The aim of this study was to investigate lipofuscin origin in brown adipocytes of hyperinsulinaemic rats and the possible role of lipid peroxidation and iron in this process. Ultrastructural examination revealed hyperinsulinaemia-induced enhancement in the lipofuscin production, accompanied by an increase of mitochondrial damage in brown adipocytes. Extensive fusions of lipid droplets and mitochondria with lysosomes were also observed. Confocal microscopy showed lipofuscin autofluorescence emission in brown adipose tissue (BAT) after excitation at 488 nm and 633 nm, particularly in the insulin-treated groups. The presence and distribution of lipid peroxidation product, 4-hydroxy-2-nonenal (4-HNE), in brown adipocytes was assessed by immunohistochemical examination revealing its higher content after treatment with insulin. The iron content was quantified by electron dispersive X-ray analysis (EDX) showing its higher content in the hyperinsulinaemic groups. The ultrastucture of the majority of lipofuscin granules suggests their mitochondrial origin, which was additionally confirmed by their co-localization with ATP synthase. In conclusion, our results suggest that increased lipofuscino genesis in the brown adipocytes of hyperinsulinaemic rats is a consequence of lipid peroxidation, mitochondrial damage and iron accumulation.

**Key words:** Brown adipocyte, Lipofuscin, Hyperinsulinaemia, Iron

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**Introduction**

Brown adipose tissue (BAT) has a unique ability to produce heat by a mechanism called non-shivering thermogenesis. Lipids accumulated in brown adipocytes are oxidized in mitochondria, generating a proton electrochemical gradient that is dissipated in the form of heat by the mitochondrial uncoupling protein-1 (Nicholls and Locke, 1984; Nedergaard et al., 2001). Among the positive signals known to regulate BAT function, noradrenalin, insulin and insulin-like growth factor I are probably the most physiologically relevant (Rothwell and Stock, 1981; Rothwell et al., 1981; Mur et al., 2003). Insulin role is still controversial, exerting a completely different role depending on applied dose and treatment duration. For years, insulin has been regarded as an important mitogen with a potent adipogenic effect (Porras et al., 2003) that is able to protect cells from apoptosis (Navarro et al., 1998). At high doses (Korac et al., 1999) or after a long-term insulin treatment (Porras et al., 2003) insulin is also able to induce the apoptosis of brown adipocytes. In addition, hyperinsulinaemia, which often precedes obesity and diabetes leading to insulin resistance, can be the link to mitochondrial dysfunction, which can reduce ATP production (Rains and Jain, 2011).

The relationship between iron and insulin resistance is well established and it was shown that insulin can cause rapid and pronounced stimulation of iron uptake by adipocytes (Le Guenno et al., 2007). Our previous studies revealed that hyperinsulinaemia also causes erythropagosomal activity (Radovanovic et al., 1999; Grubic et al., 2008) and iron loading in brown adipocytes (Korac et al., 2003). Iron is known to be a possible co-factor in cell injury potentiation. A modest
tissue concentration of iron induces lipid peroxidation and accumulation of damaged macromolecules (Powell and Yapp, 2000; Hohn et al., 2010), but excess iron is extremely harmful and may even promote cell death (Halliwell and Gutteridge, 1999).

Several investigations have shown molecular damage to cell organelles by reactive oxygen species (ROS) generating lipofuscin as an end product (Sohal et al., 1989) and this process is enhanced by iron. Although lysosomal degradation is both rapid and effective, it is not completely successful (Terman et al., 2007), so even under normal conditions, degradation is followed by some iron-catalyzed peroxidation that results in slow accumulation of the non-degradable, autofluorescent, polymeric, plastic-like lipofuscin within lysosomes (Brunk and Terman, 2002a). Lipofuscin accumulates within cells primarily because it is non-degradable and cannot be removed to any significant degree via exocytosis (Terman and Brunk, 1998a,b).

Our preliminary results documented enhanced lipofuscinogenesis in brown adipocyte induced by hyperinsulinemia (Korac et al., 2009). The ultrastructural appearance of lipofuscin granules resembled secondary lysosomes, but direct evidence for the contribution of lipid peroxidation to lipofuscin accumulation and their origin has not been shown.

Therefore, the aim of the present study was to examine the correlation between hyperinsulinaemia and lipofuscin accumulation in brown adipocytes and to reveal the possible origin of lipofuscin in these cells.

