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Nitric oxide and thermogenesis - challenge in molecular cell physiology

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1. ABSTRACT

Only recently we can link thermogenesis, mitochondria, nitric oxide, and redox regulation in biochemical terms. Currently, we are discussing these processes from the aspect of fundamental principles of molecular physiology. Thus, the present article highlights both cell physiology and the principles of the maintenance of energy homeostasis in organisms. Energy homeostasis means much more than simple combustion; adipose tissues at this point of evolution development are related to a broad spectrum of metabolic disturbances and all aspects of cellular remodeling (*i.e.* structural, metabolic and endocrine changes). Therefore, this paper addresses not only thermogenesis but also energy homeostasis, oxidative phosphorylation and ATP production, proliferation and differentiation of brown adipocytes, their life and death, mitochondriogenesis and angiogenesis. These processes will be united by molecular players of oxidation/reduction reactions, thus creating the principles based on the redox regulation.

2. INTRODUCTION

It would be quite justified and responsible to the same extent to write this text over 100 years ago when the first cognitions about L-arginine, nitric oxide (•NO) and mitochondria appeared. This amino acid, gas (free radical) and a cell organelle, since 50s of 20th century, and especially during the last 2-3 decades came into the focus of fundamental researches in medicine and biology. Brown adipose tissue (BAT) had the same destiny, and at present it is discussed not only from the aspect of thermogenesis with mitochondria as the principal players, but as an integrator of the energy homeostasis in health and disease.

Therefore, the processes of hyperplasia and differentiation, cell death, uncoupling, mitochondriogenesis and angiogenesis are significant not only for brown adipocyte physiology during thermogenesis, but they represent the main molecular principles of cell and energy homeostasis maintenance from embryonal development to cancer.

On the other hand, above-mentioned processes underlying BAT thermogenesis are subject of redox regulation. One of key players in redox regulation is •NO derived from amino acid L-arginine. In 2002, Reutov suggested that the study and analysis of properties of one of the smallest regulatory molecules of the living organisms, •NO, allowed us to substantiate the concept of nitric oxide cycle and to propose the existence of the common principle reflecting the existence of connections at various structure-functional levels, which we call the cyclicity principle (1). This cyclicity principle enables us to create new Descartes' cycles of "life", as wide as focused on molecule and united in molecular physiology.

People were used lupine plants (Fabaceae family) in medical practice thousands years ago (cultivation of lupines was practiced in Egypt as early as 2000 BC). Comparison of physiological effects in pathophysiological conditions when lupines are recommended, as well as conditions when they should be avoided, will reveal absolute coincidence of •NO-provoked effects. This is not surprising, because L-arginine is a common denominator of lupines and •NO, keeping in mind that these plants are known to be extremely L-arginine-rich, and •NO represents the final L-arginine-originating effector molecule (2, 3).

Metabolism of L-arginine is complex, tissue-specific and still insufficiently explained in many cell types. In addition to •NO, L-arginine represents the precursor of urea, ornithine and agmatine, and this amino acid is used in the synthesis of polyamines, citrulline and glutamate. L-arginine -originating biomolecules signaling is limited to a great extent by its availability (in mammals L-arginine is a semiessential or conditionally essential amino acid, depending on the developmental stage and health status), as well as by expression and activity of multiple enzymes involved in its metabolism (for the details on L-arginine metabolism see excellent review articles 4 and 5).

In the present review, within the scope of thermoregulation and bioenergetics, we were focused on brown adipocytes and BAT, *i.e.* on L-arginine /•NO-producing pathway, particularly on the mechanisms of redox regulation mediated by •NO during structural and metabolic remodeling of BAT.

Without overlooking other important aspects of L-arginine action, we were concentrated on •NO-signaling pathway, especially in the cases when supplementation with this amino acid leads to the induction of the •NO-synthesising enzymes.

3. BROWN ADIPOSE TISSUE: STRUCTURE AND FUNCTION

In mammalian species, there are two kinds of adipose tissue, white and brown adipose tissue (WAT and BAT, respectively), which differ not only morphologically, but also have completely distinct biological functions. The crucial morphological differences are related to the number and shape of lipid droplets and mitochondria. Typically, white adipocytes contain one large lipid droplet, relatively few small and elongated mitochondria, with cristae mitochondriales widespread in various directions. These structural differences correspond to different functional roles of WAT and BAT. Namely, WAT plays a role in energy storing in the form of lipids, while the function of BAT is heat production with energy dissipation. The function of BAT is critically related to uncoupling protein 1 (UCP1), situated in the inner mitochondrial membrane and uniquely expressed in BAT (6). The UCP1 uncouples phosphorylation from respiration and dissipates proton gradient as heat (7). Until relatively recently, it has been proposed that WAT and BAT form adipose organ where both types of tissues can undergo white-to-brown-to-white transdifferentiation, induced by specific stimuli, enabling plasticity and phenotype changes of the organ (8).

BAT is a very dynamic tissue, characterised by an amassing degree of plasticity and remodeling (9, 10). BAT remodeling consists of two opposing processes - tissue hyperplasia and regression, which serve to ensure new homeostasis in different tissue metabolic states. Namely, tissue mass and its metabolic activity increase in response to the different stimuli, *e.g.* cold, nutrition, hormones, postnatal development, hibernation, *etc*. Accordingly, cessation of above stimuli induces BAT regression that transforms active, hyperplastic tissue into inactive tissue undergoing atrophy (11).

Brown adipocytes are also more vascularised than adipocytes in WAT and this vascularisation ensures a sufficient supply of oxygen and metabolic substrates to the tissue and transfer of the produced heat through the organism (12). In addition, BAT is densely innervated by sympathetic nerves that mediate the central control of tissue thermogenesis (13). Noradrenaline released by sympathetic nerve fibers in BAT binds to multiple alpha and beta adrenergic receptors, stimulating hyperplasia of preadipocytes through $beta_1$ and mediating lipolytic and thermogenic action through $beta_3$-subclass of these receptors (6). Beta-signaling is coupled to the adenylate cyclase-mediated rise of intracellular cAMP, which as a second messenger acts propagating BAT thermogenic events. In essence, cold exposure activates the sympathetic nervous system that triggers BAT thermogenic program. This is a complex process comprising series of biochemical, morphological and cytological events that rapidly transforms "dormant" tissue into a hyperplastic, active organ. Precisely, cold was shown to increase the UCP1 content and to induce proliferation and differentiation of precursor cells, as well as hypertrophy of mature brown adipocytes. It also results in increased mitochondriogenesis, angiogenesis, peroxisomal biogenesis, protein and DNA synthesis, lipolysis, mitochondrial beta-oxidation and a decreased rate of apoptosis (11-14). Besides, brown adipocytes undergo morphological transformations becoming multilocular, with numerous mitochondria whose cristae become straighter and parallel (13, 15) (Figure 1). Those events enable an increase in tissue metabolic rate and oxygen consumption, *i.e.* drive increased thermogenic capacity and make BAT suitable for thermogenesis. It is impressive that during thermogenesis, BAT consumes more than one half of total

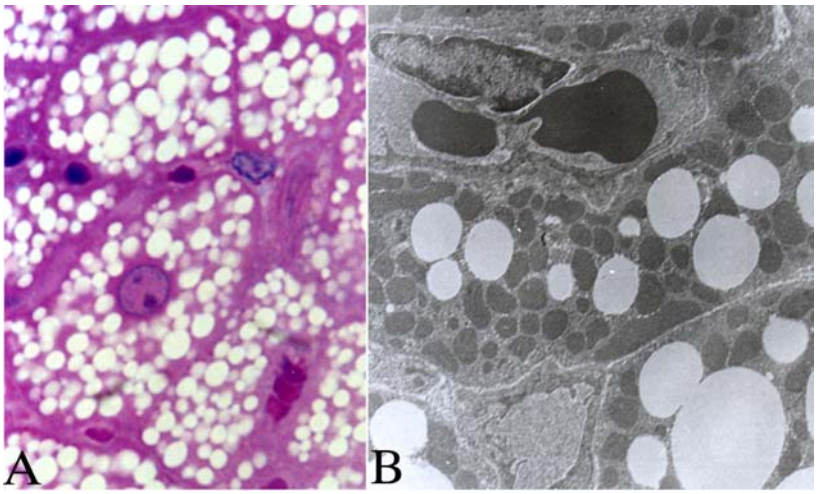

Figure 1. Brown adipose tissue of rats acclimated to cold (A-light and B-electron microscopy). Magnification: A-x100, orig.; B-x6900.

oxygen, *i.e.* it holds more than 50% of total body metabolic activity and consumes the same percent of nutrients (6).

At the molecular level, activation of the BAT thermogenic program is achieved by variety of thermogenic factors that coordinate biochemical, structural and cellular changes aimed at sufficiently providing BAT for the metabolic demand. Among them, the UCP1, peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgamma -coactivator-1alpha (PGC-1alpha), are of particular importance because of their role in increasing thermogenic capacity by intensifying uncoupling, fatty acid metabolism and differentiation, and affecting oxidative metabolism and mitochondriogenesis (7, 16, 17). Some of these aspects will be discussed later.

In essence, the mitochondria occupy the central position in the cascade of events involved in BAT thermogenesis. To understand better thermogenic function of BAT mitochondria, we have provided an outline of mitochondrial physiology.

3.1. Overview of mitochondrial physiology

Due to their role in the generation of ATP from metabolic fuels through oxidative phosphorylation, mitochondria are designated as the "power station of the cell". The oxidation of substrates produces free energy stored in special reduced carriers, such as NADH and $FADH_2$. These carriers donate electrons to the mitochondrial respiratory complexes (I-IV) localised in the inner mitochondrial membrane, resulting in the establishment of an electrochemical gradient of protons across the mitochondrial inner membrane. Three respiratory complexes I, III and IV serve as the sites of proton pumping from the matrix. In addition, complex V, ATP synthase, dissipates the proton gradient in the ATP synthesis. Inner mitochondrial membrane of brown adipocytes is equipped with UCP1 that functionally dissociates electron transport from oxidative phosphorylation, allowing the energy released in this process to be dissipated as a heat (7, 11). When the tissue energy demand is chronically increased, mitochondrial biogenesis takes place in metabolically active tissues. Exposure to cold is a metabolic stimulus that induces mitochondrial biogenesis in BAT, resulting in an improved thermogenesis. However, biogenesis of mitochondria represents a complex process that encompasses not only an increase in the number of mitochondria, but also mitochondrial remodeling at structural and molecular level.

