induction of inflammation. Therefore, the health related benefits of caloric restriction, which has been repeatedly demonstrated in animal studies, cannot be found in patients with anorexia nervosa. We conclude that caloric restriction will only decrease ROS associated diseases and prolong lifespan if the function of the antioxidant systems is guaranteed and the physiological changes of caloric restriction are not harmful.

Key words: caloric restriction, anorexia nervosa; antioxidants; oxidative stress

Zusammenfassung

Es ist hinreichend bekannt, dass reduzierte Nahrungszufuhr die Produktion von reaktiven Sauerstoffspezies (ROS) im Körper verringert, das Auftreten von Krankheiten, welche mit der Überproduktion von ROS im Zusammenhang stehen, vermindert und die Lebensdauer von Tieren in Versuchen verlängert. Im Gegensatz dazu kann krankhaftes Fasten, wie es bei Patienten mit Anorexia nervosa (AN)

auftritt, zu massiven Gesundheitsschäden führen. Im Rahmen des vorliegenden Artikels sollen die Effekte von reduzierter Nahrungszufuhr (auch bei PatientInnen mit AN) auf die körpereigenen ROS-Abwehrsysteme und ROS-Schäden an Makromolekülen diskutiert werden. In der Literatur finden sich Berichte einer Einschränkung der antioxidativen Kapazität einschließlich der von niedermolekularen Antioxidantien und antioxidativen Enzymen bei AN. Dies kann zu einer Erhöhung der Sensitivität gegenüber oxidativem Stress mit nachfolgender Steigerung des Spiegels von inflammatorischen Enzymen (wie z.B. Interleukin 6) und damit zu Entzündungen in PatientInnen mit AN führen. Man kann deshalb davon ausgehen, dass die gesundheitlichen Vorteile, welche in Tierversuchen mit verminderter Nahrungszufuhr beobachtet wurden, im Falle der AN nicht gelten. Daraus kann geschlossen werden, dass verminderte Nahrungszufuhr nur dann das Auftreten von Krankheiten, die mit ROS-Überproduktion in Verbindung stehen verringert und die Lebenszeit verlängert, wenn körpereigene ROS-Abwehrsysteme durch die Mangelernährung nicht betroffen und die dadurch ausgelösten physiologischen Veränderungen nicht abträglich sind.

Schlagworte: Anorexia nervosa, verminderte Nahrungszufuhr, Antioxidantien, oxidativer Stress

1. Introduction

The importance of reactive oxygen species (ROS) as second messenger and indicator for cell fate such as proliferation and apoptosis is well known since several years. An imbalance between ROS production and antioxidant defense systems leads to oxidant stress resulting in mutation of DNA, protein damage, and peroxidation of membrane lipids. These cell damages might be responsible for the pathogenesis of a variety of diseases and degenerative processes such as neurological disorders, carcinogeneses and ageing. A number of studies have investigated the effect of caloric restriction (CR) on oxidative damages and lifespan in animal trials. The results show that CR reduces ROS production during oxidative metabolism, diminishes cellular damages and increases lifespan of animals.

 CR therefore might also be of benefit for human beings while high caloric intake increases the incidence of ROS associated diseases. Since our knowledge about CR and ROS is mostly based on animal studies, our aim was to discuss the impact of anorexia nervosa (AN) on redox homeostasis and oxidative stress on the basis of the data of human studies. AN is associated with a very severe reduction of caloric intake, therefore it can be regarded as a form of CR. In case of CR we deal with the reduction of caloric intake which results in physiological changes, and in case of AN with the reduction of food intake which has mostly pathophysiological consequences. The working hypothesis of the present review was that the effects of caloric intake can be described by a bell-shaped curve, which is a well described phenomenon of hormesis, and we assume that for this mechanism in CR and AN, ROS are playing a prominent role. The hormesis theory claims that biological systems respond in form

of this curve type to the exposure of chemicals, toxins, and radiation. In toxicology, hormesis is a dose response phenomenon characterized by a low dose stimulation and high dose inhibition, resulting in either a J-shaped or an inverted U-shaped dose response, named non-monotonic curves (CALABRESE & BALDWIN, 2003). According to our suggestion, moderate restriction of caloric intake results in enhancement of the activity of antioxidant and oxidative damage repairing enzymes (RADAK et al., 2005), which benefits health, but severe restriction of caloric intake as in AN can seriously damage the body. The current review will focus on AN since this is poorly investigated in respect of ROS. For CR, see the reviews of MASORO et al. (1998) and YU & CHUNG (2001).