Material and methods

Experimental design

All experiments were approved by the Ethical Committee for the Treatment of Experimental Animals of the Faculty of Biology, University of Belgrade. Adult male rats of Wistar strain were used in the experiment. The animals were acclimated to 22±1°C, maintained under 12 h light/dark cycle and given commercial rat food (Subotica, Serbia), and water ad libitum. The rats were divided into four groups, each consisting of six animals. The first two groups were treated acutely (1 day) or chronically (3 days) with high dose (4 IU/kg, intraperitoneally; one injection daily) of insulin (ICN Galenika, Belgrade). The second two groups served as control and were treated with 0.9% saline intraperitonealy over 1 day or 3 days (one injection per day, 1 ml/kg). Three hours after the last injection animals were sacrificed and an interscapular portion of BAT was removed and prepared for electron and light microscopic examinations.

Electron microscopy

Half of each BAT sample was cut into small pieces, fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) and postfixed in 1% osmium tetroxide in the same buffer. The specimens were dehydrated through serial ethanol solutions of increasing concentration and were embedded in Araldite (Fluka, Germany).

For electronmicroscopic examination, the tissue blocks were trimmed and cut with diamond knives (Diatome, Switzerland) on an UC6 ultramicrotome (Leica Microsystems, Germany). The thin sections were mounted on copper grids, stained with uranyl acetate/lead citrate (EM Stain system, Leica Microsystems, Germany) and examined with a Philips CM12 transmission electron microscope (Philips/FEI, The Netherlands).

Lipofuscin autofluorescence detection - confocal laser scanning microscopy (CLSM)

The remaining half of each BAT sample was fixed for 48 h in neutral phosphate buffer solution containing 10% formaldehyde. After fixation, the tissue was dehydrated, xylene-cleared and embedded in paraffin. Five µm-thick sections were obtained with a Spencer microtome (American Optical, USA). Unstained paraffin sections were observed with a LSM 510 confocal laser scanning microscope (Carl Zeiss, Germany) and the autofluorescence was detected at the following excitation/emission wavelength settings: channel 1 (Ch1) - λex 488 nm /λem between 505-530 nm; channel 2 (Ch2) - λex 543 nm /λem between 560-615 nm; and channel 3 (Ch3) - λex 633 nm /λem over 650 nm. Unstained sections were studied by sequential (multi-track) excitations with each laser separately, and individual images were superimposed to give a combined three-color image. Channel signals were displayed in pseudo-color blue (Ch 1), green (Ch2) and red (Ch3).

Immunohistochemical staining

Paraffin-embedded 5 µm-thick BAT sections were deparaffinized by xylene and rehydrated in graded ethanol. After antigen retrieval in 10mM citrate buffer (5 minutes in microwave oven) and washing with phosphate buffer saline (PBS), endogene peroxidase blocking was performed in 3% hydrogen peroxide (H2O2) in methanol, for 10 min. After thorough rinsing, sections were incubated overnight at 4°C with mouse monoclonal anti-4-HNE-Michael-adducts-antibody (dilution 10 µg/ml, Abcam, UK) in PB S, followed by PBS rinsing. Immunoreactivity was detected by a streptavidin-biotin-peroxidase method according to the manufacturer’s protocol (LSAB detection kit; Dako, USA). The final reaction product was visualized with 3,3’-diaminobenzidine - tetrahydrochloride (Sigma-Aldrich, Germany). After counterstaining with hematoxylin, slides were mounted and examined with the light microscope (Leica Microsystems, Germany).

Co-localization of mitochondrial ATP synthase subunit beta and lipofuscin

In order to study the mitochondrial origin of
lipofuscin, co-localization of lipofuscin and ATP synthase was examined. Paraffin-embedded 5 µm-thick BAT sections were deparaffinized and rehydrated as described. ATP synthase was detected with mouse monoclonal antibody to mitochondrial ATP synthase subunit beta (1:1000; Abcam, UK) in combination with rhodamine-conjugated secondary antibody (1:200; Abcam, UK). Confocal images were acquired with a Carl Zeiss LSM510 confocal laser scanning microscope. Rhodamine fluorescence was detected with $\lambda_{\text{ex}}$ 543 nm / $\lambda_{\text{em}}$ between 560-615 nm, while lipofuscin autofluorescence was detected as previously described.