From the very start of mitochondrial function examinations, it was clear that understanding of the processes such as oxidative phosphorylation and its uncoupling, apoptosis or biogenesis will inevitably take into account their structural context, including the influence of mitochondrial membrane topology, internal diffusion and compartmentation. Precisely, the complexity of mitochondrial structure is coupled with its functional state. Mitochondria are dynamic organelles, able to interchange their morphology between the two distinct arrangements (activation or inactivation) by undergoing the processes of mitochondrial fusion and fission to generate either an elongated interconnected mitochondrial network or a fragmented discrete phenotype, respectively (18). Under normal physiological conditions, the basic structure contains a smooth outer membrane that envelops an inner membrane with a considerably larger surface area enveloping a protein-rich matrix. Namely, mitochondrial inner membrane has numerous invaginations, the cristae. The shape of the inner membrane can vary tremendously, *i.e.* it rapidly adjusts in response to physiological stimuli, *e.g.* in the case of BAT - cold exposure.

Precisely, cold-induced BAT mitochondrial remodeling consists of a series of fine timely-tuned mitochondrial changes that begin with mitochondrial hypertrophy and appearance of newly-formed organelles with a simple inner structure (13, 19) followed by the changes in mitochondrial inner structure. At first, the cristae rearrange into a more regular, parallel pattern and

begin to become more closely positioned one to another and more numerous. During cold acclimation the trend toward cristae parallelism and strengthening continues and the ratio of cristae to matrix increases. It has been shown that at times when cristae tend to be most closely packed, mitochondria become able to produce the most heat. Since the inner membrane is the site of enzymes of electron transport chain, it is reasonable to assume that the progressively greater abundance of cristae during cold exposure represents structural and metabolic basis for the increase in oxidative capacity (13, 20). Mitochondrial structure changes and uncoupling of oxidative phosphorylation are in accordance with increase in UCP1 content (11). In contrast, when cold stimulus terminates, BAT mitochondria undergo opposite changes - decrease in number and size, followed by simplification of cristae organisation (13). In general, structural remodeling of BAT mitochondria (membrane and shape changes) is caused by metabolic state transition. So far, numerous factors that control mitochondrial shape and dynamics and thus their function have been identified, and among these factors, $^{\bullet}$NO is the most intriguing (19, 21-23).

In that context, it is important to note that during the last decade, the results of several research groups including our own demonstrated that the majority of aforementioned processes governing BAT thermogenic response have been shown to be redox sensitive (24-26). Specifically, reactive oxygen and nitrogen species (ROS and RNS, respectively), primarily $^{\bullet}$NO, were found to be a part of the signal transduction pathways that regulate BAT nonshivering thermogenesis events. To understand better the redox regulation, we have given here an overview on the basic principles of redox-biology.

4. INSIGHTS INTO REDOX REGULATION

The classic point of view on reactive species as initiators and propagators of damage of biomolecules, described in 1956 by Harman, has dramatically changed during the following three decades. Starting from the discovery that hydroxyl radical ($^{\bullet}$OH) can activate guanylate cyclase and subsequently formation of the second messenger cGMP (27), a large body of evidence clearly demonstrated "purposeful" roles of both ROS and RNS as signaling molecules and regulators of cell function. So, nowadays the concept of redox regulation includes controlled and programmed production of redox active molecules and the reversibility of redox modification is widely accepted and constantly expanding with novel mediators and targets.

4.1. ROS, RNS and oxidation products as redox mediators

At present, it is well documented that ROS and RNS can affect overall activity of a number of signaling pathways affecting various redox-sensitive targets, from membrane receptors through intracellular kinases and phosphatases to transcription factors and thus, numerous cellular processes and functions. Thiols of signaling proteins in the form of cysteine residues represent direct targets for post-translational protein modification by ROS and RNS, in terms of redox sensing and signaling. The reversibility of such protein modifications is provided by two powerful redox buffers, glutathione (GSH) and thioredoxin systems. Besides well-established mechanism of GSH-mediated regulation of thiol-redox status by classic thiol-disulfide interchange between GSH and a protein, during the last decade S-glutathionylation has been considered to be an important tool for post-translational protein modifications (28).

During the last years, it has been shown that lipid peroxidation products can exert biological effects either through induction of adaptive response and thus tolerance against oxidative stress or directly reacting with proteins, enzymes and nucleic acids (29). Till date, many proteins, including mitochondrial uncoupling proteins, have been reported to be regulated by lipid peroxidation products (30).

It seems likely that the concentration of the reactive species and their oxidation products determines final cellular response and that different steps, induction and execution of signals, are regulated in concentration-dependent manner. These aspects of redox regulation can be seen in regulation of transcriptions factors such as nuclear factor-kappa B (NF-kappaB), activator protein-1 (AP-1) and nuclear factor erythroid 2-related factor 2 (Nrf-2). Namely, the overall activation of these transcription factors requires a delicately balanced compartmentation of redox state, pro-oxidative conditions in cytoplasm for their activation, *i.e.* nuclear transport, but reducing conditions in the nucleus for the binding to the gene promoter. The integrity of transcription factor signaling seems to be provided by GSH in the cytoplasm and thioredoxin in the nucleus (31). Also, the effect of $^{\bullet}$NO on transcription factors depends on its concentration, *i.e.* low concentration inducing the activation, while high ones resulting in the suppression (32).

Fine intracellular redox tone is regulated by the producing and removing systems of ROS and $^{\bullet}$NO due to their capability to sense and response to modulation of reactive species level.

4.2. Cellular sources and regulation of ROS level

Besides mitochondrial respiratory chain, well established as an important source of superoxide anion radical ($O_2^{\bullet-}$) involved in many signaling pathways, xantine oxidase, lipoxygenase, peroxidase represent important contributors to redox regulation. In that context, NADPH oxidase (Nox) family of $O_2^{\bullet-}$ and hydrogen peroxide (H_2O_2)-producing proteins are now recognised to play essential role in many tissues. A surprisingly large number of nonphagocytic Nox isoforms and the fact that individual Nox isoforms differs from one to another tissue, as well as among the species, are strong indicators pointing to their physiological relevance in redox signaling (33).

To satisfy the regulatory aspect, ROS production must be equilibrated with their removal. Thus, the antioxidative defense can be considered as one of the most important factors for maintaining the redox homeostasis,

not only in the view of its protective role, but also from the aspect of maintenance of intracellular steady state level of reactive species (34).

4.3. Signaling by $^{\bullet}NO$: role of $^{\bullet}NO/O_2^{\bullet-}$ system in redox regulation

$^{\bullet}NO$ mediates signaling either through cGMP-dependent or independent pathways. A large majority of $^{\bullet}NO$-associated biological signaling is due to the second messenger cGMP, since the enzyme, soluble guanylate cyclase represents the most established target of $^{\bullet}NO$ action (35).

Acting in cGMP-independent manner, $^{\bullet}NO$ interacts with other hemoproteins such as cytochrome ***c*** oxidase (COX) and oxyhemoglobin (36). However, the rich chemistry of $O_2^{\bullet-}$ and $^{\bullet}NO$, based on the spectrum of redox conditions determined by the concentration of each and their ratio, together with the peroxynitrite formation seem to be in the core of $^{\bullet}NO$-mediated signaling through protein S-nitrosylation and tyrosine nitration.

S-nitrosylation of protein thiol is mediated by $^{\bullet}NO$ itself but also by other $^{\bullet}NO$ species, metal-$^{\bullet}NO$ complexes, peroxynitrite or nitrite. Almost all classes of proteins, including receptors, enzymes and transcriptional factors, are targets of S-nitrosylation. So, during the recent years S-nitrosylation is reasonably considered as the mechanism of signal transduction, as ubiquitous as phosphorylation/dephosphorylation (37-38). The results of recent studies highlighted the special importance of nitrosoglutathione (GSNO). GSNO, formed by interaction of $^{\bullet}NO$ with GSH, represents a stable and mobile molecule and can therefore serve as both reservoir of $^{\bullet}NO$ bioactivity and $^{\bullet}NO$ donor (39-40). The important regulatory roles of GSNO are acknowledged by the recent findings of GSNO receptor, as well as of a special enzyme mediating its denitrosylation, known as S-nitrosoglutathione reductase (40).

On the other hand, the protein modifications by tyrosine nitration, mainly mediated by peroxynitrite, potentially result in alteration, loss or gain of function but their reversibility has not yet been demonstrated, and hence, a clear assignment to redox regulation cannot be made.