2. Symptoms of AN

Anorexia nervosa is characterized by psychological changes, which lead to nutritional deficiencies due to self-induced reduction in caloric intake, a significant loss in body weight and, subsequently amenorrhea and further physiological alterations.

Three subtypes of AN, types with restriction of food intake in order to obtain low body weight can be distinguished: (i) the restricting type without self-induced vomiting or the misuse of laxatives or diuretics, (ii) the binging/purging type with binge eating and purging behavior (i.e., self-induced vomiting, abusing laxatives or diuretics) and (iii) the third type, AN athletica, above all with excessive physical exercise.

The symptoms of AN are either consequences of malnutrition or an adaptation to nutrient restriction. Caloric restriction does not only alter secretions of peptides implicated in control of hunger and saturation, neurotransmitters and other compounds which are directly related to the situation of starvation, but also affect the redox-status in cells. Despite the main role of nutrition in AN, especially for the antioxidant status, only a few studies report on the vitamin and mineral status (LANGAN & FARRELL, 1985: THIBAULT & ROBERGE, 1987; VAISMAN et al., 1992). Some authors determined the redox-balance and oxidant/antioxidant status directly (ROCK & VASANTHARAJAN, 1995; SOFIC et al., 2002) but no further research has been done on the influence of the oxidant stress on the psychological and physiological state in patients with AN. Based on the present knowledge, only extrapolation of effects of oxidant stress in other diseases to pathogenesis and progression of AN is possible.

3. Consequences of low caloric intake in AN

Diminished food intake triggers several adaptive processes in AN patients. Firstly, an insufficient caloric intake causes a breakdown of adipose and muscle tissues. Free fatty acids (FFA) are mobilized and directly utilized as substrate for ketone body production in the liver. Gluconeogenesis in the liver ensures a minimum glucose supply and protects against hypoglycemia. Furthermore lactate, glycerol as well as

amino acids deriving from structural proteins of muscles and - in advanced stages of malnutrition - from functional proteins such as membrane proteins (RICHARD et al., 1982) are used for gluconeogenesis.

Secondly, diminished caloric intake causes dietary deficiencies as it will be shown below. Remarkably the levels of some micronutrients are not as low as expected. Specific food choices, adaptive processes and the catabolic processes occurring in bone and muscles liberating vitamins and micronutrients from fat and muscle tissues may help to maintain more or less normal plasma concentrations.

Table 1: Vitamin status (plasma or serum concentrations) in patients with AN

Vitamin	Status in relation to healthy subjects	Authors
Vitamin E	lower reduced reduced no difference	LANGAN & FARRELL, 1985 MOYANO et al., 1999 VAISMAN et al., 1992 VAN BINSBERGEN et al., 1988
Retinol (plasma)	higher no difference	VAN BINSBERGEN et al., 1988 ROCK & VASANTHARAJAN, 1995
Alpha-carotene (plasma)	higher	ROCK et al., 1996
Beta-carotene	high normal	BOLAND et al., 2001 MEHLER et al., 1998
Vitamin B1 (thiamin)	reduced normal	WINSTON et al., 2000 ROCK & VASANTHARAJAN, 1995
Vitamin B6	reduced or normal reduced or normal normal	ROCK & VASANTHARAJAN, 1995 VAN BINSBERGEN et al., 1988 ZENGER et al., 2004
Riboflavin Riboflavin (Erythrocyten) FAD (blood)	reduced normal higher reduced	ROCK & VASANTHARAJAN, 1995 HADIGAN et al., 2000 CAPO-CHICHI et al., 1999 VAN BINSBERGEN, 1988
Vitamin C	no difference	VAN BINSBERGEN et al., 1988
Folic acid (plasma)	reduced	CAPO-CHICHI et al., 1999 CASPER et al., 1980 ROCK & VASANTHARAJAN, 1995 VAN BINSBERGEN et al., 1988
Niacin	reduced	ROCK & VASANTHARAJAN, 1995

In most studies on AN found in the literature the vitamin status was determined by measuring the serum concentration of vitamins. When measuring the micronutrient status in AN one should consider the influence of (i) the degree of AN, (ii) the phase of treatment and (iii) neuroendocrine abnormalities on the results. Different results of the vitamin status could be due to the heterogeneity of the population, the cross-sectional nature of these investigations, and the reference data used for defining the reference range. The results obtained for the antioxidant vitamins are shown in Table 1. In Table 2 the mineral status is represented.

