*Original Article*

# CHANGES IN FREE FATTY ACIDS IN RAT BRAIN NUCLEAR AND MICROSOMAL SUBCELLULAR FRACTIONS IN EXPERIMENTAL MODEL OF CEREBRAL HYPOXIA

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## Summary
The aim of the present investigation was to establish the changes of the level of total and individual free fatty acids (FFAs) in nuclear and microsomal subcellular fractions from hypoxic rat brains. Twenty male Wistar rats at the age of three months were subjected to sodium nitrite-induced cerebral hypoxia (intravenously, 20 mg/kg body weight, 2 ml/kg dosing volume). Nuclear and microsomal fractions were isolated and lipids were extracted. The free fatty acid content was measured by gas-liquid chromatography. In control rats, stearic, linoleic and arachidonic acids were the most prominent components in both fractions. Hypoxia caused a weak increase of total FFAs in nuclei and microsomes - 1.4- and 1.04-fold, respectively. Among the individual free fatty acids, stearic acid increased 4.7-fold in nuclei, and arachidonic acid increased 1.2-fold in both fractions. A notable observation was the accumulation of $C_{16:1}$, $C_{18:1}$ and $C_{20:2}$, which were absent in control rats. In conclusion, sodium nitrite-induced cerebral hypoxia causes various changes of FFAs in brain nuclei and microsomes. There is a tendency to synthesize long-chain unsaturated fatty acids. The high concentration of unsaturated FFAs is probably due to their neuroprotective effect.

**Key words:** Cerebral hypoxia, rat brain, microsomes, nuclei, free fatty acids

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## Introduction
The brain is highly oxidative organ and although it constitutes only 2% of body weight, it accounts for 20% of the total body oxygen consumption. Brain is of special interest for hypoxia studies as it is extremely sensitive to reductions in oxygen supply.

Hypoxia, as well as ischemia, provokes alterations in the lipid metabolism. Most of the conducted studies in hypoxia concern the brain as a whole with no attempt of obtaining information on how sensitive to hypoxia brain subcellular fractions are [1].

The aim of the present investigation is to establish the changes of the level of total and individual free fatty acids in brain nuclei and microsomes from hypoxic rat brains.

## Materials and Methods
The animal experiments were performed in accordance with animal protection guidelines approved by the Ethics Committee for experimental animal use at IEMAM - BAS.

Twenty male Wistar rats at the age of three months, each weighing 190-220 g, were subjected to sodium nitrite-induced cerebral hypoxia. Sodium nitrite was

administered intravenously at 20 mg/kg body weight (2 ml/kg dosing volume). Hypoxic rats were killed by decapitation.

Brain nuclear and microsomal subcellular fractions were isolated as described by Venkov [2] using two-step sucrose gradient. Lipids were extracted according to the method of Kates [3] using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v). The fatty acids were converted to fatty acyl methylesters (FAME) by the addition of methanol and HCl. The FAME were extracted by petroleum ether, then concentrated in a rotary vacuum evaporator and subjected to gas-liquid chromatographic analysis. A gas-liquid chromatograph with flame ionization detector, SE-35 column and connected with Trio Vector computing integrator was used. The temperature was programmed from 85ºC to 205ºC (2.5ºC/min). Nitrogen was used as carrier gas at a flow-rate of 40 ml/min. We used mix computering program with inner and outer standard to determine the real quantity of FFAs.

The data were analysed with Student's t-test.

## Results

In control rats, in the microsomes the percentage of saturated FFAs was higher in comparison to unsaturated FFAs. The same tendency was observed in the composition ratio between the short-chain FFAs and long-chain FFAs. In contrast to this tendency, in nuclei unsaturated FFAs predominated over saturated FFAs and long-chain FFAs predominated over short-chain FFAs.

Nevertheless, $C_{18:0}$, $C_{18:2}$ and $C_{20:4}$ were the most prominent components in the control FFA pool in the both brain subfractions (Fig. 1 and Fig. 2).

In the brains of experimental rats subjected to cerebral hypoxia we found an increase in total FFAs - 1.4-fold in nuclei ($p<0.001$) and 1.04-fold in microsomes ($p<0.001$). The changes in the amount of total FFAs after hypoxia are shown in Fig 3.

In the nuclear fraction, there was a significant increase in the amount of $C_{18:0}$ - 4.7 times the control values, from 0.26±0.02 mg/g/ml (mg free fatty acid per g dry lipid residue per ml lipid extract) to 1.2±0.03 mg/g/ml ($p<0.001$). After hypoxia, some monounsaturated FFAs released - palmitoleic ($C_{16:1}$) and $C_{18:1}$ acids. Arachidonic acid comprised of 59% of the total free fatty acid pool ($p<0.001$) (Fig. 1).

In the microsomal fraction, the following mono- and polyunsaturated FFAs appeared after hypoxia: $C_{16:1}$, $C_{18:1}$ and $C_{20:2}$, with concentrations 5.29±0.03 mg/g/ml, 5.16±0.03 mg/g/ml, and 4.19±0.04 mg/g/ml, respectively. The microsomal free fatty acid pool contained only unsaturated FFAs (Fig. 2).