4.4. $^{\bullet}NO$-producing system

$^{\bullet}NO$ represents a simple diatomic gas that functions as a cell signaling molecule in mammalian cells, controlling vital functions such as neurotransmission, blood vessel tone, host defense, immunity (42) *etc*. It is synthesised in virtually all mammalian cells *via* L-arginine oxidation by a family of $^{\bullet}NO$ synthase (NOS) isoforms (43). Three distinct NOS isoforms have been identified so far: neuronal (nNOS or NOS I), endothelial (eNOS or NOS III), originally described in neuronal tissue and endothelial cells, respectively, and inducible NOS (iNOS or NOS II), originally identified in macrophages (42, 44). All characterised mammalian NOS isoforms are heme-containing proteins that are dimeric in native conditions with monomer molecular mass of about 126-160 kDa. Until recently, it has been considered that nNOS and eNOS are constitutively expressed, while iNOS is solely inducible form. However, extensive research in this field has undoubtedly revealed that all these NOS isoforms can be induced by different appropriate stimuli and can be expressed in various tissues and cells (42, 44). On the other hand, NOS isoforms display different affinity for calmodulin, whereas nNOS and eNOS are Ca^{2+}-calmodulin dependent, while iNOS is almost Ca^{2+}-calmodulin independent. iNOS forms a complex with calmodulin at very low concentrations of Ca^{2+} and due to that, its activity is not regulated by Ca^{2+}. Another distinction between the NOS isoforms has been related to their level of $^{\bullet}NO$ production, since iNOS was shown to produce higher $^{\bullet}NO$ levels (microM-mM) and to remain active for a longer time period comparing to nNOS and eNOS ($^{\bullet}NO$ concentrations from nanoM-microM). The NOS isoforms eNOS, nNOS or iNOS may be found attached to or within the mitochondria, in which case the $^{\bullet}NO$ synthase is referred to as fourth isoform, *i.e.* as mitochondrial NOS (mtNOS) (45-47). However, its full characterization is still in progress. Namely, the cross-reaction of mitochondria with antibodies to Ca^{2+}-sensitive eNOS was reported almost simultaneously by two research groups (48-50). In addition, $^{\bullet}NO$ production absent in nNOS-knockout mice was found to occur in individual mitochondria (51) and NOS isolated from mitochondria was shown to be identical in sequence to the main nNOS isoform, but covalently modified (52). A proportion of nNOS was subsequently observed to physically interact with cytochrome ***c*** oxidase (53).

$^{\bullet}NO$ exhibits its effects in different ways, firstly through the activation of soluble guanylate cyclase leading to an increase in intracellular cGMP level, or through the reaction with its common intracellular targets $O_2^{\bullet-}$, GSH and various heme proteins (42).

The effects of $^{\bullet}NO$ depend on its local concentration, which is in turn determined by the rate of its synthesis and cellular redox milieu. Thus, its signaling capacity must be controlled at the levels of biosynthesis and local availability. Accordingly, NOSs are tightly controlled enzymes being regulated at transcriptional and translational levels, through co- and post-translational modification, by substrate availability and subcellular compartmentation, which enable close proximity to the target proteins of $^{\bullet}NO$ (44, 54).

4.5. NOS isoforms in BAT

BAT is expressing two NOS isoforms - eNOS and iNOS (55), localised in the cytoplasm and nuclei of brown adipocytes (26, 56, 57). $^{\bullet}NO$ mediates increased blood flow in BAT following short noradrenergic stimulation (55, 58) and regulates lipid and glucose metabolism (5). Also, we have found that after chronic exposure to cold, $^{\bullet}NO$ participates in hyperplasia, proliferation and differentiation of brown adipocytes, as well as in UCP1 protein content increase, apoptosis (26), remodeling of capillaries (57) and mitochondriogenesis (19), and in GSH synthesis (59) in interscapular BAT (IBAT). In addition, $^{\bullet}NO$ regulates molecular basis of the IBAT thermogenic program, especially during the multiple

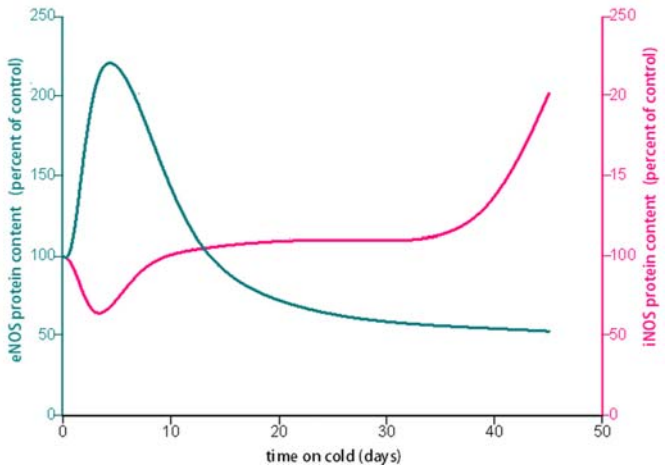

Figure 2. Time-course of eNOS and iNOS protein expression in IBAT during cold acclimation. Adapted with permission from Petrovic *et al* (23).

stages of cold acclimation (23). In that regard, we have shown that different time-dependent activations of eNOS and iNOS in brown adipocytes during cold acclimation translate into distinct •NO effects on the tissue thermogenic program (23).

4.6. Expression profiles of NOS isoforms in BAT during cold acclimation

During cold acclimation, we have observed different time-dependent induction of eNOS and iNOS protein expression (23). An increased eNOS protein expression was recorded early in the course of cold acclimation, whereas the iNOS protein content exceeded control level in the late phase of acclimation to cold (Figure 2). In addition, L-arginine prolonged and/or accelerated periods when both eNOS and iNOS protein expressions were higher than the control values, but both isoforms still have retained their characteristic time-dependent expression profiles. These different expression patterns during cold acclimation indicate subtle functional differences between these two NOS isoforms and their engagement in different phases of cold acclimation. The molecular basis of the early eNOS response and the late response of iNOS could be explained by their differential regulation. For example, in acute cold exposure, almost immediate •NO production is required for the increased blood flow in BAT (55), which could be achieved by the activation of "constitutively" expressed eNOS. Accordingly, our results showed that vascular endothelial growth factor (VEGF) immunopositivity and vascularisation increased in parallel with the eNOS expression from day 1 of L-arginine treatment, strongly suggesting eNOS involvement in cold-induced angiogenesis (23). These data are consistent with the previously shown key role of eNOS in capillary network remodeling (57). Also, the involvement of iNOS in the late phase of cold acclimation is suggested by the additional increase in protein content and IBAT mass after 45 days of L-arginine treatment, when iNOS protein expression increased. Generally, •NO-producing enzymes in IBAT during acclimation to cold are likely to be finely tuned, so that optimal timing of NOSs activation results in optimal production of •NO required to achieve an adequate response during cold exposure.

5. •NO IN BAT THERMOGENESIS

5.1. •NO and BAT hyperplasia

In animals exposed to cold, BAT undergoes hyperplasia that is fundamental for an increased tissue thermogenic capacity and survival in cold environment. BAT hyperplasia comprises proliferation and differentiation of brown adipocytes precursor cells and hypertrophy of mature brown adipocytes (11-13). At the molecular level, hyperplastic BAT recruitment is regulated by a variety of thermogenic factors, UCP1, PPARgamma, PGC-1alpha and proliferating cell nuclear antigen (PCNA), which are of a particular importance because of their role in increasing thermogenic capacity by intensifying uncoupling, fatty acid metabolism and differentiation, promoting oxidative metabolism and mitochondriogenesis, as well as cell proliferation (7, 16, 17, 60).

These thermogenesis-related molecules are in a complex mutual transcription network where PPARgamma is involved in cold-induced UCP1 transcriptional activation (17), while PGC-1alpha acts as a coactivator required for the interaction of PPARgamma with the UCP1 promoter (16). As stated above, UCP1 plays an indispensable role in thermogenesis, while a ligand-activated nuclear receptor family member - PPARgamma apart from its activation of UCP1 expression, plays important roles in BAT proliferative and differentiation programs (17, 61). In addition, BAT of transgenic mice expressing dominant-negative PPARgamma was found to display a reduced thermogenic function (62).

It has been observed by several others and our research group, that BAT hyperplasia represents a redox-sensitive process. Precisely, Saha *et al* (24) found that N^{omega}-nitro-L-arginine-methyl ester (L-NAME) solution administered as drinking liquid for 4-6 weeks decreased the

IBAT mass, while Nisoli *et al* (25) reported that •NO takes part in differentiation and proliferation in brown adipocyte culture. Also, Jobgen *et al* (63) have shown increase in BAT mass by L-arginine in diet-induced obese rats. Accordingly, we have shown that in rats acclimated to cold, L-arginine increased both IBAT mass and protein content, while L-NAME expressed an opposite effect (26). However, precise molecular mechanisms underlying •NO-stimulated BAT hyperplasia were elusive until recently. Nevertheless, our latest data is offering an explanation of this event. Namely, we have shown time-coordinated cold-induced transcriptional activation of UCP1, PGC-1alpha and PPARgamma, and also nuclear PCNA translocation that correlates well with induction of eNOS gene and protein expression, strongly suggesting the •NO involvement in the activation of thermogenic factors (23). Moreover, these results have shown that L-arginine accelerated and prolonged cold-induced UCP1, PPARgamma, PGC-1alpha and PCNA activation, while L-NAME expressed opposite effects. This is the first time that •NO-dependent activation of PCNA in IBAT during acclimation to cold has been demonstrated, and it is of the utmost importance for determination of the mechanisms underlying the previously shown improvements of cold-induced IBAT hyperplasia by •NO (26). In addition to their broad significance, our results contributed to the understanding of our previous finding on •NO-stimulated BAT hyperplasia, revealing promoting •NO effects on BAT hyperplasia through stimulation of UCP1, PPARgamma, PGC-1alpha and PCNA gene and protein expression. Namely, Nisoli *et al* (25) reported that •NO acted arresting cell growth and initiating cell differentiation program in brown adipocyte culture. However, our data clearly showed that •NO promotes both brown adipocyte differentiation and proliferation *in vivo*, acting as a stimulator of overall BAT hyperplasia process.

5.2. BAT uncoupling: role of •NO

"New story for the long evolutionary history", probably represents the best beginning for considering uncoupling processes and uncoupling proteins. This process, which is likely as old as aerobic life, attracted scientific attention during the 20th century (64) and underwent a strong expansion during the last 2-3 decades when UCP1 was purified (65) and characterised as a 32 kDa member of the mitochondrial carrier protein family. From historical aspect, physiological uncoupling in BAT mitochondria represents one of the first independent proofs of the Mitchel's hemiosmotic theory. Today, it is considered as the most essential bioenergetic regulator of mitochondria in all tissues under physiological and pathophysiological conditions (66).