Table 2: Mineral status in patients with AN

Mineral	Status in relation to healthy subjects	Authors
Cu	reduced	CASPER et al., 1980
Fe	reduced	THIBAULT & ROBERGE, 1987
Total iron binding capacity	reduced	CASPER et al., 1980
		VAN BINSBERGEN et al., 1988
	normal	CASPER et al., 1980
Transferrin saturation		VAN BINSBERGEN et al., 1988
Mg	reduced	HADIGAN et al., 1999
Zn (plasma, urin)	reduced	CASPER et al., 1980
		MCCLAIN et al., 1992
Zn (plasma)	normal	VAN BINSBERGEN et al., 1988

3.1 Caloric restriction in animal studies compared to AN

CR over a longer period of time decreases the resting energy expenditure (REE) of an organism (DE ZWAAN et al., 2002). Mitochondrial processes are down regulated and the ROS production by the respiratory chain reaction is diminished. Animal studies has shown that CR decrease oxidative cellular damages by a reduced ROS production (ZAINAL et al., 2000; GREDILLA et al., 2001a; GREDILLA et al., 2001b). Patients with AN have a lower REE and therefore if an adequate supply of essential macro- and micronutritients is guaranteed, decreased oxidative cellular damages and a lower risk of e.g. atherosclerosis, diabetes mellitus and cancer might be suggested. The effect of caloric restriction on occurrence of malignant tumors was investigated by measuring the cancer incidence of patients with AN and of the general population in a long-term-study from 1970-1993 (MELLEMKJAER et al., 2001). Women with AN had a slight reduction of occurrence of malignant tumors, indicating that low energy intake might lower the cancer risk. One possible explanation could be that cell division is a strongly energy consuming mechanism and low level energy uptake therefore provides an advantage. A similar phenomenon was observed, when tumor-

bearing mice were exposed to exercise and the development of the tumors was retarded by this training, most likely due to redirecting the use of available energy (RADAK et al., 2002). The cancer reducing effect of AN is a very important finding, and longer follow-up and control studies are needed to obtain more convincing evidence and to investigate whether the oxidant/antioxidant status may play a role on this finding.

A lower REE in AN effects more systems than the ROS production, which makes the comparison very complex. Multiple changes dependent on the nutrition intake and the general condition of patients are reported in literature. Generally, a low metabolic process is associated with a low level of ROS production. On the other hand, for instance organs with high energy demand have a higher level of antioxidant defence to cope with the increased risk of ROS generation. Therefore, decreasing the level of the metabolic rate to a certain degree could be beneficial, but on the other hand, severe decrease could disturb the redox homeostasis resulting in increased ROS production. Malnutrition and lack of necessary minerals would cause malfunction of antioxidant and oxidative damage repairing enzymes leading to oxidative modification of biomolecules and altered physiological function. While recent investigations determined the total antioxidant capacity using an oxygen absorbance capacity assay in serum of patients with neurological, psychiatric, renal diseases and cardiomyopathy (SOFIC et al., 2002), only few studies have investigated the oxidant/antioxidant status in AN. The total antioxidant capacity in serum of AN patients was decreased ($p < 0.01$) to 24% of the normal value. The drop in other diseases was 20% for HIV encephalopathy, 13% for diabetic polyneuropathy, and 17% for cardiomyopathy.