**Electron dispersive X-ray analysis (EDX)**

Resin-embedded 2 µm-thick tissue blocks were mounted on stubs, carbon-coated and analyzed with an EDAX Genesis X-ray microanalysis system (AMETEK, USA) in combination with a Philips XL 20 scanning electron microscope (Philips/FEI, The Netherlands). EDX analysis was performed at 1000X magnification, with a beam voltage of 15.0 kV. The results were collected and statistically processed by two-way analysis of variance (ANOVA). Statistical significance was accepted at p<0.05.

**Results**

**Transmission electron microscopy (TEM) – lipofuscin origin and formation**

Ultrastructural analysis of brown adipocytes showed that a high dose of insulin increases the number of lipofuscin granules without regard to treatment duration. Lipofuscin granules were also visible in the brown adipocyte cytoplasm from control rats, although they appeared less frequently than in the hyperinsulinaemic groups. Brown adipocyte profiles of control animals generally had only two or three lipofuscin granules (Fig. 1A). Lipofuscin granules were characterized by the presence of osmiophilic electron-dense material, lipid droplets and occasional fibrillar structures (Fig. 2A-C), surrounded by a typical lysosomal membrane. Their proportions varied among the brown adipocytes and between the experimental groups.

The most striking differences were in the BAT after acute insulin treatment, where various signs of cellular degeneration were observed. The number of lysosomes was increased, along with lipofuscin accumulation. Lipofuscin was especially abundant around mitochondria and was closely attached to lipid droplets (Fig. 1B). The fusion of primary lysosomes with lipid droplets was also visible in brown adipocytes from the hyperinsulinaemic rats, with subsequent appearance of one large lipid droplet or numerous small ones inside the lipofuscin granules (Fig. 3).

In addition, mitochondria were also affected after acute insulin treatment. They showed different stages and types of degradation, including: a) cristae disorganization or accumulation at one pole of the organelle, and formation of lamellar (myelin-like) structures that gradually led to transformation of the mitochondria into lipofuscin granules (Fig. 4A-C); b) swelling of the mitochondria with lightening of the matrix and loss of cristae, sometimes leading to bursting and flattening of mitochondria with content outflow (Fig. 4D-F); c) mitochondrial matrix condensation (Fig. 4G); d) fusion of lysosome with mitochondria (Fig. 4I);

![Fig. 1. Transmission electron microscopy of lipofuscin accumulation in brown adipocytes from control (A) and hyperinsulinaemic group (B). Compared to brown adipocytes of controls, brown adipocytes of insulin-treated rats have a larger number of lipofuscin granules (arrows), scattered around mitochondria (m) and lipid droplets (ld). Scale bars: 2 µm.](image-url)
and e) mitochondrial autophagocytosis (Fig. 4H).

**Lipofuscin localization and quantity**

CLSM revealed autofluorescent material corresponding to lipofuscin granules by its localization, quantity and size, in BAT of all experimental groups (Figs. 5, 6). The material showed autofluorescent emission between 505-530 nm after excitation at 488 nm, and over 650 nm after excitation at 633 nm. In addition to emission in these wavelength ranges, erythrocytes also autofluoresced between 560-615 nm with a 533 nm laser line (Fig. 6B,G), enabling us to distinguish lipofuscin from red blood cells in the tissue.

The highest amount of lipofuscin was detected in BAT of the group that received acute insulin treatment (Fig. 6A-D). Lipofuscin was localized in brown adipocytes primarily in the cytoplasm near cellular membrane, with multiple granules gathered in smaller or larger groups. The approximate size of the lipofuscin granules was 1-2 µm.

Brown adipocytes of chronically treated rats also showed significant amounts of lipofuscin, with localization and granule size similar to acutely treated rats, but with slightly decreased quantities of granules (Fig. 6E-H). Many granules could be seen near lipid droplets.