In cold-acclimated animals UCP1 makes 14% of the total proteins of the inner mitochondrial membrane (67). Since the UCP1 plays an indispensable role in thermogenesis, the mechanisms of activation of its expression and regulation have been rigorously investigated and characterised. It has been pointed out that long-chain acyl-CoA, fatty acids and cytoplasmic pH activate UCP1, while purine nucleotides, *e.g.* GDP act as inhibitors (7). However, a full and generally accepted understanding of the proton transport control and the mechanism of this transport has not been reached. This opens a question could fatty acids as intact molecules or their derivatives act as allosteric regulators, cofactors, or proton shuttles during the UCP1 activation? On the other hand, during the past several years a growing body of evidence on the redox regulation of the UCP1 was rapidly accumulated. In fact, it is known that thermogenesis-induced uncoupling acts decreasing $O_2^{\bullet-}$ production (64, 68). Moreover, Skulachev (69) emphasised that in mitochondria UCPs-mediated uncoupling so-called "mild uncoupling" takes place in the basal state as well, aimed at attenuating mitochondrial proton motive force and at reducing $O_2^{\bullet-}$ production in respiratory chain. Some authors suggested that protection against $O_2^{\bullet-}$ represents the oldest function of mitochondrial uncoupling proteins (70). This theory is in agreement with our data demonstrating decreased activity of both manganese- and copper, zinc- superoxide dismutase (Mn- and CuZn-SOD, respectively) in BAT of animals acclimated to cold (26). This decrease was explained in terms of adaptive responses of enzyme activities to decreased $O_2^{\bullet-}$ production by uncoupling. Later on, we have shown also that cold generally decreased both Mn- and CuZn-SOD mRNA expression during the 45-day cold acclimation (71) suggesting that adaptive response in SODs activities on uncoupling-induced decrease in $O_2^{\bullet-}$ production was achieved already at the level of SOD's gene transcription.

Furthermore, relationship between UCP1 and $O_2^{\bullet-}$ is more complex and directed toward maintenance of redox equilibrium. Namely, Talbot *et al* (72) have shown that at a high mitochondrial ROS production, $O_2^{\bullet-}$ activates UCP1 and that resulting decrease of the membrane potential leads to decline of its production. On the contrary, inhibition of the UCP1 activity in brown adipocytes increases proton motive force and H_2O_2 production. Besides, activation of uncoupling was confirmed by inducing this process by lipid peroxidation products (73). Our data showing that L-arginine increases UCP1 protein content in BAT clearly confirmed that UCP1 is under redox control (23, 26). In addition, our very recent results revealed molecular mechanisms underlying this •NO effect, showing that •NO acted inducing UCP1 gene transcription and that this increase had a significant functional consequence considering that it was followed by an increased UCP1 protein level (23) and decreased protein content of mitochondrial MnSOD, as well as of total SOD activity (71).

5.2.1. Transcriptional control of UCP1 gene

Since UCP1 plays an essential role in thermogenesis, during recent years a considerable progress has been achieved in the recognition of the mechanisms controlling the UCP1 gene expression, particularly after acute cold exposure (74-76). UCP1 activation occurs within hours after cold exposure (6), when noradrenaline released from activated sympathetic nerve endings stimulates BAT production of cAMP, which binds to the cAMP response element binding protein (CREB) both in UCP1 promoter and enhancer and initiates its transcription as a part of the BAT thermogenic program (77). The UCP1 enhancer also

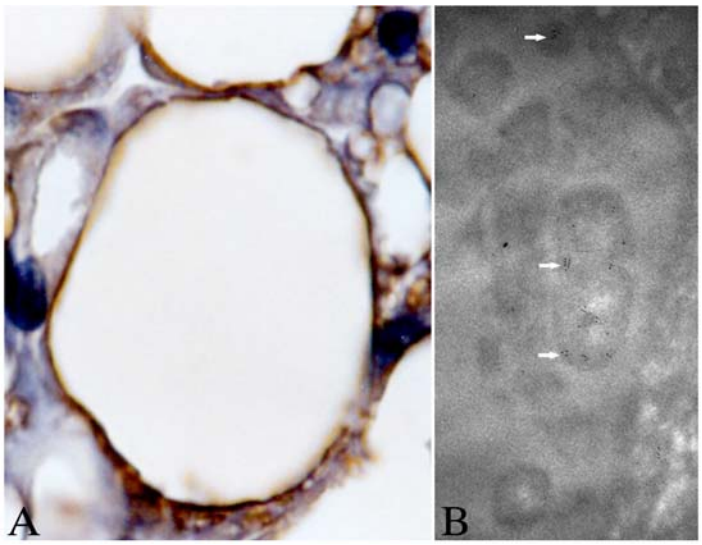

Figure 3. UCP1 expression in retroperitoneal WAT of L-arginine treated rats acclimated to cold. Immunohistochemistry (A) and immunogold (B) revealed UCP1-positivity (arrows) in white adipocytes mitochondria. Magnification: A-x100, orig.; B-x19500.

harbors several nuclear receptors, including PPARgamma, the retinoic X receptor (RXR), the retinoic acid receptor (RAR) and the thyroid receptor (TR) which must be coactivated by PGC-1alpha for efficient interaction with the UCP1 promoter (16, 78). In addition, recent results showed that $^{\bullet}$NO increased UCP1 transcription concomitantly with an increase of BAT noradrenaline supply and tissue sympathetic innervation (23). These data suggests that this $^{\bullet}$NO-induced increase of noradrenaline supply could be responsible for $^{\bullet}$NO-activated UCP1 transcription, considering well known fact that noradrenaline activates expression of numerous thermogenic factors including UCP1 by increasing BAT production of cAMP. However, the precise mechanisms underlying this L-arginine /$^{\bullet}$NO acting remain to be elucidated.

5.2.2. Whether UCP1 is uniquely expressed in brown adipocytes?

BAT has traditionally been considered to be absent and physiologically irrelevant in adult humans. Recently, this view has changed dramatically, when the presence of functional BAT in adult humans was demonstrated (79-81). In addition, it has been shown that brown-like multilocular adipocytes expressed UCP1 within human WAT, and that in intraperitoneal depot of human WAT, one *per* 100-200 adipocytes expressed UCP1 (82). Above authors suggested that WAT depots contained brown adipocytes and brown-like adipocytes, originating from brown adipocytes and myogenic progenitors, respectively (83, 84). Besides, these data also pointed to the existence of brown-like adipocytes appearing in WAT upon adrenergic stimulation, originating from cell lineage, closer to white adipocytes. Our results showed that L-arginine induces UCP1 protein expression in rat retroperitoneal WAT depot additionally to cold (to be published). Furthermore, we demonstrated the UCP1 presence in mitochondria by immunocytochemistry in retroperitoneal WAT (Figure 3). On the other hand, there have been other reports on the presence of UCP1 in tissues other that BAT. Namely, Adams *et al* (85) by using wide array of techniques demonstrated the presence of UCP1 in thymus mitochondria. Also, Nibbelink *et al* (86) found UCP1 in uterine longitudinal smooth muscle cells, while Mori *et al* (87) reported UCP1 expression in human skin - in the granular layer of epidermis, sweat glands, hair follicles and sebaceous glands. Nevertheless, Frontini *et al* (88) suggested that any detection of UCP1 in thymus is solely due to associated BAT, while Rouset *et al* (89) disproved that uterine smooth muscle cells express UCP1 demonstrating that the above finding was due to uncoupling protein 2. These informations require further examinations, but certainly shed a new light on UCP1 distribution and function, especially in humans.

5.3. $^{\bullet}$NO and mitochondria

Each organelle has individual responsibilities required for a life. As such, the mitochondria occupy a strategic position in the hierarchy of cellular organelles to either promote the healthy life of the cells, or to terminate it. Namely, mitochondria perform crucial functions necessary for homeostasis and are arbiters of cell death and survival. During the past decade, multiple regulatory effects of $^{\bullet}$NO on mitochondria functioning became evident. $^{\bullet}$NO stimulates mitochondrial biogenesis, regulates mitochondrial respiration, increases O_2 and substrate supply to mitochondria, stimulates reactive species production, and regulates mitochondria-dependent apoptosis and necrosis. Recent findings demonstrate that mitochondria may produce $^{\bullet}$NO and reveal that mitochondrial NOS-derived $^{\bullet}$NO and its reactive derivatives participate in regulation of the functions of mitochondria, cells and organs (90).

5.3.1. $^{\bullet}$NO as mitochondrial substrate supplier

$^{\bullet}$NO represents a potent vasodilator, expressing its action through an increase of intracellular cGMP (43). In this way, $^{\bullet}$NO increases O_2 and respiratory substrate supply

to mitochondria. Besides, by increasing tissue blood flow, •NO indirectly enhances transfer of heat produced in BAT throughout the body (55, 57).