3.2 Protein deficiency and its effect on the endogenous antioxidant system

MOYANO et al. (1999) have investigated the balance and the enzymatic defense systems in AN. They reported reduced activity of superoxid dismutase (CuZn-SOD) in AN patients compared to control subjects. The activity of catalase was increased in AN patients which suggests an adaptive response to the increasing demands of its scavenging action. The activities of enzymes of the glutathione system did not show significant differences between patients and controls (MOYANO et al., 1999) (Tab.3)

Table 3: Antioxidant enzymes in AN

Antioxidant enzymes	Status in relation to healthy subjects	Authors
Superoxid dismutase	reduced activity	MOYANO et al., 1999 (3)
Catalase	higher activity	MOYANO et al., 1999
Gluthation system reduced/oxidized GSH/GSSG	no difference	MOYANO et al., 1999
Plasma concentration of GSH	lower	ZENGER et al., 2004

In addition to antioxidant enzymes mentioned above small peptides (e.g. GSH and carnosine), some amino acids (e.g. arginine, citrulline, glycine, histidine and taurine) and nitrogenous metabolites (e.g. creatine and uric acid) can directly scavenge ROS, and this group is called: non-enzymatic antioxidant system. Decreased serum concentrations of valine, isoleucin and tryptophan were reported in one study by Yap et al. (YAP et al., 1975), while reduced concentrations of arginine and cysteine and significantly increased plasma concentrations of taurine, asparagines, glutamine, glycine, methionine, phenylalanine, ornithine, and histidine compared to reference values were found by MOYANO et al. (1998). These data show that only some of the ROS-scavenging amino acids are increased while arginine is reduced. A recent study (ZENGER et al., 2004) showed a lower circulating concentration of free cysteine and free and total glutathione in patients with AN while their plasma concentration of homocysteine, glycine and glutamine were significantly higher. ZENGER et al. (2004) suggest that the utilization of these amino acids for the glutathione synthesis and therefore also the detoxification of ROS through glutathione is decreased. Whether the changes in concentrations of the scavenging amino acids effect oxidant damages in AN is still unclear.

Research on animal model systems (e.g. rats) has shown that insufficient protein intake can also result in zinc deficiency (FANG et al., 2002). Zinc is an integral part of CuZn SOD. Reduced concentrations of zinc as it will be demonstrated below are also common in patients with AN. The lower activity of SOD in patients with AN might be due to reduced zinc availability and a negative protein balance.

Albumin represents another potent antioxidant protein which scavenges lipid hydroperoxides and hypochlorous acid and ensures the bilirubin-protected transport of unsaturated fatty acids in blood. Albumin is found at high concentrations in extracellular fluid and is impaired in diseases with oxidant stress, e.g. in cystic fibrosis (ABMAN et al., 1985). For AN patients no difference was found in plasma albumin concentration, in the rate of albumin catabolism, or in the fractional exchange rates between extravascular and intravascular pools compared to control subjects (SMITH et al., 1996). In contrast to this UMEKI et al. (1988) found a significant decrease in total serum protein and albumin in AN patients. In summary, albumin concentrations are not substantially reduced in AN but there is strong evidence that albumin synthesis is reduced in semi-starvation when amino acids are in short supply (JAMES & HAY, 1968).

3.3 Micronutrients involved in the antioxidant defense and their status in AN

Malnutrition especially as an inadequate intake of protein and micronutrients during a prolonged period of time might cause a certain degree of oxidant stress that will aggravate AN and its possible neurologic sequelae (MOYANO et al., 1999). As mentioned above several components of the antioxidant defense system are dietary micronutrients (e.g. carotenoides, vitamin C, E) or depend on them (e.g. CuZn and Mn superoxid dismutase).

Vitamin E:

In one study on AN vitamin E concentrations were within normal ranges (VAN BINSBERGEN et al., 1988). However, reduced concentrations of erythrocyte tocopherol were reported by Moyano (MOYANO et al., 1999). Circulating concentrations of alpha- (VAISMAN et al., 1992) beta- and gamma-tocopherol (LANGAN & FARRELL, 1985) were reduced in AN patients; alpha-tocopherol was close to lower limit of the normal range (VAISMAN et al., 1992). Vitamin E has been recognized as one of the most important fat-soluble antioxidants by inhibiting ROS-induced generation of lipid peroxyl radicals. Dietary vitamin E deficiency induces liver lipid peroxidation, and affects the upregulation of the activities of antioxidant enzymes such as hepatic catalase, GSH peroxidases, and glutathione reductase.