Compared to BAT from the hyperinsulinaemic groups, control animals showed a considerably lower content of lipofuscin granules, especially after chronic treatment, when only erythrocytes were visible, and almost no signs of lipofuscinogenesis were seen (Fig. 5B). The number of brown adipocytes containing lipofuscin in acutely treated control animals was slightly higher compared to chronic controls. The granules were significantly smaller (under 1 µm), and sparsely

![Fig. 2. Transmission electron microscopy of lipofuscin granules appearance in rat brown adipocytes after acute insulin treatment. Lipofuscin granules in brown adipocytes are usually round (A, B), surrounded by a typical lysosomal membrane and filled with osmiophilic, electron-dense material and lipid droplets and occasionally, with fibrillar, myelin-like structures (C). Scale bars: 0.5 µm.](image)

![Fig. 3. Transmission electron microscopy of lipofuscin formation from lipid droplets in brown adipocytes after acute insulin treatment: lysosomes (arrows) in close contact with lipid droplet (ld) (A); lysosomal fusion with lipid droplet (B); and lipid droplets inside of lipofuscin granule (C). Scale bars: 0.5 µm.](image)
distributed in the brown adipocyte cytoplasm (Fig. 5A).

**Immunohistochemical detection of lipid peroxidation**

4-HNE-Michael adducts were highly expressed in the BAT of groups treated with high doses of insulin, with no significant differences in reaction intensity between the acute and chronic treatment groups (Figs. 7B,C). Immunoreactivity was visible in the cytoplasm of a majority of brown adipocytes, around lipid droplets. In acutely treated animals, 4-HNE-Michael adducts were also detected around the nuclei. In BAT samples from the control groups (Fig. 7A) immunorepression was almost undetectable.

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**Fig. 4.** Different stages and types of mitochondrial degradation in brown adipocytes of hyperinsulinaemic rats: cristae swirling and myelin-like structures (A, B), leading to lipofuscin formation (C); mitochondrial matrix lightening and swelling (D), leading to organelle rupture (E) and content outflow (F); mitochondrial condensation (G); mitochondrial autophagocytosis (H); and fusion of mitochondrion with lysosome (I). Scale bars: 0.5 μm.
Co-localization of ATP synthase and lipofuscin

The immunofluorescence detection of ATP synthase subunit beta (Fig. 8A) combined with lipofuscin autofluorescence detection (Fig. 8B) in BAT of hyperinsulinaemic rats revealed their co-localization in the brown adipocyte granules corresponding to lipofuscin (Fig. 8C).

Iron quantification in BAT

Quantitative elemental analysis of brown adipose tissue sections was carried out by EDX analysis combined with scanning electron microscopy. The given iron weight percentage showed significant differences (p<0.001) between control groups and groups treated with a high dose of insulin, and no significant differences were seen between the acutely and chronically treated groups (p>0.05). In other words, groups treated with high doses of insulin showed a very significant increase in iron content compared to control groups, without regard to treatment duration (Fig. 9).

Discussion

The results of this study demonstrate that hyperinsulinaemia induces massive lipofuscin accumulation in rat brown adipocytes. This is shown at ultrastructural level and confirmed by specific lipofuscin autofluorescence.

In brown adipocytes, lipofuscin granules showed autofluorescence after excitation with blue (488 nm) and red (633 nm), but not with green light (543 nm). The photosensitizing property of lipofuscin depends on the nature of the material contained and may vary in different cell types. Thus, Nakae et al. (2001) demonstrated autofluorescence of lipofuscin in the cells of the diaphragm muscle after excitation with blue and green light. Also, Brunk and Terman (2002a) showed autofluorescence of lipofuscin granules using blue, but also UV and green excitation light. Although fluorescent materials in lipofuscin (fluorophore) derive mainly from lipid peroxidation of organelle membranes, their exact chemical structure in different cell types is still unknown.

In brown adipocytes, we found lipofuscin granules as complexes of lipids and pigment surrounded by a typical lysosomal membrane. Lipofuscin appears to be derived from damaged cellular components, mainly mitochondria and lipid droplets, through the peroxidation of their unsaturated lipids. The numerous mitochondrial structural alterations we observed (swelling, cristae disorganization and swirling), mitophagy and fusion with lysosomes, clearly demonstrated a mitochondrial origin of lipofuscin, which we additionally confirmed by the co-localization of mitochondrial ATP synthase subunit beta and lipofuscin granules. These findings are in accordance with data.
Fig. 6. Autofluorescence detection of lipofuscin in unstained paraffin sections of BAT from acutely treated (A-D) and chronically treated hyperinsulinaemic (E-H) rats, with the following CLSM excitation/emission wavelengths: channel 1 (Ch1) - 488 nm/505-530 nm; channel 2 (Ch2) - 543 nm/560-615 nm; and channel 3 (Ch3) - 633 nm/over 650 nm. Images are shown in pseudo-color blue (Ch1) (A, F), green (Ch2) (B, G) and red (Ch3) (C, H), and as superposed (D, E), multi-channel images. Compared to control group samples, larger quantities of autofluorescent material are detectable in Ch1 and Ch3, corresponding to lipofuscin (white arrows) in brown adipocytes of both hyperinsulinaemic groups. Inserts: Enlarged brown adipocytes showing lipofuscin-like autofluorescence in the cytoplasm and around lipid droplets. Scale bars: 20 µm.
previously published by other laboratories (Ezaki et al., 1995a; Elleder et al., 1997) showing that lipofuscin contains the ATP synthase subunit c.