5.3.2. •NO and mitochondrial biogenesis

Biogenesis of mitochondria is a hallmark of BAT thermogenic program. It is regulated by numerous factors, such as external stimuli, cold being the strongest one, then some hormones (primarily noradrenaline) and numerous autocrine factors, •NO representing one of the most fascinating among them. Nisoli *et al* (21) showed that •NO triggers mitochondrial biogenesis in brown adipocytes and that eNOS-deficient mice have reduced mitochondrial biogenesis associated with reduced energy expenditure and increased body weight. The same observation was based on the experiments with hepatic tissue and gastrocnemius muscle, as well as with brown adipocytes and 3T3-L1 cell cultures (91). Thus, it has been emphasised that •NO-induced mitochondrial biogenesis is a general phenomenon that occurs in cell cultures and animal tissues, most likely through the same cascade of signaling events. Precisely, it was shown that •NO induces mitochondrial biogenesis in a similar manner in three cell types of different species and lineages (human monocytic U937, rat L6 myoblasts and neurosecretory PC12 cells), through cGMP-dependent induction of PGC-1alpha, the principal regulator of mitochondrial biogenesis (21). PGC-1alpha activates an extensive program of mitochondrial biogenesis through coactivation of nuclear respiratory factor 1 and 2 which activate nuclear genes encoding mitochondrial proteins, as well as mitochondrial transcription factor A, essential for mitochondrial DNA replication. Besides, PGC-1alpha greatly increases the transcriptional activity of PPARgamma and thyroid hormone receptor on the UCP1 promoter (16). PGC-1alpha in this way promotes "oxidative phenotype in BAT", *i.e.* coordinates expression of genes involved in oxidative metabolism and mitochondriogenesis (16). Blockade of •NO production leads to a lack of PGC-1alpha induction, and results in an impaired thermogenesis followed by inability of animals to survive at low temperature (91). Our results demonstrating L-arginine -related intensification of cold-induced increase of mitochondria number (19) are in accordance with the above report. However, mitochondriogenesis is a complex process comprising not only an increase in the number of mitochondria but also an increase of their volume, then the UCP1 content increase and its incorporation into mitochondria, as well as increased number of cristae and their specific organisation, necessary for mitochondrial functional activation. Our recent data extended knowledge about effects of •NO on mitochondrial physiology showing that besides increasing the number of mitochondria in BAT, •NO acted as a stimulator of mitochondrial structure remodeling (19). Namely, in BAT of cold-acclimated animals, •NO increased cristae number, UCP1 protein content, mitochondria/cristae volume, and also induced ultrastructural mitochondrial remodeling (23). Thus, it seems likely that •NO influences all aspects of biogenesis of mitochondria acting as a unique molecular switch that triggers the entire mitochondriogenic process operating at gene, protein and structural levels. In addition, our results, apart from well characterised role of eNOS in mitochondriogenesis, implicate the involvement of •NO produced by iNOS isoform in the regulation of all above aspects of BAT mitochondriogenic program.

5.3.3. •NO, ROS production and mitochondrial respiration

It has been recognised that •NO increases the mitochondrial rates of $O_2^{\bullet-}$ and H_2O_2 production (92, 93). Generally, •NO triggers mitochondrial free radical production through different ways: (a) •NO at moderate levels inhibits mitochondrial respiration and actually increases $O_2^{\bullet-}$ and H_2O_2 production and (b) •NO in higher concentrations rapidly reacts with $O_2^{\bullet-}$ producing peroxynitrite that in turn irreversibly inhibits respiration and further increases ROS production (22). In a latter case, production of H_2O_2 is decreased, due to decreased availability of $O_2^{\bullet-}$ because of its interaction with •NO. In this manner •NO actually modifies mitochondrial redox-state and participates in the regulation of numerous redox-sensitive cellular pathways such as cell growth, differentiation, apoptosis and senescence (94).

Also, •NO regulates mitochondrial function by direct binding to COX, that leads to inhibition of mitochondrial respiration. This •NO-provoked inhibition might be rapid and reversible inhibition of COX, or slow, non-selective and irreversible inhibition of many mitochondrial components (22).

In a former case, •NO at nanomolar concentrations inhibits COX by binding to the cytochrome $\boldsymbol{a_3}$-Cu_B center of reduced COX, while dissociation of •NO from COX allows COX reactivation (95). So, at physiologically relevant concentrations of •NO and O_2, COX inhibition by •NO is reversible and competitive, in a manner representing a pharmacological competitive antagonism between •NO and O_2 (47). Thus, •NO is considered as a potential physiological regulator of respiration and •NO/COX system has been proposed to act as the acute oxygen sensing system in the cells (22).

In addition, •NO exerts its effects on other mitochondrial components, as well. So, •NO in the concentrations from 0.3.-0.5. microM acts inhibiting electron transfer between cytochromes $\boldsymbol{b}$ and $\boldsymbol{c_1}$; moreover, it has been shown that exposure of rat liver or skeletal muscle mitochondria to •NO markedly increased the production rate of $O_2^{\bullet-}$ and H_2O_2 (92). At •NO concentrations from 20-50 nanoM, most of mitochondrial •NO induces the formation of H_2O_2 (96), relying on the inhibition of COX and complex III. At higher •NO concentrations, •NO is reacting with derived $O_2^{\bullet-}$, and •NO utilisation is mainly driven to the evolvement of peroxynitrite, representing a powerful oxidant which inhibits mitochondrial complexes I (97, 98), II (98, 99), III (99) and IV (100) *via* nitrosylation or nitration leading to reduced oxygen uptake.

However, in highly specialised tissue such as BAT, effects of •NO are strongly dependent on tissue functional state, that determines the rate of uncoupling,

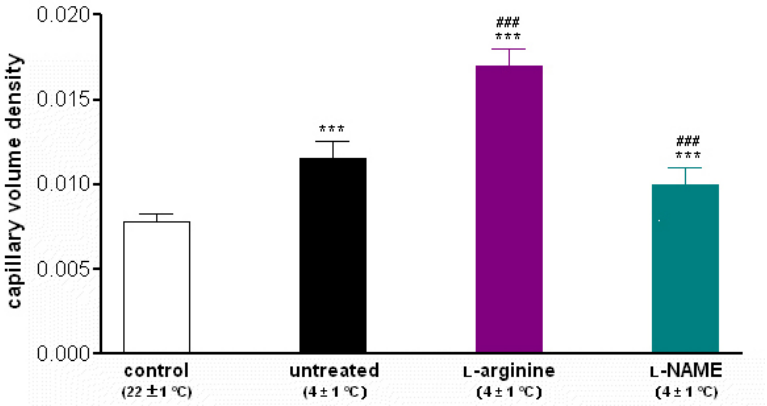

Figure 4. The capillary volume density in IBAT of rats acclimated to room temperature (control) and acclimated to cold (untreated, L-arginine - or L-NAME-treated). * comparison with the control, *** p<0.0.01; # comparison of L-arginine - or L-NAME-treated group with untreated group, ### p<0.0.01. Adapted with permission from Korac *et al* (57).

production of reactive species, cellular redox state and finally, the rate of $^{\bullet}$NO production. BAT functional state at low temperature is characterised by a high rate of uncoupling, known to act reducing $O_2^{\bullet-}$ production (66, 69). Also, it has been shown that $^{\bullet}$NO acts intensifying BAT uncoupling (23, 26), practically meaning that $^{\bullet}$NO in addition to cold decreases $O_2^{\bullet-}$ production. On the other hand, apart from well known increase in UCP1 protein content, changes in protein content of mitochondrial respiratory chain components during acclimation to cold, have not been studied in detail. Namely, only a few studies demonstrated that prolonged cold acclimation lead to an increased protein/mRNA expression of complexes I, III (101), and COX (16). However, subtle changes in their expression occurring during different time-periods of cold acclimation are still unknown. Our recent results (to be published) clearly showed cold-induced time-dependent increase in protein levels of respiratory chain components - complex I, cytochrome ***c*** and COX. Furthermore, these results also unravel stimulatory effect of L-arginine /$^{\bullet}$NO producing pathway on the expression profiles of those components of mitochondrial electron transport chain. Taken together, these data extended our knowledge about $^{\bullet}$NO effects on BAT mitochondria demonstrating for the first time stimulatory effects of $^{\bullet}$NO on mitochondrial molecular status in thermogenically active tissue.

5.4. $^{\bullet}$NO and BAT capillary network

Thermogenic activation of BAT is followed by a strong angiogenesis and increase in tissue blood flow, aimed at both to supply BAT with thermogenic substrates and to transfer produced heat throughout the body. Blood flow in BAT depends on the tissue thermogenic state, it is angiogenically dependent, and numerous autocrine, paracrine, and endocrine factors are included in its regulation. During cold exposure, blood flow in BAT increases, presumably because of the increased circulating noradrenaline levels (58). It has been shown that $^{\bullet}$NO, endogenously produced in brown adipocytes, can directly modulate not only BAT thermogenic capacity, but also BAT blood flow (9, 58, 102, 103). It was found that L-NAME treatment acted preventing cold-induced increase of blood flow in rat BAT (58), while reducing the local IBAT temperature both *in vivo* and *in vitro* (24, 104). In addition, Kikuchi-Utsumi *et al* (55) showed that sympathetic nerves, *i.e.* noradrenaline, mediate vasodilatation through the stimulation of $^{\bullet}$NO production, resulting in an increase of BAT blood flow, which is completely abolished with L-NAME treatment.

On the other side, it has been observed that noradrenaline stimulates the VEGF expression and secretion in brown adipocytes cultures, and that VEGF expression in BAT during cold exposure is activated *via* adrenergic mechanisms (105-107). VEGF is one of the most important angiogenic factors, the expression of which is enhanced during angiogenesis in various physiological and pathological processes (108). It is a specific mitogen for vascular endothelial cells *in vitro* and induces angiogenesis *in vivo* (108). Inhibition of *in vivo* $^{\bullet}$NO production results in inhibition of VEGF-induced angiogenesis and vascular permeability, but underlying mechanisms are still elusive. Some authors single out eNOS as the main NOS isoform that mediates VEGF-induced angiogenesis (55, 103).

Our results are in agreement with the studies reported so far, which indicate that $^{\bullet}$NO, in addition to modulating various functions in BAT, also plays a role in angiogenesis. Precisely, we demonstrated that L-arginine treatment acted increasing capillary volume density and capillary count per brown adipocyte, while L-NAME reduces them, either in thermogenically active or inactive tissue (57) (Figure 4). We have also proposed that $^{\bullet}$NO mediates its vasostimulatory effect by interacting with other component of the redox system, primarily $O_2^{\bullet-}$ and that interaction between these reactive species and adaptive regulation of CuZnSOD play the roles in the regulation of BAT angiogenic response (57). In addition, our very recent data extended knowledge on $^{\bullet}$NO role in BAT angiogenesis (23). Namely, opposite to rapid and transient VEGF mRNA induction in BAT after cold exposure/adrenergic stimulation (109), it has been shown that, different from the other tissues, including WAT, low oxygen levels (hypoxia)

do not contribute to cold-induced VEGF expression in BAT *in vivo* (110). Our recent results offer the potential to unlock the regulation of the VEGF signaling pathway in BAT during cold acclimation. Namely, we have revealed that expression of VEGF is under redox regulation, *i.e.* that •NO stimulates both mRNA and protein expression of VEGF (23). Moreover, such •NO-induced molecular basis of BAT vascularisation had a functional consequence, *i.e.* it was translated into increased tissue blood flow and capillary network remodeling (23).