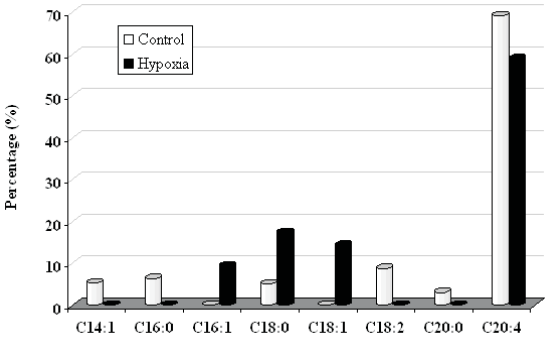


**Figure 1.** Changes of the free fatty acid content in the nuclear fraction after hypoxia. P<0.001

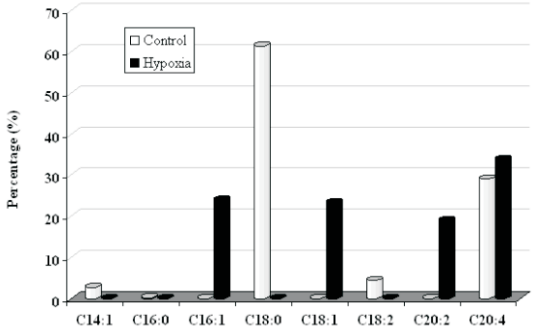


**Figure 2.** Changes of the free fatty acid content in the microsomal fraction after hypoxia. P<0.001

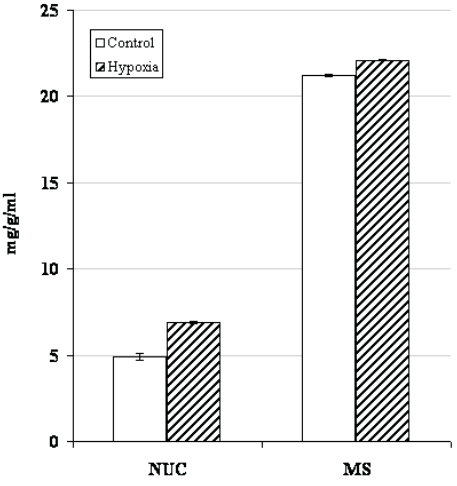


**Figure 3.** Changes of total free fatty acid content in nuclei and microsomes after hypoxia. P<0.001

## Discussion

Limitation of oxygen supply to the brain below a "critical" level quickly results in reduced oxidative phosphorylation, decreased cellular ATP and a consequent increase in adenosine and collapse of ion gradient, thereby affecting the function of the central nervous system.

It is known that elevated levels of methemoglobin can lead to anemic hypoxia, a condition in which there is inadequate supply of oxygen to tissues. Rats exposed to sodium nitrite achieve elevated concentrations of methemoglobin in their blood [4]. Unlike the ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. The oxidation of oxyhemoglobin by nitrite to produce methemoglobin is a complex process and it is characterized by a lag phase followed by an autocatalytic phase [5].

Lipids are particularly sensitive to hypoxia, in comparison to other membrane components. Although significant efforts have been directed at evaluating changes in various metabolic and physiological functions [6] lipid metabolism in response to hypoxia in the brain subcellular fractions has not been fully evaluated. Considering the role of some fatty acids in stimulation of cellular processes, establishing the changes in their content may be of basic significance for understanding the involved pathomechanism.

Brain free fatty acids are normally present in very small amounts, but are found to accumulate during hypoxic conditions in tissues. The increase of the FFA pool is considered as a result of their release from the membrane phospholipids and it is due to the inhibition of phospholipid synthesis and disturbances in the dynamic equilibrium between FFAs and the acyl groups of membrane phospholipids.

To our knowledge, an increase in FFA content is documented in brain mitochondria, synaptosomes and myelin [7]. There are no data about FFA changes in nuclei and microsomes.

In our experiments on rats subjected to hypoxia, we have found increased levels of total and some individual FFAs in brain nuclei and microsomes. The major components of the increased FFA pool were $C_{18:0}$ and some mono- ($C_{16:1}$, $C_{18:1}$) and polyunsaturated ($C_{20:2}$, $C_{20:4}$) FFAs.

It is known that lysoglycerophospholipids are generated along with free fatty acids during glycerophospholipid hydrolysis by phospholipases $A_1$ and $A_2$. It is considered that the increased sensitivity of these enzymes to the stimulatory action of calcium ions may represent one of the factors, responsible for the high levels of unsaturated FFAs. Another possible factor is the decreased activity of acylCoA ligase [7, 8]. Alterations in brain polyunsaturated FFAs have also been associated with other changes in physicochemical function, for example, behaviours such as learning ability [9].

Our results showed that the hypoxic brain nuclei and microsomes tended to have higher concentrations of long-chain fatty acids. This tendency reflects a process of membrane repair by brain cells. There is a tendency to synthesize long-chain and polyunsaturated FFAs. The high concentration of unsaturated FFAs is probably due to their neuroprotective effect. Whether the increase affects neural function and development is an important issue to be considered.

## Conclusions

The results of the present study reveal that brain nuclei and microsomes are sensitive to cerebral hypoxia.

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