REVIEW

Effects of Long-Term Thyroid Hormone Level Alterations, n-3 Poly-unsaturated Fatty Acid Supplementation and Statin Administration in Rats

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Summary

Thyroid hormones (THs) play multiple roles in the organism and alterations of their levels can result in many pathological changes. Currently, we use hyperthyroid and hypothyroid rats as "models of a diseased organism" and analyze whether n-3 polyunsaturated fatty acids (n-3 PUFA) administration can ameliorate TH-induced pathophysiological changes. We investigate myosin heavy chain composition, calsequestrin levels, changes in cardiac tissue remodeling and cell-to-cell communication, expression of protein kinases, mitochondrial functions, oxidative stress markers and cell death, changes in serum lipid levels, activities of key enzymes of thyroid hormone metabolism, activity of acetylcholine esterase and membrane anisotropy, as well as mobile behavior and thermal sensitivity. Additionally we also mention our pilot experiments dealing with the effect of statin administration on skeletal muscles and sensory functions. As THs and n-3 PUFA possess multiple sites of potential action, we hope that our complex research will contribute to a better understanding of their actions, which can be useful in the treatment of different pathophysiological events including cardiac insufficiency in humans.

Key words

Thyroid hormones • n-3 polyunsaturated fatty acids (n-3 PUFA) • Statins • Rat muscle proteins • Cardiac remodeling

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Introduction

Thyroid hormones (THs) play an important role in cell growth, development and differentiation and represent one of the major endocrine regulators of cell and tissue metabolic activity. Alterations of their levels in experimental animals can induce different changes including muscle fiber type transitions, alterations of cardiac rhythm, myosin heavy chain (MyHC) and serum lipid level alterations, modification of calcium handling, ion channels, transporters, exchangers and enzyme activities (for review see Soukup and Jirmanová 2000, Hudecova *et al.* 2004, Kahaly and Dillmann 2005, Bielecka-Dabrowa *et al.* 2009, Tribulová *et al.* 2010, Novák and Soukup 2011).

On the other hand, omega n-3 polyunsaturated fatty acids (n-3 PUFA) have been suggested in many clinical trials and animal models (McLennan 2001) to possess multiple effects, including reduction of lipid levels, metabolic effects, direct interactions with cytosolic or membrane bound proteins, alteration of membrane fluidity (after being incorporated into the phospholipid bilayer) or cardiac tissue remodeling and cell-to-cell communications (for review see Den Ruijter *et al.* 2007, Rupp 2009, Tribulová *et al.* 2008, 2010, Richardson *et al.* 2011, Rauch and Senges 2012, von Schacky 2010, 2012), although the data demonstrating improvement remain contradictory. Their effects are usually tested using preparations from fish oil containing a high amount of eicosapentaenoic (EPA) and docosahexaenoic (DHA) n-3 PUFA. These preparations

are supposed to help in post myocardial infarction states as well in the reduction of hypertriglyceridemia, often as a supplementary treatment to statins.

Statins are drugs used in human therapy to reduce high levels of serum lipids (cholesterol, LDL and triglycerides) and to prevent cardiovascular disease. They are used when dietary regimens and life style changes (body mass reduction and exercise) are not sufficient. On the other hand, it is well known that chronic administration of statins can induce in humans a range of side effects, including skeletal muscle weakness and pain, myopathies or even muscle breakdown (including a life threatening form termed rhabdomyolysis), decreased sensitivity to touch and various neurological problems, such as polyneuropathies or nerve damage resulting in loss of sensitivity in the fingers or toes or the development of neuropathic pain.

The goal of this review is to compare our experiments that have been using hyperthyroid and hypothyroid rats as "a model of a diseased organism" with known literary data. We particularly reviewed whether i) n-3 polyunsaturated fatty acids (n-3 PUFA) administration can ameliorate TH-induced pathophysiological changes such as skeletal muscle protein alterations, cardiac tissue remodeling and cell-to-cell communication changes, alterations in expression of protein kinases, mitochondrial functions, oxidative stress markers and cell death, changes in serum lipid levels, in activities of key enzymes of TH metabolism and acetylcholine esterase or in membrane anisotropy, as well as in mobile behavior and thermal sensitivity, and ii) whether chronic statin administration affects fiber type composition and structure of skeletal muscles, as well as hind paws sensitivity to heat stimuli.