6. BAT REGRESSION: IMPLICATION OF •NO-MEDIATED APOPTOSIS

BAT is an extremely dynamic tissue, characterised by a high degree of plasticity and remodeling. Remodeling of BAT consists of two opposing processes - tissue hyperplasia and regression, which serve to ensure new homeostasis in different tissue metabolic states. Tissue hyperplasia is directly related to thermogenic state and it is presumably induced by thermogenic requirements (26, 111, 112). Cessation of thermogenic stimulus induces BAT regression that rapidly reduces the thermogenic capacity and transforms hyperplastic into a proliferatively resting tissue, in which the decrease in brown adipocyte size and number are accompanied by a decrease in IBAT mass and reductions of DNA, protein, and mitochondrial content (113, 114).

A variety of intracellular and extracellular signals are integrated in the processes that provide BAT remodeling. In that regard, apoptotic process plays a significant role in tissue turnover (14, 115). Namely, it has been shown that cold rapidly decreased the rate of BAT apoptosis, and this decrease was taken as an adaptive change that contributes to cell survival during tissue hyperplasia and enhancement of tissue thermogenic capacity. The above authors emphasized that with respect to the antiapoptotic role of cold in BAT, noradrenaline acting and increased uncoupling take place. Accordingly, during BAT regression, termination of cold stimuli followed by cessation of noradrenaline action and decreased uncoupling, were accompanied by a conspicuous increase in the rate of apoptosis. In other words, during transition from thermogenically active to inactive state, BAT is losing cells (undergoes atrophy) by apoptosis (14, 115). We have recently observed that GSH and reactive species, primarily •NO play important roles as regulatory mediators in BAT apoptosis (26, 116).

In the first case, it has been shown that GSH depletion triggers common pathways of apoptosis induced by physiological stimuli, such as BAT reacclimation, and initiates tissue regression (117). Moreover, our earlier results demonstrated that GSH amount is significantly increased during cold acclimation, strongly suggesting involvement of GSH in the regulation of essential hyperplasic processes in BAT (112, 113). Also, it has been observed that •NO induces IBAT glutathione synthesis *in vivo* through the activation of glutamate-cysteine ligase mRNA and protein expression (59). In addition, we have revealed that during reacclimation such GSH depletion is synchronized with increase in both iNOS and heme oxygenase 1 (HO-1) expression, indicating their participation in the regulation of brown adipocytes apoptosis. Time dependent increase of iNOS and HO-1 expression followed by increased apoptosis during the process of reacclimation strongly support the implication of •NO and carbon monoxide producing system in IBAT cell death (118).

Hence, it seems likely that production of •NO in IBAT is regulated in that way that at low temperature •NO is produced in physiological concentrations which play regulatory roles in IBAT hyperplasia (induction of UCP1 and tissue proliferation). In contrast, high •NO production in unstimulated tissues acts to induce apoptosis and can be unfavorable.

7. COMMENTS AND PERSPECTIVES

At present, it is quite obvious that besides fundamental examinations, studies on the central role of BAT in the maintenance of energy homeostasis are of utmost significance for the understanding of pathophysiological conditions characterized by disturbed energy homeostasis, from obesity and metabolic syndrome to diabetes and cancer. This was the reason for attempting to functionally integrate numerous elements involved in the regulatory system controlling BAT remodeling. Because of that, this review was aimed not only at providing a survey of already known facts and integrating different aspects of redox regulation in BAT, but also at emphasising and opening new fields for the research of redox regulation in cell physiology.

This opens a new space to search for the responses to the following questions. Which roles BAT play in humans? Whether the UCP1 in other cell types but brown adipocytes has some other functions? To what extent nutrition could improve mitochondrial functioning, influence angiogenesis, or decide on life and death of brown adipocytes? Which other L-arginine potentials could be involved in redox-dependent control of energy homeostasis.

On the other hand, current understanding of energy homeostasis does not change in essence and it is based on the equilibrium between the intake and consumption of calories. If we decide to place BAT into a focus and direct our attention to the potential of its remodeling, two research approaches would be available: pharmacological and non-pharmacological one. The former is employing potent molecular tools directed not only toward induction of BAT function, but also toward increase of its depots within the body and prevention of its involution during ageing. Even approaches in molecular biology are leading to transgenic UCP1 expression. The goal of consuming the excess is clear. The latter, more moderate approach insists on the change of life style and diet. Incorporated in this approach, L-arginine (•NO) and redox regulation occupy a very significant position for the studies in the years to come. It is evident that redox regulation in brown adipocytes represents an unavoidable,

new tool for the studies of fundamental cellular processes integrated in BAT, the tissue with a high remodeling potential.

Probably the most important questions requiring the answers remain to be mentioned in the end. What are the chances and possibilities to take into account the prevailing life style where the words eating, sitting down, watching TV, dressing warmly are dominant? Is there a space for intervening with this rapid evolution change of the life style? However, we have to be aware that even for attempts of changing the life style we must understand better key molecular events and cellular processes situated in the very core of bioenergetics with BAT as a leading conductor of a biological orchestra.

DaVincian credo "*Nulla dies sine experimento*" was a vital motto of the late Prof. Jean Giaja (1884-1957), the founder of the Belgrade School of Physiology exactly 100 years ago. The present work, dedicated to this distinguished scientist and this significant event, represents a continuation of researches in thermoregulation and bioenergetics.

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9. REFERENCES

1. VP Reutov: Nitric oxide cycle in mammals and the cyclicity principle. *Biochemistry (Moscow)* 67, 293-311 (2002)

2. M Duranti, P Cerletti: Amino acid composition of seed proteins of Lupinus albus. *J Agric Food Chem* 27, 977-978 (1979)

3. G Wu, WF Bazer, TA Davis, WS Kim, P Li, J Marc Rhoads, M Carey Satterfield, SB Smith, TE Spencer, Y Yin: Arginine metabolism and nutrition in growth, health and disease. *Amino Acids* 37, 153-168 (2009)

4. G Wu, MS Morris Jr: Arginine metabolism: nitric oxide and beyond. *Biochem J* 336, 1-17 (1998)

5. WS Jobgen, SK Fried, WJ Fu, CJ Meininger, G Wu: Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. *J Nutr Biochem* 17, 571-588 (2006)

6. B Cannon, J Nedergaard: Brown adipose tissue: function and physiological significance. *Physiol Rev* 84, 277-359 (2004)

7. DG Nicholls, RM Locke: Thermogenic mechanisms in brown fat. *Physiol Rev* 64, 1-64 (1984)

8. S Cinti: The adipose organ. *Prostaglandins Leukot Essent Fatty Acids* 73, 9-15 (2005)

9. DO Foster, ML Frydman: Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can J Physiol Pharmacol* 57, 257-270 (1979)

10. NJ Rothwell, MJ Stock: A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31-35 (1979)

11. J Himms-Hagen: Brown adipose tissue thermogenesis: Interdisciplinary studies. *FASEB J* 4, 2890-2898 (1990)

12. LJ Bukowiecki, A Géloën, AJ Collet: Proliferation and differentiation of brown adipocytes from interstitial cells during cold-acclimation. *Am J Physiol* 250, 880-887 (1986)

13. ER Suter: The fine structure of brown adipose tissue I. Cold-induced changes in the rat. *J Ultrastruct Res* 26, 216-241 (1969)

14. MJ Lindquist, S Rehnmark: Ambient temperature regulation of apoptosis in brown adipose tissue. *J Biol Chem* 273, 30147-30156 (1998)

15. P Trayhurn: Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiol Scand* 184, 285-293 (2005)

16. P Puigserver, Z Wu, CW Park, R Graves, M Wright, BM Spiegelman: A cold inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92, 829-839 (1998)

17. J Nedergaard, N Petrovic, EM Lindgren, A Jacobsson, B Cannon: PPARgamma in the control of brown adipocyte differentiation. *Biochim Biophys Acta* 1740, 293-304 (2005)

18. M Liesa, M Palacin, A Zorzano: Mitochondrial dynamics in mammalian health and disease. *Physiol Rev* 89, 799-845 (2009)

19. V Petrovic, A Korac, B Buzadzic, A Vasilijevic, A Jankovic, K Micunovic, B Korac: Nitric oxide regulates remodeling in interscapular brown adipose tissue: ultrastructural and morphometric-stereologic studies. *J Microsc* 232, 542-548 (2008)

20. JC Roberts, RE Smith: Time-dependent responses of brown fat in cold-exposed rats. *Am J Physiol* 212, 519-525 (1967)

21. E Nisoli, E Clementi, C Paolucci, V Cozzi, C Tonello, C Sciorati, R Bracale, A Valerio, M Francolini, S Moncada, MO Carruba: Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 299, 896-899 (2003)

22. GC Brown: Nitric oxide and mitochondria. *Front Biosci* 12, 1024-1033 (2007)

23. V Petrovic, A Korac, B Buzadzic, A Vasilijevic, A Jankovic, B Korac: NO modulates the molecular basis of rat interscapular brown adipose tissue thermogenesis. *Comp Biochem Physiol C* 152, 147-159 (2010)

24. SK Saha, H Ohinata, A Kuroshima: Effects of acute and chronic inhibition of nitric oxide synthase on brown adipose tissue thermogenesis. *Jpn J Physiol* 46, 375-382 (1996)

25. E Nisoli, E Clementi, C Tonelo, C Sciorate, L Briscini, MO Carruba: Effects of nitric oxide on proliferation and differentiation of rat brown adipocytes in primary cultures. *Br J Pharmacol* 125, 888-894 (1998)