The intracellular formation of lipofuscin is a very complex process involving mitochondria, the lysosomal system, and various products of protein oxidation and lipid peroxidation (Brunk and Terman, 2002b; Terman et al., 2010). The accumulation of lipid droplets in lipofuscin granules in hyperinsulinaemic rat brown adipocytes, as we have demonstrated at the ultrastructural level, could be a consequence of lipid removal, which could lead to lipotoxicity and lipid peroxidation. Therefore, considering the well-known lipogenic effect of insulin (Deshaies et al., 1991; Wang and Sul, 1998), it is possible that brown adipocytes treated with high doses of insulin accumulate large amount of lipids that cannot be oxidized in the absence of signals activating thermogenesis, thus altering mitochondrial structure and function.

Immunohistochemical detection of higher content of 4-HNE-Michael adducts in brown adipocytes of hyperinsulinaemic rats confirmed our suggestions about

![Fig. 7. Immunohistochemical localization of 4-HNE-Michael adducts in brown adipose tissue from control and hyperinsulinaemic rats: control (A); acute insulin treatment (B); chronic insulin treatment (C). Higher immunoreactivity is visible in both hyperinsulinaemic groups compared to the controls. Immunoreactivity is visible in the cytoplasm, close to lipid droplets, and in nuclei. Scale bars: 20 µm.](image)

![Fig. 8. Co-localization of ATP synthase subunit beta (A) and lipofuscin granules in brown adipocytes of hyperinsulinaemic rats (B, C). Merged image reveals superposition of ATP synthase subunit beta rhodamine signal (excitation/emission: 543 nm/560-615 nm) and autofluorescence signal corresponding to lipofuscin (excitation/emission: 488 nm/505-530 nm) inside brown adipocytes (arrows). Scale bar: 20 µm.](image)
increased lipid peroxidation. Therefore, 4-HNE along with malondialdehyde (MDA) belongs to the group of the final aldehyde products of lipid peroxidation which tend to cross-link various protein residues in the lysosomes, forming non-degradable polymers of lipofuscin (Brunk and Terman, 2002a). It was recently shown that, at least in retinal pigment epithelial cells, 4-HNE and MDA can directly inactivate lysosomal cysteine cathepsins by covalent binding to the active site simultaneously converting the proteolytic substrates of lysosomal enzymes into their competitive inhibitors (Krohne et al., 2010). In addition, a significant proportion of lysosomal hydrolases was suggested to be largely associated with the lipofuscin-loaded vesicles in such lipofuscin-loaded cells.

Increased lipid peroxidation levels could also be one of the consequences of increased ROS production, especially H$_2$O$_2$, which can react with iron in its ferrous (Fe (II)) state, leading to lipid peroxidation in the reducing, acidic conditions of lysosomes (Terman et al., 2007; Kurz et al., 2008). Many studies on the rapid production of H$_2$O$_2$ by various cell types, including brown adipocytes after insulin stimulation (May and de Haen, 1979), have shown its important physiological role in the insulin signaling cascade (Mahadev et al., 2001; Pomytkin and Kolesova, 2003). In addition, increased H$_2$O$_2$ production shows cytotoxic effects, especially on membranes (Zdolsek et al., 1995; Sheridan et al., 1996) and mitochondria (Maechler et al., 1999). The finding that H$_2$O$_2$ in concentrations that normally induce apoptosis or necrosis has no influence on cells in the presence of potent iron chelators (Kurz and Brunk, 2009) suggests that H$_2$O$_2$ per se is not particularly toxic but rather works in concert with iron to damage cells.