Skeletal muscles

MyHC isoforms and calcium handling proteins

Skeletal muscles contain variable proportion of four fiber types, marked slow type 1 and fast 2A, 2X/D and 2B fibers (Pette and Staron 2001, Schiaffino 2010). These types can be recognized by immunostaining with specific monoclonal antibodies against individual MyHC isoforms (Fig. 1), using histochemistry, e.g. by myofibrillar ATPase reaction or using RT-PCR to determine the level of MyHC isoform mRNA expression (Schiaffino *et al.* 1986, Soukup *et al.* 2002, 2009, Zacharova *et al.* 2005, Zurmanova *et al.* 2007, 2008, Smerdu and Soukup 2008, Novák *et al.* 2010, Zurmanova

and Soukup 2013). On the other hand, mammalian heart muscle cells express only two MyHC isoforms, α and β (Mahdavi *et al.* 1982), the latter corresponding to slow type 1 isoform in skeletal muscles and being a product of the same gene. The molecular masses of the rat α and β isoforms are both about 223 kDa (Rat Gene Database: http://rgd.mcw.edu/) and their amino acid sequences are 93 % identical (McNally *et al.* 1989) and their separation using SDS-PAGE is not simple (Arnostova *et al.* 2011). However, they differ in their ATPase activity and effect on heart contractility, as MyHCα is a part of a „fast myosin" with higher ATPase activity and faster contraction, whereas MyHCβ is contained in a „slow myosin" with lower ATPase activity and slower contraction (Pope *et al.* 1980).
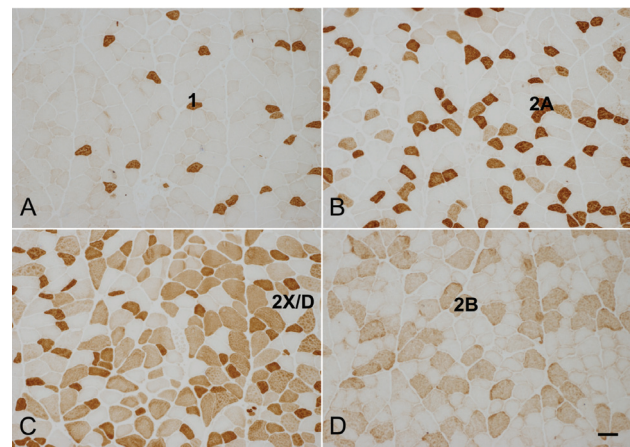

Fig. 1. Examples of cross sections demonstrating immune reactivity of the extensor digitorum longus muscle of adult Lewis euthyroid rats. **A:** BA-D5 staining slow type 1 fibers, **B:** SC-71 staining fast 2A fibers, **C:** BF-35 staining all fibers except fast 2X/D and **D:** BF-F3 staining fast 2B. Bar indicates 100 µm (from Soukup *et al.*, *Physiol Res* **61**: 575-586, 2012, with the kind permission of the Journal).

It is generally supposed that the *MyHC* mRNA levels define the amount of subsequently synthesized MyHC protein isoforms and decisively contribute to fiber type contractile characteristics (e.g. Schiaffino and Reggiani 1996, Pette 2002, Schiaffino 2010). Skeletal muscles react to TH alteration by modifying their MyHC content and fiber type composition (d´Albis and Butler-Browne 1993, Larsson *et al.* 1994, Caiozzo *et al.* 1997, Soukup and Jirmanová 2000, Soukup *et al.* 2001, 2012, Vadászová *et al.* 2004, 2006a,b, Vadászová-Soukup and Soukup 2007, Novák and Soukup 2011, Soukup and Zurmanova 2012) and this reaction is different from that occurring in the heart muscle (Dillmann 1990, Tribulová *et al.* 2010). It is generally supposed that elevated levels