26. V Petrovic, A Korac, B Buzadzic, B Korac: The effects of L-arginine and L-NAME supplementation on redox-regulation and thermogenesis in interscapular brown adipose tissue. *J Exp Biol* 208, 4263-4271 (2005)

27. CK Mittal, F Murad: Activation of guanylate cyclase by superoxide dismutase and hydroxyl radical: a physiological regulator of guanosine 3',5'-monophosphate formation. *Proc Natl Acad Sci U S A* 74, 4360-4364 (1977)

28. MM Gallogly, JJ Mieyal: Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress. *Curr Opin Pharmacol* 7, 381-391 (2007)

29. E Niki: Lipid peroxidation: physiological levels and dual physiological effects. *Free Radic Biol Med* 47, 469-484 (2009)

30. KS Echtay, TC Esteves, JL Pakay, MB Jekabsons, AJ Lambert, M Portero-Otin, R Pamplona, AJ Vidal-Puig, S Wang, SJ Roebuck, MD Brand: A signalling role for 4-hydoxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J* 22, 4103-4110 (2003)

31. JM Hansen, WH Watson, DP Jones: Compartmentation of Nrf-2 redox control: regulation of cytoplasmic activation by glutathione and DNA binding by thioredoxin-1. *Toxicol Sci* 82, 308-317 (2004)

32. IM Grumbach, W Chen, SA Mertens, DG Harrison: A negative feedback mechanism involving nitric oxide and nuclear factor kappa-B modulates endothelial nitric oxide synthase transcription. *J Mol Cell Cardiol* 39, 595-603 (2005)

33. DI Brown, KK Griendling: Nox proteins in signal transduction. *Free Radic Biol Med* 47, 1239-1253 (2009)

34. B Korac, B Buzadzic: Oxidative stress and antioxidative protection in skin. *Yugoslov Med Biohem* 19, 363-379 (2000)

35. JR Stone, MA Marletta: Spectral and kinetic studies on the activation of solubile guanylate cyclase by nitric oxide. *Biochemistry* 35, 1093-1099 (1996)

36. MW Cleeter, JM Cooper, VM Darley-Usmar, S Moncada, AH Schapira: Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implication for neurodegenerative diseases. *FEBS Lett* 345, 50-54 (1994)

37. JB Mannick, CM Schonhoff: Nitrosylation: the next phosphorylation. *Arch Biochem Biophys* 408, 1-6 (2002)

38. J Abat, P Saigal, R Deswal: S-nitrosylation-another biological switch like phosphorylation? *Physiol Mol Biol Plants* 14, 119-130 (2008)

39. JS Stamler, DI Simon, JA Osborne, ME Mullins, O Jaraki, T Michel, DJ Singel, J Loscalzo: S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc Natl Acad Sci U S A* 89, 444-448 (1992)

40. C Lindermayr, G Saalbach, J Durner: Proteomic identification of S-nitrosylated proteins in Arabidopsis. *Plant Physiol* 137, 921-930 (2005)

41. L Liu, A Hausladen, M Zeng, L Que, J Heitman, JS Stamler: A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. *Nature* 410, 490-494 (2001)

42. S Moncada, RM Palmer, EA Higgs: Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43, 109-142 (1991)

43. S Moncada, RM Palmer, EA Higgs: The biological significance of nitric oxide formation from L-arginine. *Biochem Soc Trans* 17, 642-644 (1989)

44. WK Alderton, CE Cooper, RG Knowels: Nitric oxide synthases: structure, function and inhibition. *Biochem J* 357, 593-615 (2001)

45. P Ghafourifar, C Richter. Nitric oxide in mitochondria: Formation and consequences. In: From Symbiosis to Eukaryotism-Endocytobiology VII. Eds: E Wagner, J Normann, H Greppin, JHP Hackstein, RG Herrmann, KV Kowallik, HEA Schenk, J Seckbach, University of Geneva (1999)

46. C Giulivi, JJ Poderoso, A Boveris: Production of nitric oxide by mitochondria. *J Biol Chem* 273, 11038-11043 (1998)

47. P Ghafourifar, E Cadenas: Mitochondrial nitric oxide synthase. *Trends Pharmacol Sci* 26, 190-195 (2005)

48. L Kobzik, B Stringer, JL Balligand, MB Reid, JS Stamler: Endothelial type nitric oxide synthase in skeletal muscle fibers: mitochondrial relationships. *Biochem Biophys Res Commun* 211, 375-381 (1995)

49. TE Bates, A Loesch, G Burnstock, JB Clark: Immunocytochemical evidence for a mitochondrially located nitric oxide synthase in brain and liver. *Biochem Biophys Res Commun* 213, 896-900 (1995)

50. TE Bates, A Loesch, G Burnstock, JB Clark: Mitochondrial nitric oxide synthase: A ubiquitous regulator of oxidative phosphorylation? *Biochem Biophys Res Commun* 218, 40-44 (1996)

51. AJ Kanai, LL Pearce, PR Clemens, LA Birder, MM VanBibber, SY Choi, WC de Groat, J Peterson: Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. *Proc Natl Acad Sci U S A* 98, 14126-14131 (2001)

52. V Haynes, S Elfering, N Traaseth, C Giulivi: Mitochondrial nitric-oxide synthase: enzyme expression, characterization, and regulation. *J Bioenerg Biomembr* 36, 341-346 (2004)

53. T Persichini, V Mazzone, F Polticelli, S Moreno, G Venturini, E Clementi, M Colasanti: Mitochondrial type I nitric oxide synthase physically interacts with cytochrome c oxidase. *Neurosci Lett* 384, 254-259 (2005)

54. R Govers, S Oess: To NO or not to NO: 'where?' is the question. *Histol Histopathol* 19, 585-605 (2004)

55. K Kikuchi-Utsumi, B Gao, H Ohinata, M Hashimoto, N Yamamoto, A Kuroshima: Enhanced gene expression of endothelial nitric oxide synthase in brown adipose tissue during cold-exposure. *Am J Physiol* 282, 623-626 (2002)

56. A Giordano, C Tonello, A Bulbarelli, V Cozi, S Cinti, MO Carruba, E Nisoli: Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus. *FEBS Lett* 514, 135-140 (2002)

57. A Korac, B Buzadzic, V Petrovic, A Vasilijevic, A Jankovic, K Micunović, B Korac: The role of nitric oxide in remodeling of capillary network in rat interscapular brown adipose tissue after long-term cold-acclimation. *Histol Histopathol* 23, 441-450 (2008)

58. T Nagashima, H Ohinata, A Kuroshima: Involvement of nitric oxide in noradrenaline induced increase in blood flow through brown adipose tissue. *Life Sci* 54: 17-25 (1994)

59. V Petrovic, B Buzadzic, A Korac, A Vasilijevic, A Jankovic, B Korac: L-arginine supplementation induces glutathione synthesis in interscapular brown adipose tissue through activation of glutamate-cysteine ligase expression: the role of nitric oxide. *Chem Biol Interact* 182, 204-212 (2009)

60. LS Cox: Who binds wins: competition for PCNA rings out cell-cycle changes. *Trends Cell Biol* 7, 493-498 (1997)

61. ED Rosen, BM Spiegelman: Peroxisome proliferator-activated receptor gamma ligands and atherosclerosis: ending the heartache. *J Clin Invest* 106, 629-631 (2000)

62. SL Gray, E Dalla Nora, EC Backlund, M Manieri, S Virtue, RC Noland, S O'Rahilly, RN Cortright, S Cinti, B Cannon, A Vidal-Puig: Decreased brown adipocyte recruitment and thermogenic capacity in mice with impaired peroxisome proliferator-activated receptor (P465L PPARgamma) function. *Endocrinology* 147, 5708-5714 (2006)

63. W Jobgen, CJ Meininger, SC Jobgen, P Li, MJ Lee, SB Smith, TE Spencer, SK Fried, G Wu: Dietary L-arginine supplementation reduces white fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. *J Nutr* 139, 230-237 (2009)

64. A Boveris, N Oshino, B Chance: The cellular production of hydrogen peroxyde. *Biochem J* 28, 617-630 (1972)

65. CS Lin, M Klingenberg: Characteristics of the isolated purine nucleotide binding protein from brown fat mitochondria. *Biochemistry* 21, 2950-2956 (1982)

66. VP Skulachev: Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. *FEBS Lett* 294, 158-162 (1991)

67. DG Nicholls, E Rial: A history of the first uncoupling protein, UCP1. *J Bioenerg Biomembr* 31, 399-406 (1999)

68. M Cino, RF Del Maestro: Generation of hydrogen peroxide by brain mitochondria: the effect of reoxygenation following postdecapitative ischemia. *Arch Biochem Biophys* 269, 623-638 (1989)

69. VP Skulachev: Decrease in the intracellular concentration of O2 as a special function of cellular respiratory system. *Biokhimiia* 59, 1910-1912 (1994)

70. P Jezek: Possible physiological roles of mitochondrial uncoupling proteins-UCPn. *Int J Biochem Cell Biol* 34, 1190-1206 (2002)

71. V Petrovic, B Buzadzic, A Korac, B Korac: Antioxidative defense and mitochondrial thermogenic response in brown adipose tissue. *Genes Nutr* 5, 225-235 (2010)

72. DA Talbot, AJ Lambert, MD Brand: Production of endogenous matrix superoxide from mitochondrial complex I leads to activation of uncoupling protein 3. *FEBS Lett* 556, 111-115 (2004)

73. KS Echtay, E Winkler, M Klingenberg: Coenzyme Q is an obligatory cofactor for uncoupling protein function. *Nature* 408, 609-613 (2000)

74. D Ricquier, F Bouillaud, P Toumelin, G Mory, R Bazin, J Arch, L Pénicaud: Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. Evidence for a rapid beta-adrenoreceptor-mediated and transcriptionally regulated step during activation of thermogenesis. *J Biol Chem* 261, 13905-13910 (1986)