Vitamin A:

Lower circulating vitamin A (retinol) concentrations than in normal subjects were reported in AN patients (VAISMAN et al., 1992). Routine clinical chemical variables and parameters of the vitamin status showed that patients with AN had higher (VAN BINSBERGEN ET AL., 1988) or normal (ROCK & VASANTHARAJAN, 1995) plasma retinol concentrations.

Carotenoids:

The plasma concentration of alpha-carotene was reported to be increased (ROCK & VASANTHARAJAN, 1995) and the concentration of beta-carotene was increased (BOLAND et al., 2001) or normal (MEHLER et al., 1998) in AN compared to controls. Depending on eating habits of AN patients data of vitamin concentrations can differ. However, a common finding in AN patients, especially in the restricting subgroup, is hypercarotenemia. High concentrations of carotenoids can exert pro-oxidant activity (ZHANG & OMAYE, 2001a; ZHANG & OMAYE, 2001b; LOWE et al., 2003) and therefore cause – instead of reduce - oxidant stress in AN.

Vitamin C:

Concentrations of vitamin C were reported to be in normal ranges (VAN BINSBERGEN et al., 1988). Only one case of early scurvy complicating long-standing AN was reported by Christopher (CHRISTOPHER et al., 2002). Despite the poor intake of nutrients scurvy is an extremely rare complication of AN.

Vitamin B group:

The intake of thiamin (Vitamin B1) (THIBAULT & ROBERGE, 1987; HADIGAN et al., 2000) of patients with AN is significantly lower than that of normal subjects. Different B vitamin plasma concentrations were determined by several of studies. Thiamin concentrations were reduced in patients with AN (WINSTON et al., 2000) whereas no significant difference was found for vitamin B1 in other studies (VAN BINSBERGEN et al., 1988; ROCK & VASANTHARAJAN, 1995). Low concentrations of vitamin B1 may account for some of the neuropsychiatric symptoms of AN like depression, instability of mood and low appetite (ROCK & VASANTHARAJAN, 1995;

WINSTON & JAMIESON et al., 2000). AN patients had increased serum vitamin B12 (VAN BINSBERGEN et al., 1988). A reduced concentration of FAD in blood has been reported in AN by Van Binsbergen (VAN BINSBERGEN et al., 1988). The plasma concentration of riboflavin in AN was reduced or normal (ROCK & VASANTHARAJAN, 1995; HADIGAN & ANDERSON et al., 2000) but increased in erythrocytes (CAPO-CHICHI et al., 1999).

Folic acid:
The plasma concentration of folic acid was reduced in several studies (CASPER et al., 1980; VAN BINSBERGEN et al., 1988; ROCK & VASANTHARAJAN, 1995; CAPO-CHICHI et al., 1999). Niacin also seems to be reduced in AN patients (ROCK & VASANTHARAJAN, 1995).

Iron:
Serum concentrations of iron of AN patients have been reported to be significant reduced (THIBAULT & ROBERGE, 1987) or similar (CASPER & KIRSCHNER et al., 1980) compared to control subjects. The total iron binding capacity was lower while the iron saturation seemed not to be significantly different in patients with AN (CASPER & KIRSCHNER et al., 1980; VAN BINSBERGEN et al., 1988).
Based on these studies it can be suggested that free iron does not give rise to increased ROS production via Fenton reaction in AN. If iron supplementation is given in cases of low iron concentrations in patients with AN it has to be taken into account that dietary protein deficiency might additionally increase iron concentrations and promote ROS production, lipid peroxidation, and oxidant stress (DABBAGH et al., 1994).