of THs, as transcriptional factors acting *via* the thyroid hormone response element (Yen 2001, Fondell 2013), stimulate the expression of fast genes and thus increase expression of fast MyHC isoforms and number of fast fibers with high mATPase activity (Fig. 2). Furthermore, muscle fiber type characteristics can flexibly react to physiological demands within their given genetic range (Erzen *et al.* 1996, Snoj-Cvetko *et al.* 1996a,b). We presented quantitative evidence of corresponding proportions between *mRNA* level, protein content and fiber type composition in the rat soleus and EDL muscles and suggested that Real Time RT-PCR could be used as a routine method for analyzing muscle composition changes and could thus be advantageous for the analysis of scant biological samples such as muscle biopsies in humans (Zurmanova and Soukup 2013). Alteration of TH status, as well as many other experimental approaches, can lead to mismatch of mRNA, MyHC isoform and fiber type characteristics and may increase the incidence of s.c. mixed fibers expressing more *MyHC* mRNAs and protein isoforms within a single fiber (Stevens *et al.* 1999). Hyperthyroid (HT) status significantly increased the number of mixed 2C (1C) fibers in the slow soleus muscle, while the fast EDL muscle was more affected by hypothyroid (HY) status compared to euthyroid (EU) status (Novák and Soukup 2011). Increased numbers of hybrid fibers were also observed after suspension hypokinesia (Asmussen and Soukup 1991, Caiozzo *et al.* 1997), weightlessness during space flight (Kraemer *et al.* 2000) or during increased mobility (Asmussen *et al.* 2003).
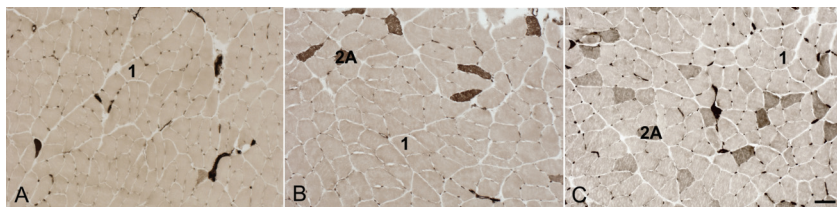

Fig. 2. Examples of cross sections demonstrating alkali-stable mATPase reaction after pH 10.3 pre-incubation of the soleus muscles of adult hypothyroid **(A)**, euthyroid **(B)** and hyperthyroid **(C)** Lewis strain rats. The same image was achieved using the SC-71 monoclonal antibody. Slow type I fibers are marked as 1 and fast type 2A fibers as 2A. Note the great difference in the number of darkly stained 2A fibers. Bar indicates 50 μm (from Soukup *et al.*, *Physiol Res* **61**: 575-586, 2012, with the kind permission of the Journal).

Calsequestrin expression

For the physiological change of muscle performance one should expect changes of both contractile and excitation-contraction-coupling (ECC) machinery, namely changes of calcium binding proteins (CaBPs), including calsequestrin (CSQ). CSQ is the most abundant CaBP of skeletal and cardiac muscle. It maintains free Ca^{2+} concentrations relatively low, which is important for easier and more efficient transport of released calcium by SERCA pumps. CSQ is a component of the macromolecular complex involved in ECC, the process linking surface membrane depolarization to Ca^{2+} release from the SR (Berchtold *et al.* 2000, Dulhunty 2006, Franzini-Armstrong 2009). CSQ is produced as a skeletal (CSQ1) isoform found in fast-twitch and slow-twitch muscles and a cardiac (CSQ2) isoform, considered to be the only transcript present in cardiac and a minor transcript in adult slow-twitch muscle (Beard *et al.* 2004, Wei *et al.* 2009). Functional changes of the CSQ complex and its mutations can result in pathology, including impairment of ECC, skeletal muscle myopathies or cardiac arrhythmias (for review see Marks *et al.* 2002, Tomelleri *et al.* 2006, for detailed literature survey see Novák and Soukup 2011). We investigated the effects of altered TH levels on the expression of CSQ1 in relation to simultaneously induced changes in fiber type composition. Both features were analyzed in normal and regenerated fast and slow skeletal muscles and in hearts of EU, HT and HY adult inbred Lewis strain rats. We found that the extent of changes in CSQ1 levels after TH alterations corresponded to the changes of the fiber type composition both in normal and regenerated muscles. This "correlation" was most remarkable after grafting of the soleus muscle into the EDL and vice versa, as the CSQ1 level and fiber type composition corresponded to the level typical for the host muscle and not to that of the graft source (Soukup *et al.* 2012). The higher TH level thus increased both the level of CSQ1 and the percentages of fast 2X/D and 2B fibers in the EDL, while HY status lead to opposite changes. This suggests that the observed minor changes in CSQ1 level are probably related to complex fiber type changes occurring during muscle fiber type TH induced transformation. We observed no significant effect of n-3 PUFA supplementation (unpublished data).