Free iron can be destructively redox-active, causing damage to almost all cellular components, killing both cells and organisms. This may explain why iron uptake and metabolism is tightly controlled and why most cells cannot efficiently excrete excess iron. When even a small amount of intracellular free iron occurs, most of it is safely stored in a non-redox-active form in ferritins (Kurz et al., 2011). Within cells, iron is constantly being recycled from aged iron-rich organelles such as mitochondria and used for new organelle construction. Most of this recycling occurs within the lysosome, and for that reason these organelles are unusually susceptible to oxidant-mediated destabilization or rupture (Kurz et al., 2011). Most cells, including macrophages, contain a certain amount of reducing iron in lysosomes (Miyawaki, 1965; Brun and Brunk, 1970; Zdolsek et al., 1993a; Radisky and Kaplan, 1998; Persson et al., 2003). Further iron accumulation in this compartment makes cells more susceptible to oxidative stress and lipid peroxidation (Sakaida et al., 1990; Baynes, 1996; Yuan et al., 1996; Yu et al., 2003). In a previous study (Korac et al., 2003), we used histochemical analysis to detect the presence of iron in BAT under hyperinsulinaemic conditions, and showed an increase of its content in brown adipocytes. Our results also revealed iron accumulation-associated induction of heme-oxygenases, sensitive indicators of oxidative stress (unpublished results). Lipofuscin formation can be significantly increased by the addition of iron, and conversely, lipofuscin production is substantially hampered after treatment with the iron-chelators (Marzabadi and Llvaas, 1996). Also, sources of Fe (II) in brown adipocytes include various metalloproteins, such as cytochrome c from mitochondria that are degraded inside autophagosomes (Ezaki et al., 1995b; Elleder et al., 1997). The additional sources of iron might be hemoglobin and ferritin, since we showed ferritin accumulation and erythropagosomal activity in brown adipocytes, especially under chronic hyperinsulinaemic conditions (Grubic et al., 2008). Recently, it was shown that increased erythropagocytosis leads to iron accumulation and ferritin release (Otogawa et al., 2007). Iron stored in ferritin is extracted in the lysosome for utilization by cells, which is an important component of cellular iron homeostasis (Asano et al., 2011). Since cancer-delivered cells are resistant to iron toxicity, it is postulated that loss of ferritin delivery to the lysosome under iron-rich conditions may protect against cell death induced by iron-induced oxidative insults (Asano et al., 2011).

Insulin administration appears to affect the lysosomal enzyme system in the BAT of rats by changing enzyme levels (Ohno et al., 1996). This could lead to irregular lysosomal activity, which would lead to lipofuscin accumulation. Because lipofuscin is not degradable, it permanently blocks lysosomes, although they continue to receive lysosomal enzymes, in an attempt to decompose lipofuscin. This leads to

**Fig. 9.** Weight percentage of iron (mean ± SEM) in BAT of control and hyperinsulinaemic rats; treated acutely or chronically. The results demonstrate a significant increase in BAT iron content of hyperinsulinaemic groups compared to control groups (**p<0.001).**
increasing lysosomal blockade, forcing the cell to maintain damaged organelles and macromolecules (Terman et al., 2007). Ultrastructural analysis showed an increased number of swollen and damaged mitochondria that failed to be autophagocytosed, since the lysosomes were permanently occupied by lipofuscin (Ermini, 1976; Beregi et al., 1988; Terman et al., 2003). Also, lipofuscin 

\[ \text{per se} \]

can contribute to reduced cell proteolytic capacity (Szwedz et al., 2003; Hohn et al., 2011). As reported earlier, lipofuscin can sensitize cells to oxidative stress (Terman et al., 1999a), inducing lysosomal breach and apoptosis, apparently due to a high content of iron in pigment granules (Terman et al., 1999b).

In conclusion, our results highlight the potential importance of mitochondria in controlling oxidative stress under hyperinsulinaemic conditions. Increased lipofuscinogenesis in brown adipocytes of hyperinsulinaemic rats is a consequence of lipid peroxidation, iron accumulation and subsequent mitochondrial damage. Modulation of mitochondrial oxidant protection could lead to amelioration of insulin resistance. Further studies will focus on the elucidation of possible mechanism(s).

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References


Mitochondrial origin of lipofuscin in brown adipocytes

hydrogen peroxide production in rat epididymal fat cells. J. Biol. Chem. 254, 2214-2220.

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