Copper, zinc and magnesium:
Plasma concentrations of copper were reduced in AN patients compared to control subjects (CASPER & KIRSCHNER et al., 1980). Dietary zinc intake in vegetarian AN patients was reported to be significantly lower than in non-vegetarian patients, resulting in higher risk of zinc deficiency in vegetarian AN patients (BAKAN et al., 1993). In several studies plasma and urinary zinc concentrations were reduced (CASPER & KIRSCHNER et al., 1980; MCCLAIN et al., 1992) while plasma zinc concentrations were reported to be normal in an other study (VAN BINSBERGEN et al., 1988). Using rats as a model system,
HAMMERMUELLER et al. (1987) shows that a deficiency of copper or zinc also enhances cytochrome P-450 activity in microsomes of lung and liver, stimulates ROS generation, and increases intestinal NOS expression which render the animal more susceptible to lipid peroxidation and gastrointestinal infection. Recent publications focusing on the magnesium intake showed that 50% of AN patients failed to meet the recommended dietary allowance (RDA) for magnesium (HADIGAN & ANDERSON et al., 2000). Data on plasma concentrations are missing. A deficiency of dietary magnesium reduces glutathione reductase activity and results in radical-induced protein oxidation.

Selenium:

The significant lower intake of selenium of patients with AN compared to normal subjects (HADIGAN & ANDERSON et al., 2000) might therefore decrease the activity of the glutathion peroxidase (XIA et al., 1985).

3.4 Sources of oxidant stress in AN

Although it is not well known, CR is regarded as a mild stressor (MASORO, 1998), which in the long term results in increased activity of antioxidant and oxidative damage repairing enzymes, resulting in decreased level of oxidative damage (SREEKUMAR et al., 2002) . On the other hand, AN, which is an extreme model of human CR, appears to cause more significant stress, including the oxidative one, which could be originating from a variety of sources.

3.4.1 Psychological stress in AN

Patients with AN suffer from psychological stress. First, the period of adolescence is a situation of psychological stress itself. Personal, social and/or family problems may lead to tension, anxiety and full-blown stress reactions. Second, an extreme fear of weight gain and an obsession with body image are additional stress factors. The psychological situation of patients with AN might therefore be an important cause of oxidant stress (MOYANO et al., 1999), together with overproduction of cytokines, which are potential generators of ROS, like interleukin 6 (IL-6) (NISHIDA et al., 2002). Moreover, catecholamines, which are secreted at higher concentration during AN can undergo auto-oxidation to form ROS.

3.4.2 Physiological stress in AN

The electron transport associated with the mitochondrial respiratory chain is considered the major process leading to ROS production. It is widely assumed that the increased electron flow through the mitochondrial electron transport chain leads to an increased rate of ROS production during exercise. Recent animal and human studies showed that regular exercise results in adaptation, which not only involves a wide range of specific changes in different organs (improved cardiovascular system, better cognitive processing e.g. better short- (24 h) and long-term (72 h) memory) but also adaptation of antioxidant and oxidant damage repair systems (RADAK et al., 2001; RADAK et al., 2002). In AN requirements of substrates and components which, under normal conditions, would respond to increased demands of antioxidant processes, are not met. It is therefore questionable if an adaptation to regular physical exercise may occur in patients with AN. Many patients with AN exercise excessively. This and the absence of adaptation processes may lead to oxidant stress and may also be one factor for the higher concentration of oxidant stress reported in AN.

In addition, physical inactivity could also cause very serious problems to AN patients. Physical inactivity, as a general phenomenon is against our evolutionary determination, since we are not designed to be sedentary; it increases the sensitivity to oxidative stress and makes to the body more vulnerable for oxidative challenge (RADAK et al., 2008). Since the dietary uptake of vitamins and minerals is very limited for AN patients, it is suggested that physical inactivity would be a further factor to increase sensitivity of the body to oxidative stress. In addition, alcohol intake or smoking, which are potential generators of ROS, would result for patients with AN in an increased risk of oxidative stress due to the down-regulated antioxidant system.

3.4.3 Proinflammatory cytokines (IL-6)

IL-6 gene expression is modulated by oxidant stress through activation of the transcription factor nuclear factor NF-kB. As a proinflammatory cytokine, IL-6 also leads to an overproduction of free radicals e.g. by activating leukocytes. Exercise has been shown to induce higher concentrations of IL-6 (NIEMAN et al., 2001; TOFT et al., 2002). Therefore an increased IL-6 concentration caused by excessive exercise and/or physiological changes in AN may directly affect the anti/proinflammatory balance and also the oxidant/antioxidant balance. The profil of circulating cytokines of AN patients has also been related to several factors including decreased food intake (CORCOS et al., 2003), psychopathological and neuroendocrine factors (POMEROY et al., 1994; BRAMBILLA, 2001; NOVA et al., 2002). However, the consequences of increased IL-6 concentrations on the oxidant/antioxidant status and the effect of an altered oxidant/antioxidant balance on psychopathologic and neuroendocrine factors has not been investigated in AN.