Pravastatin effect

We are currently studying two effects of long term statin application known in human pathology, i.e. muscle and sensory perception impairment. We are testing whether muscle structure or fiber type composition as well as MyHC isoform composition, thermal sensitivity and mobile behavior will also change in rats. We use pravastatin, which is one of so-called acid statins (fluvastatin, pravastatin, cerivastatin) that directly inhibit the activity of 3-hydroxy-3-methyl-glutaryl- CoA (coenzyme A) (HMG-CoA), which is a key enzyme in the cholesterol synthesis pathway. Pravastatin can be easily dissolved in water (lactone statins – simvastatin and lovastatin must first be metabolized in the organism and are practically insoluble in water) and can be commercially purchased as a chemical, although it was already used in human therapy. We applied pravastatin beginning from the 4th postnatal week for maximally 21 months. Comparing the life span of rat and man, this corresponds to the administration in man from about the age of 3 to 75 years. The minimal dose was 50 μg/100 g at the beginning of the experiments; the maximal dose applied from the 6th month onward was 300 μg/100 g of body weight. In man, the minimal dose used is 10 and the maximum 80 μg/100 g of body weight. Thus, both the length of administration and dose greatly surmount those used in humans. Nonetheless, our preliminary experiments did not show any significant changes either in the structure of muscle fibers or changes in fiber type or MyHC isoform composition. Also, the thermal sensitivity tested with radiant heat applied to the plantar surface of each hind paw at room temperature (Pospíšilová and Paleček 2006) did not change significantly after chronic pravastatin application. Still, it has been reported that the combined effect of n-3 PUFA and atorvastatin suppresses ventricular fibrillation inducibility in hypertriglyceridemic rat hearts (Bacova *et al.* 2010).

Heart

Heart remodeling

Apart from pathophysiological factors such as hemodynamic overload and diabetes, THs represent the most potent regulator of cardiac MyHC gene expression leading to MyHC transitions and changes of heart contraction (Lompre *et al.* 1984, Izumo *et al.* 1986, Ojamaa and Klein 1993, Fletcher and Weetman 1998, Stevenson 2002, Danzi *et al.* 2008, for review see

Swynghedauw 1986, Morkin 2000, Gupta 2007, Tribulova *et al.* 2010). We have recently shown that in the left ventricles of EU and HT adult inbred Lewis strain rats, MyHCα was the predominant isoform, while in HY rats it was the MyHCβ isoform (Arnostova *et al.* 2011). We also showed that the HT status led in Lewis strain rats to cardiac hypertrophy (both absolute and relative), while HY status resulted in heart atrophy. Unfortunately, it is difficult to draw any parallels with the situation in humans, as human hearts contain MyHCβ as the major isoform. Nonetheless, it is known that chronic exercise induces physiological hypertrophy in humans characterized by the increased expression of MyHCα, while pathological hypertrophy caused by pressure and volume overload leads to the opposite effect (for review see Gupta 2007). Based on the differences in the ATP requirement of the two isoforms, it is accepted that hearts expressing mostly MyHCβ have a more economical metabolism than those expressing predominantly MyHCα. Still, data obtained from studies on transgenic rabbit hearts have indicated that moderate expression of the MyHCα isoform was advantageous for preserving heart function under stress conditions, suggesting that the benefit to heart function presented by this isoform may outweigh its higher energy demands (James *et al.* 2005, Gupta 2007). Maybe even more importantly, changes in cardiac MyHC isoforms expression (i.e. shift to MyHCβ) are supposed to be the major cause of heart failure (Gorza *et al.* 1984, Spann 1984, Miyata *et al.* 2000, Reiser *et al.* 2001, Tribulova *et al.* 2002). Alterations of TH levels thus contribute to various pathological changes, including some of the most life threatening cardiac events, such as atrial and ventricular fibrillations or malignant ventricular arrhythmias (Kahaly and Dillmann 2005, Bielecka-Dabrowa *et al.* 2009, Tribulová *et al.* 2010). Since it was shown that many cardiovascular diseases are associated with a shift between cardiac α and β MyHC isoforms, their unequivocal determination by SDS-PAGE is extremely important, especially for human pathology (Arnostova *et al.* 2011). Malignant cardiac arrhythmias including atrial and ventricular fibrillation represent, as already said, major problems in humans. Certain therapies, such as the administration of statins and n-3 PUFA, are supposed to provide additive antiarrhythmic efficacy by reducing risk factors involved in the development of the arrhythmogenic substrate (myocardial remodeling). Previous experiments suggested that intercellular Cx43 gap junction channels are involved in the increased susceptibility of the heart to arrhythmias