75. A Jacobsson, B Cannon, J Nedergaard: Physiological activation of brown adipose tissue destabilizes thermogenin mRNA. *FEBS Lett* 224, 353-356 (1987)

76. S Enerbäck, A Jacobsson, EM Simpson, C Guerra, H Yamashita, ME Harper, LP Kozak: Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90-94 (1997)

77. JE Silva, R Rabelo: Regulation of the uncoupling protein gene expression. *Eur J Endocrinol* 136, 251-264 (1997)

78. IB Sears, MA MacGinnitie, LG Kovacs, RA Graves: Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. *Mol Cell Biol* 16, 3410-3419 (1996)

79. MC Zingaretti, F Crosta, A Vitali, M Guerrieri, A Frontini, B Cannon, J Nedergaard, S Cinti: The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J* 23, 3113-3120 (2009)

80. M Saito, Y Okamatsu-Ogura, M Matsushita, K Watanabe, T Yoneshiro, J Nio-Kobayashi, T Iwanaga, M Miyagawa, T Kameya, K Nakada, Y Kawai, M Tsujisaki: High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526-1531 (2009)

81. KA Virtanen, ME Lidell, J Orava, M Heglind, R Westergren, T Niemi, M Taittonen, J Laine, NJ Savisto, S Enerbäck, P Nuutila: Functional brown adipose tissue in healthy adults. *N Engl J Med* 360, 1518-1525 (2009)

82. H Oberkofler, G Dallinger, YM Liu, E Hell, F Krempler, W Patsch: Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans. *J Lipid Res* 38, 2125-2133 (1997)

83. P Seale, B Bjork, W Yang, S Kajimura, S Chin, S Kuang, A Scimè, S Devarakonda, HM Conroe, H Erdjument-Bromage, P Tempst, MA Rudnicki, CR Beier, BM Spiegelman: PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961-977 (2008)

84. JA Timmons, K Wennmalm, O Larsson, TB Walden, T Lassmann, N Petrovic, DL Hamilton, RE Gimeno, C Wahlestedt, K Baar, J Nedergaard, B Cannon: Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci U S A* 104, 4401-4406 (2007)

85. AE Adams, AM Carroll, PG Fallon, RK Porter: Mitochondrial uncoupling protein 1 expression in thymocytes. *Biochim Biophys Acta* 1777, 772-776 (2008)

86. M Nibbelink, K Moulin, E Arnaud, C Duval, L Penicaud, L Casteilla: Brown fat UCP1 is specifically expressed in uterine longitudinal smooth muscle cells. *J Biol Chem* 276, 47291-47295 (2001)

87. S Mori, N Yoshizuka, M Takizawa, Y Takema, T Murase, I Tokimitsu, M Sato: Expression of uncoupling proteins in human skin and skin-derived cells. *J Invest Dermatol* 128, 1894-1900 (2008)

88. A Frontini, S Rousset, AM Cassard-Doulcier, C Zingaretti, D Ricquier, S Cinti: Thymus uncoupling protein 1 is exclusive to typical brown adipocytes and is not found in thymocytes. *J Histochem Cytochem* 55, 183-189 (2007)

89. S Rousset, MC Alves-Guerra, S Ouadghiri-Bencherif, LP Kozak, B Miroux, D Richard, F Bouillaud, D Ricquier, AM Cassard-Doulcier: Uncoupling protein 2, but not uncoupling protein 1, is expressed in the female mouse reproductive tract. *J Biol Chem* 278, 45843-45847 (2003)

90. P Ghafourifar, CK Sen: Mitochondrial nitric oxide synthase. *Front Biosci* 12, 1072-1078 (2007)

91. E Nisoli, E Clementi, S Moncada, MO Carruba: Mitochondrial biogenesis as cellular signaling framework. *Biochem Pharmacol* 67, 1-15 (2004)

92. JJ Poderoso, JG Peralta, CL Lisdero, MC Carreras, M Radisic, F Schöpfer, E Cadenas, A Boveris: Nitric oxide regulates oxygen uptake and hydrogen peroxide release by the isolated beating rat heart. *Am J Physiol* 274, 112-119 (1998)

93. V Borutaite, GC Brown: Nitric oxide induces apoptosis via hydrogen peroxide, but necrosis via energy and thiol depletion. *Free Radic Biol Med* 35, 1457-1468 (2003)

94. MC Carreras, JJ Poderoso: Mitochondrial nitric oxide in the signaling of cell integrated responses. *Am J Physiol* 292, 1569-1580 (2007)

95. RS Blackmore, C Greenwood, QH Gibson: Studies of the primary oxygen intermediate in the reaction of fully reduced cytochrome oxidase. *J Biol Chem* 266, 19245-19249 (1991)

96. JJ Poderoso, C Lisdero, F Schöpfer, N Riobó, MC Carreras, E Cadenas, A Boveris: The regulation of mitochondrial oxygen uptake by redox reactions involving nitric oxide and ubiquinol. *J Biol Chem* 274, 37709-37716 (1999)

97. R Radi, M Rodriguez, L Castro, R Telleri: Inhibition of mitochondrial electron transport by peroxynitrite. *Arch Biochem Biophys* 308, 89-95 (1994)

98. I Lizasoain, MA Moro, RG Knowles, V Darley-Usmar, S Moncada: Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem J* 314, 877-880 (1996)

99. JP Bolanos, SJ Heales, JM Land, JB Clark: Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurons and astrocytes in primary culture. *J Neurochem* 64, 1965-1972 (1995)

100. MA Sharpe, CE Cooper: Interaction of peroxynitrite with mitochondrial cytochrome oxidase. Catalytic production of nitric oxide and irreversible inhibition of enzyme activity. *J Biol Chem* 273, 30961-30972 (1998)

101. R Navet, G Mathy, P Douette, RL Dobson, P Leprince, E De Pauw, C Sluse-Goffart, FE Sluse: Mitoproteome plasticity of rat brown adipocytes in response to cold acclimation. *J Proteome Res* 6, 25-33 (2007)

102. SW Ma, DO Foster: Redox state of brown adipose tissue as a possible determinant of its blood flow. *Can J Physiol Pharmacol* 62, 949-956 (1984)

103. D Fukumura, T Gohongi, A Kadambi, Y Izumi, J Ang, CO Yun, DG Buerk, PL Huang, RK Jain: Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proc Natl Acad Sci U S A* 98, 2604-2609 (2001)

104. SK Saha, T Ohno, H Ohinata, A Kuroshima: Effects of nitric oxide synthase inhibition on phospholipid fatty acid composition of brown adipose tissue. *Jpn J Physiol* 47, 477-480 (1997)

105. JM Fredriksson, JM Lindquist, GE Bronnikov, J Nedergaard: Norepinephrine induces vascular endothelial growth factor gene expression in brown adipocytes through a beta - adrenoreceptor/cAMP/protein kinase A pathway involving Src but independently of Erk1/2. *J Biol Chem* 275, 13802-13811 (2000)

106. A Asano, K Kimura, M Saito: Cold-induced mRNA expression of angiogenic factors in rat brown adipose tissue. *J Vet Med Sci* 61, 403-409 (1999)

107. A Asano, Y Irie, M Saito: Isoform-specific regulation of vascular endothelial growth factor (VEGF) family mRNA expression in cultured mouse brown adipocytes. *Mol Cell Endocrinol* 174, 71-76 (2001)

108. KA Thomas: Vascular endothelial growth factor, a potent and selective angiogenic agent. *J Biol Chem* 271, 603-606 (1996)

109. A Asano, M Morimatsu, H Nikami, T Yoshida, M Saito: Adrenergic activation of vascular endothelial growth factor mRNA expression in rat brown adipose tissue: implication in cold-induced angiogenesis. *Biochem J* 328, 179-183 (1997)

110. JM Fredriksson, H Nikami, J Nedergaard: Cold-induced expression of the VEGF gene in brown adipose tissue is independent of thermogenic oxygen consumption. *FEBS Lett* 579, 5680-5684 (2005)

111. B Buzadzic, M Spasic, ZS Saicic, R Radojicic, VM Petrovic, B Halliwell: Antioxidant defenses in the ground squirrel Citellus citellus. 2. The effect of hibernation. *Free Radic Biol Med* 9, 407-413 (1990)

112. V Petrovic, B Buzadzic, A Korac, A Vasilijevic, A Jankovic, B Korac: Free radical equilibrium in interscapular brown adipose tissue: relationship between metabolic profile and antioxidative defense. *Comp Biochem Physiol C* 142, 60-65 (2006)

113. B Buzadzic, B Korac, VM Petrovic: The effect of adaptation to cold and readaptation to room temperature on the level of glutathione in rat tissues. *J Thermal Biol* 24, 373-377 (1999)

114. M Desautelles, J Himms-Hagen: Parallel regression of cold-induced changes in ultrastructure, composition, and properties of brown adipose tissue mitochondria during recovery of rats from acclimation to cold. *Can J Biochem* 58, 1057-1068 (1980)

115. B Korac, A Korac, B Buzadzic. Interscapular brown adipose mass regression during re-adaptation after cold-induced hyperplasia: contribution of apoptosis. In: Basic and Applied Thermophysiology. Ed: V N Gourine, Polibig, Minsk (2002)

116. V Petrovic, B Buzadzic, A Korac, A Vasilijevic, A Jankovic, B Korac: The role of heme oxygenase 1 and NF-kappaB in interscapular brown adipose tissue: the influence of nitric oxide. *Acta Physiol Pharamacol Serb* 42, 186-191 (2006)

117. B Buzadzic, A Korac, V Petrovic, B Korac: Glutathione content, rate of apoptosis, and brown adipose tissue mass in rats exposed to different ambient temperatures. *J Thermal Biol* 29, 503-507 (2004)

118. B Buzadzic, A Korac, V Petrovic, B Korac: Redox regulation of brown adipocytes: molecular and cellular targets in tissue remodeling. *Acta Physiol Pharamacol Serb* 42, 141-159 (2006)

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