3.4.4 Glucose depletion

In case of moderate CR, the decrease of blood sugar level, could be even beneficial, since it can result in decreased level of sugar-protein interaction, glycation, which impairs protein function (GILLERY, 2006) and could have physiological consequences as well. On the other hand, in case of AN, the sugar depletion could be pathophysiological. Chang et al. (CHANG et al., 2003) showed that glucose depletion in HepG2 hepatoma cells results in cellular stress and ROS production. Glucose deprivation causes increased generation of pro-oxidants and decreased scavenging of free radicals, presumably *via* reduced formation of pyruvate and NADPH (SPITZ et al., 2000). In the absence of glucose, an important source of an oxidizable fuel for mitochondria is glutamine, which has been shown to enhance mitochondrial production of ROS (GOOSSENS et al., 1999).

In patients with AN cerebral glucose metabolism was reported to be lower than in control subjects (DELVENNE et al., 1997). There was a positive correlation between absolute metabolism of glucose and body mass index. It may be hypothesized that

glucose hypometabolism also increases ROS formation in patients with AN. Nevertheless in AN patients regulatory hormones prevent a hypoglycemia by controlling the carbohydrate metabolism. Growth hormones as well as cortisol advance gluconeogenesis to ensure sufficient glucose supply. Despite of these mechanisms plasma glucose and insulin concentrations were significantly lower in AN compared to control subjects (SCHREIBER et al., 1991). IL-6 is known to induce hepatic glucose output and lipolysis during strenuous exercise to compensate low plasma glucose concentrations (PEDERSEN et al., 2001). The up-regulation of IL-6 reported in some studies may therefore represent a response to low glucose concentration as a consequence of caloric restriction-related metabolic changes in AN.

4. Discussion

This review is aimed at providing a comprehensive overview over the oxidant/antioxidant balance in AN, and point out the differences between CR and AN. It can be suggested that the ROS-formation in patients with AN is reduced due to a decreased metabolic turnover and the requirements for antioxidants should be accordingly. But on the contrary, due to excessive physical activity and increased psychological stress high amounts of ROS are likely to be found in AN patients. This overproduction can cause oxidant cellular damage and long-term effects. Not only the possible increase in the generation of ROS during AN, but most likely the deficit of antioxidant and oxidative damage repairing system would result in oxidative stress. Here, we emphasize again, that the improper "feeding" of the antioxidant system, which requires nutritional uptake of wide variety of vitamins, minerals, amino acids, etc, would jeopardize the balance of redox homeostasis leading to oxidative stress and increased vulnerability to oxidative challenge.

Indeed, plasma concentrations of some vitamins and trace elements were reported to be lower in patients with AN. For instance, a low status of vitamin E is very common in AN. Dietary vitamin E deficiency is thought to affect the entire antioxidant network by reducing activities of hepatic catalase, GSH peroxidases, and glutathione reductase and may therefore significantly alter the oxidant/antioxidant status (FANG et al., 2002). Normalization of the vitamin E status in patients with AN by dietary vitamin supplementation might be required to recover the antioxidant network.

Hypercarotenemia is a common finding in AN and may result from excessive intake, decreased tissue storage capacity or pharmacologic vitamin supplements (FISHER et al., 1995; BOLAND et al., 2001). Clinical intervention trials have shown that supplemental beta-carotene at doses leading to very high plasma concentrations are associated with increased risk of lung cancer in smokers (PAISLEY, 1999). Therefore, high carotene concentrations in AN may not be beneficial for these patients.

Zink deficiency seems to be a very important link between psychological and pathophysiological changes and antioxidant constituents in AN. Studies with

athletes showed that zinc deficiency itself can lead to anorexia, significant loss in body weight, latent fatigue with decreased endurance and risk of osteoporosis (MICHELETTI et al., 2001), which can also be found in AN in many cases. Zinc therapy improves the rate of recovery in AN patients by increasing weight gain and reducing anxiety and depression. Since zinc is an essential part of the antioxidant enzyme CuZn-SOD, zinc supplementation in patients with anorexia may increase the activity of SOD.