caused by increased TH levels and also that expression of PKCε, which directly phosphorylates Cx43, is affected (Tribulová *et al.* 2002, 2005, Lin *et al.* 2008, Mitašíková *et al.* 2009, for review see Dhein 1998, Tribulova *et al.* 2008, 2010). Some experiments suggested that n-3 PUFA may exert their protective effect *via* attenuation of the arrhythmogenic substrate (Mitašíková *et al.* 2008, Bacova *et al.* 2011, Radosinska *et al.* 2011). It was also shown that left ventricle hypertrophy in SHR rats was also associated with "remodeling" of MyHC, which altered susceptibility of the heart to sustained ventricular fibrillation in experimental animals (Tribulova *et al.* 2002). In our two recent papers (Radosinska *et al.* 2013, 2014) we found that n-3 PUFA intake significantly reduced cardiovascular risk factors, as they suppressed the incidence of ventricular fibrillation and facilitated sinus rhythm restoration in SHR in early and late stages of hypertension. The antiarrhythmic effects of n-3 PUFA can be attributed to the attenuation of abnormal myocardial Cx43 distribution, expression and phosphorylation, as well as to positive modulation of PKCε and PKCδ signaling and normalization of MyHC profiles (Radosinska *et al.* 2013, 2014). These results support the prophylactic use of n-3 PUFA to minimize the risk of lethal arrhythmias in hypertensive individuals. In addition, n-3 PUFA also modify the activity of membrane bound proteins such as the fast sodium channel, the voltage-gated L-type Ca^{2+} channel, Na^{+}/Ca^{2+} exchanger, proteins regulating calcium homeostasis (e.g. SERCA), transporters and membrane receptors or molecular targets such as peroxisome proliferator activated receptors (PPARs) or mitogen activated protein kinase/extracellular signal-related kinase (MEK/ERK) that all can modulate the function of the heart as well as of other organs (e.g. Richardson *et al.* 2011, Rauch and Senges 2012).

Antioxidant system and cell death

THs affect the energy metabolism of cardiomyocytes and at physiological concentrations may exert beneficial effects on the heart. Excess of THs can enhance mitochondrial respiration, but also the production of potentially harmful reactive oxygen species (ROS) (Venditti and Meo 2006). THs affect energy metabolism by regulating the gene expression of enzymes involved in oxidative as well as glycolytic metabolism *via* interaction with TH receptors (THR). T_3-regulated genes also include transcriptional factors such as nuclear respiratory factor 1 (NRF-1) and peroxisome proliferator-

activated receptor gamma co-activator 1α (PGC-1α), which are critical for mitochondrial metabolism (Weitzel *et al.* 2011). A stimulatory effect of THs on hypoxia inducible transcriptional factor (HIF1) was also recently described (Moeller *et al.* 2005), as well as a HIF1 feedback loop controlling THs function *via* activation of local deiodinase D3 expression (Simonides *et al.* 2008). There is clear evidence indicating down-regulation of the TH signaling system demonstrated as decrease of THR in the failing heart (for review see Dillmann 2010). The n-3 PUFA that have been shown to build up TH signaling (Souza *et al.* 2011) may then ameliorate such negative effect. Energy homeostasis of cardiomyocytes and control of apoptosis are also closely connected with the function of proteins associated with outer or inner mitochondrial membranes such as Bcl-2 family proteins (Youle and Astrasser 2008), hexokinase I and II (Miyamoto *et al.* 2008, Waskova-Arnostova *et al.* 2013) or the mitochondrial creatine kinase (mtCK). The mtCK octamer complex is localized in the inter-membrane space between the voltage-dependent anion channel (VDAC) and adenine nucleotide translocase (ANT) (Wallimann *et al.* 2011). Physiological interaction of the functional mtCK octamer is dependent on cardiolipin content in the inner membrane (Schlattner *et al.* 2009) and n-3 PUFAs directly increase the membrane n-3: n-6 ratio and cardiolipin content and improve tolerance to ischemia and reperfusion (Pepe 2000). Administration of n-3 PUFA thus could stabilize the mtCK octamer function and support energy homeostasis of cardiomyocytes under altered TH states. New avenues of cardioprotection have been opened by studies of cellular homeostasis, which is regulated by mitochondria, endoplasmic reticulum or expression of cytosolic signaling molecules. Several types of cell death may appear in response to death-inducing stimuli (Fink *et al.* 2005, Chung *et al.* 2012, Li *et al.* 2012). Hypothetically, TH can also act as a stimulus of cell death, and exploring and attenuating TH induced autophagy, apoptosis and pyroptosis formation in the damaged heart or other tissues is important for ameliorating the progress of cardiac diseaseas (Chien *et al.* 2012). Activation of nuclear factor-erythroid-2-related factor 2 (Nrf2) signaling provides cardioprotection, renoprotection and an anti-inflammatory effect, whereas down-regulation or knockout Nrf2 abrogates such protection (Chen *et al.* 2011, Wu *et al.* 2011, Chung *et al.* 2012). It can be assumed that elevated TH level through its receptor overt activation may inhibit nuclear Nrf2 translocation, impair

Bcl-2/Bcl-xL dependent-mitochondrial function and reduce downstream gene translation products such as HO-1 in the damaged heart. Enhanced Bcl-2/Bcl-xL expression, using Nrf2 activator or n-3 PUFA treatment may countervail TH induced heart injury.

Serum lipids

THs are important modulators of lipid metabolism. Generally, hypothyroidism is associated with increased levels of serum triglycerides, cholesterol and LDL cholesterol and *vice versa* hyperthyroidism is associated with their decreased levels. As regards n-3 PUFAs, they can shift energy substrates away from their storage as triglycerides, suppressing lipogenesis and promoting the utilization of fatty acids as fuel by increasing lipase activity and β-oxidation in mitochondria and peroxisomes. EPA and DHA are also poor substrates for triglyceride synthesizing enzymes, thus decreasing lipid levels. The hypolipidemic effect of n-3 PUFA is not completely understood, but it seems that it is mainly exerted *via* the activation of gene expression by up-regulation of nuclear transcription factors, such as PPARα in the liver, which indicates cross-talk between n-3 PUFA and THs (Bordoni *et al.* 2006, Sugiyama *et al.* 2008, Souza *et al.* 2011). Souza *et al.* (2011) reported that EU Wistar rats maintained on a fish oil diet (n-3 PUFA) exhibited higher liver expression of TH receptor β1 (TRβ1). In contrast, in the HY rats, the ability to induce TRβ1 was lost suggesting the enhancement of THs action following n-3 PUFA supplementation. The recommended dose of n-3 PUFA for humans with established coronary heart disease is 1 g/day and 3-4 g/day for patients wishing to achieve clinical protection against lipoprotein level elevation (Davidson *et al.* 2011). In rats, however, even 6-week-supplementation at a higher dose of 0.2 g/kg body weight/day, which significantly decreased blood pressure, suppressed inducible ventricular fibrillation, improved myocardial metabolic state, preserved cardiomyocytes and the integrity of their junctions in aged male and female SHR (Mitasikova *et al.* 2008), had no significant effect on serum postprandial triglyceride, total cholesterol and LDL-cholesterol levels (Rauchová *et al.* 2013). This could be partially explained by the data of Raederstorff *et al.* (1991) who showed that the quality and consistency of n-3 PUFA were altered by THs probably due to the competition for desaturases, elongases and acyltransferases between n-3 and n-6 PUFA. Moreover, rats are a rather poor model for testing lipid metabolism because they transport most of their cholesterol in the HDL fraction (Harris 1997) and are relatively hyporesponsive to increasing cholesterol levels (Zhang *et al.* 2009). Nevertheless, many animal studies have shown that a diet with n-3 PUFA usually lowered plasma triglyceride and total cholesterol levels almost always due to a decrease in HDL cholesterol (Harris 1997).