Normalization of the redox state may balance certain cell-signaling molecules which are known to be targets for redox modification and functional alterations mediating oxidant-induced cellular responses, including nuclear factor kB (NF-kB) activation and subsequent cytokine gene expression. Normalizations on the cellular and intracellular level may help to improve the overall physical and psychological condition of AN patients. Zinc supplementation is already recommended as part of the overall therapeutic regimen of patients with AN, and it might be effective by re-establishing an oxidant/antioxidant balance in cells and tissues (SU & BIRMINGHAM, 2002).

It should be mentioned that moderately increased concentrations of ROS are required for adaptive responses to a higher oxidant stress. The latter include up-regulation of antioxidant enzymes and other lines of cellular antioxidant defense (RADAK et al., 2002). A strict prerequisite, however, for adaptation is maintenance of basic mineral nutrients such as copper, zinc and selenium, which might not be granted in AN-patients. Therefore adaptive processes might not occur and aggravate the cellular oxidant stress, especially during excessive exercise. At least for unsaturated fatty acids there might be an adaptation by the modification of the composition of the LDL (low density lipoproteins). Curatola et al. report a significant decreased sensibility for plasma fatty acids to ROS from patients with AN (CURATOLA et al., 2004).

The literature on hand does not sufficiently discuss the positive and negative effects of ROS in AN and their specific roles as second messenger, in signal transduction or in adaptive processes sufficiently. Changes in the redox balance in patients with AN have positive and negative effects by leading to alterations of the endocrine, nervous and immune system and may therefore affect several symptoms of this illness as well as adaptive responses. Dietary supplements may prevent from vitamin and mineral deficiencies and consequently inhibit alterations of the oxidant/antioxidant balance and of redox-dependent cellular functions. Correction of deficiencies of vitamins and trace elements to normal concentrations by antioxidant supplementation is usually regarded as safe and beneficial, but in the case of AN patients difficult to predict due to lacks in investigational data about the antioxidant status of patients. As mentioned above deficiencies in the dietary vitamin uptake may be – at least to some extent – counterbalanced by destruction of body tissue, but may not be compensated sufficiently to maintain certain concentrations of antioxidants which ensure a well balanced state between oxidants and antioxidants. However, dietary vitamin supplementation should be given after detailed

examination of the AN-patient's vitamin status only, since even harmfully elevated levels of some micronutrients can be found in AN.

In addition, elucidating the difference between CR in AN and in animal trials and the contributions of ROS to the pathogenesis and progression of AN might prove important for a deeper understanding of this eating disorder. Given that data on lipid peroxidation, oxidative protein modification and DNA damage in AN are lacking, the following questions need to be answered:

* Does impaired antioxidant status as found in AN due to caloric restriction cause increased oxidant damage?
* If so, which macromolecules are affected?
* Which role do ROS play in signal transduction and subsequent cytokine expression in AN?
* Could ROS be a connecting link between psychological alteration and pathophysiological changes?
* Does the antioxidant system break down in AN when stores are depleted and compensation by endogenous antioxidants is no longer possible?

Experimental data on the redox status in patients with AN, gained under highly standardized conditions may lead to a better understanding of the role of ROS and long-term consequences of oxidant stress in this special type of caloric restriction.

5. Conclusion

The well documented beneficial effects of CR such as increased activity of antioxidant and oxidative damage repairing enzymes, increased resistance to oxidative stress, decreased level of oxidative damage and increased level of mean and maximal life span can not be found in patients with AN. On the other hand, it appears that AN is only leading to the impairment of enzymatic and non-enzymatic antioxidant systems due to the inadequate uptake of food, with the possible consequence of increased generation of ROS resulting in a collapse of redox homeostasis, altered gene expression of redox sensitive transcription factors and oxidative damage. Here we propose that AN could seriously increase the vulnerability of the body to oxidative stress and oxidative stress related diseases. Therefore, patients with AN need a very special attention to re-establish the ability of their antioxidant system to decrease the potentially serious consequences of AN.

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