A good marker of the different thyroid status is mitochondrial glycerol-3-phosphate dehydrogenase (GPDH EC 1.1.99.5.), a flavin-linked enzyme, which is implicated in glycolysis, oxidative phosphorylation and lipid metabolism. It is well known that THs markedly influence GPDH activity in various mammalian organs, such as liver, skeletal muscle or heart (Lee and Lardy 1965, Dümmler *et al.* 1996). Our chronic experiments confirmed that HT status increased expression and activity of rat liver GPDH, while HY status resulted in opposite changes (Rauchová *et al.* 2004, 2011).

Metabolism of thyroid hormones

Multiple biological effects of THs depend on intracellular levels of 3,5,3'-triiodo-L-thyronine (T_3), which binds to nuclear THRs with the highest affinity. More than 80 % of circulating T_3 is generated in peripheral tissues by outer-ring 5'-deiodination of the pro-hormone thyroxin (T_4), produced entirely in the thyroid gland. This enzymatic conversion is catalyzed by iodothyronine 5'-deiodinases (IDs) type 1 and 2 (D1 and D2, respectively). D1 is mainly present in the liver, kidney, thyroid gland and pituitary gland and due to its high specific activity, hepatic D1 is considered the most important source of circulating T_3. In turn, its activity is regulated by circulating T_3 (Pavelka 2010a). As D1 enzyme activity in white adipose tissue under the conditions of changing adiposity shows pronounced changes (Macek-Jílková *et al.* 2010), it can be anticipated that supplementing the diet with n-3 PUFA may also influence adipose tissue metabolism and/or accumulation of the tissue in experimental rats by affecting their THs metabolism. Moreover, activities of thyroid peroxidase (TPO) and/or of THs conjugating enzymes (e.g. iodothyronine glucuronyl-transferase, UDP-GT) (Pavelka 2010b, 2012) may be modified by n-3 PUFA supplementation and therefore the rate of biosynthesis and the rate of excretion of metabolized THs can be altered.

Neurological effects

There is a close association between THs, brain cholinergic function and AChE activity. Dietary depletion of n-3 PUFA has been shown to adversely affect cholinergic function (Aid *et al.* 2005) and may contribute to cognitive decline in Alzheimer disease (Astarita *et al.* 2010). On the other hand, animal studies suggest that higher long-term dietary intake of n-3 PUFA can exert positive effects on various functions of the CNS (Wang *et al.* 2010) and other tissues, including rat cardiomyocytes (Leifert *et al.* 2000), apparently due to incorporation of n-3 PUFA into the cellular membrane phospholipid bilayer influencing its fluidity. Our preliminary results, however, failed to demonstrate any significant effect of n-3 PUFA supplementation on AChE activity and membrane fluidity (membrane anisotropy) measured in the cortex, striatum, hippocampus and cerebellum of EU, HT and HY rats (Říčný *et al.* 2011). Alterations of CNS caused by THs can be reflected by behavioral changes. Experiments measuring activity and response latency in the open field test showed that HT rats reacted with increased activity and shortening of response latency, while HY status yielded opposite results (Redei *et al.* 2001). Knocking down THRα in mice, mimicking HY status was manifested by decreased activity, learning and recall impairments in the Morris water maze and by increased anxiety/fear behavior in the open field test compared to control C57BL6J mice (Wilcoxon *et al.* 2007). Our pilot experiments (Petrásek *et al.* 2011) showed that HT rats are more mobile and HY less mobile than EU rats. The HY rats also spent less time by visiting central parts of the arena (increased thigmotaxis) compared to EU and HT rats. This suggests that behavior of the HY rats was less explorative and more anxious. Alterations of THs are thus involved in behavioral alterations and cognitive deficits resulting in increased anxiety and decreased exploratory behavior. Supplementation with n-3 PUFA, however, did not show any significant effect compared to changes caused by altered thyroid status.

Conclusions

Polyunsaturated fatty acids (n-3 PUFA) can help

in the prevention of adverse cardiac tissue remodeling associated with severe arrhythmias and cell-to-cell communication. Although thyroid hormones (TH) play multiple positive roles in the organism, chronic or prolonged alterations of their levels can result in pathophysiological changes affecting expression of many proteins as well as tissue remodeling. As THs and n-3 PUFA possess multiple sites of potential action, we hope that our complex research will contribute to a better understanding of their actions, which can be useful in the treatment of different pathophysiological events including cardiac insufficiency in humans.

Conflict of Interest
There is no conflict of interest.

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