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SHORT COMMUNICATION

Lactate Dehydrogenase Activity in Human Placenta Following Exposure to Environmental Pollutants

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Summary

The impact of environmental pollution at the place of residence of pregnant women and of their smoking habits on the cellular energy metabolism of placental tissue was investigated. Samples of full-term placentas were randomly collected from two environmentally different regions of Slovakia (Bratislava, Stará Ľubovňa) and the activity of lactate dehydrogenase (LDH) was measured. Our results showed enhanced LDH activity in the placenta that was dependent on both the type of environmental pollutants at the place of residence and the smoking habits during pregnancy. The enhanced LDH activity may reflect hypoxic conditions due to the accumulation of heavy metals and toxic compounds of tobacco smoke in the placental tissue. A high content of heavy metal particles, found in placental samples from Stará Ľubovňa in our previous studies, might contribute to the increased LDH activity in placentas from this region. We hypothesize that fine metal particles deposited in the placental tissue might be phagocytozed by the syncytiotrophoblast, thus contributing to the decreased oxygen level in placental tissue.

Key words

Human placenta • Lactate dehydrogenase • Environmental pollutants

Human placenta offers an outstanding opportunity to investigate the individual exposure of pregnant women to environmental pollutants. In a previous study we found that, of the placental layers, the syncytiotrophoblast had the heaviest deposition of particles containing the heavy metals lead and nickel. The syncytiotrophoblast is the epithelium of chorionic villi and is the site of oxygen exchange between mother and fetus and the site of excretion of noxious metabolites by the fetus. Therefore, the syncytiotrophoblast layer has an intense enzyme activity (Reichrtová *et al.* 1998). In the syncytiotrophoblast, accumulation of environmental pollutants and of some toxic products of tobacco smoke may cause hypoxia, resulting in changes of enzyme activities linked to cellular energy metabolism (Niweliński *et al.* 1990). During cellular respiratory suppression, glucose is metabolized by anaerobic glycolysis rather than by oxidative phosphorylation. Increased usage of the anaerobic glycolytic pathway is associated with increased activity of lactate dehydrogenase (LDH). The aim of this study was to determine the impact of environmental pollutants at the place of residence of pregnant women and of their smoking habits on cellular energy metabolism.

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Women with normal term deliveries (40±2 weeks of gestation), who had been residents in the regions of interest for at least three years prior to conception and through to delivery, were eligible to be selected for the study. Fresh samples of full-term placentas were collected from two environmentally different regions of Slovakia at delivery in the respective regional maternity hospitals. At the maternity hospitals, data on smoking habits, intensity of smoking and occupational exposure were obtained *via* a questionnaire. The two regions of interest were Bratislava and Stará Ľubovna. Bratislava is an industrial region, which is heavily polluted by chemical industries. Stará Ľubovňa is a rural region, which lacks heavy industry, but seems to be affected by pollution from the Polish coal basin area, and has a high non-domestic traffic density, since it is at the border crossing between Slovakia and Poland. Only women who had smoked more than 5 cigarettes per day during pregnancy were included in the group of smokers. Persons occupationally exposed to heavy metals and polychlorinated organic compounds were excluded from the study. Groups of smokers and non-smokers from Bratislava (n = 19 and 21, respectively), and Stará Ľubovňa (n = 27 and 22, respectively) were investigated. The study was approved by the Research Ethics Committee of the Institute of Preventive and Clinical Medicine. Informed consent was obtained from the subjects.

Placenta samples, harvested fresh and frozen at –80 °C, were uniformly sectioned from the maternal surface to the chorionic plate with emphasis on the trophoblastic tissue. Removal of blood cells was achieved by gently stirring the tissue mince for 5 min in phosphate buffer saline, followed by compressing the tissue and decanting the supernatant fluid. The above process was repeated until the supernatant fluid was clear. After disintegration and homogenization of the tissue mince, the activity of lactate dehydrogenase was evaluated using an LDH-UV kit (Imuna, Šarišské Michaľany, S.R.). The assay relied on the rate of reduction of pyruvate in the presence of NADH at 37 °C. The rate of conversion of NADH was measured spectrophotometrically and was directly proportional to the catalytic concentration of LDH in the sample. The catalytic activity of the enzyme was expressed in microkatals (μkat) per mg of protein in the sample. Microkatal represents the amount of enzyme activity that catalyzes the transformation of 1 micromole of substrate per second. Protein content in the samples was measured by the method of Lowry *et al.* (1951). The results were statistically evaluated by analysis of variance at a significance level $\alpha = 0.05$.

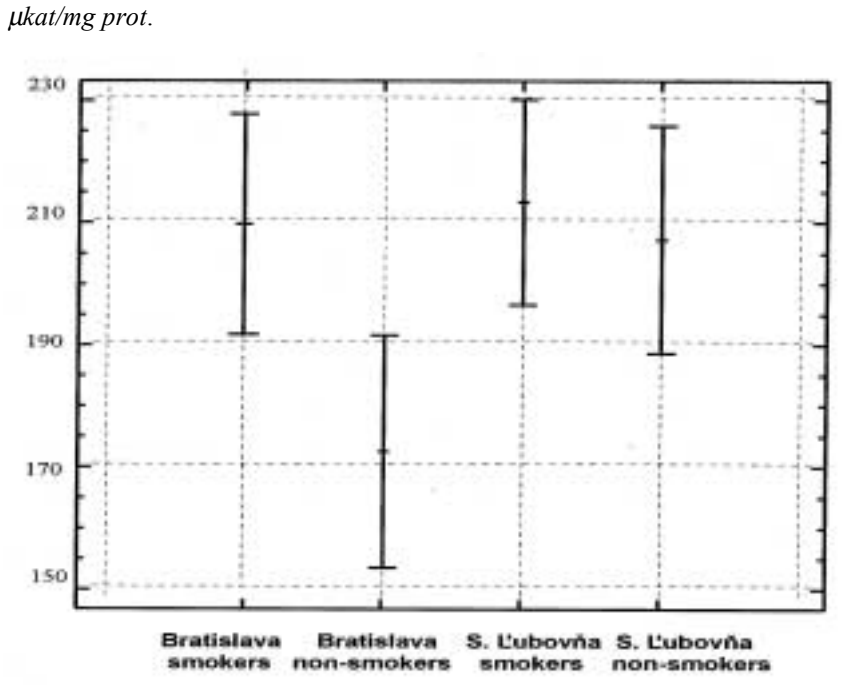

Fig. 1. *LDH activity in placental samples (95 % confidential intervals for means). Significantly elevated LDH activity was found in the group of smoking women in Bratislava in comparison with non-smoking women in this region. The activity of LDH in both groups from the region of Stará Ľubovňa was significantly elevated in comparison with non-smoking women in Bratislava.*

In our study, significantly elevated LDH activity was found in the group of smoking women in Bratislava in comparison with non-smoking women from this region. However, in comparison with non-smoking women from Bratislava, significantly elevated LDH activity was also found in both smokers and non-smokers from the region of Stará Ľubovňa (Fig. 1). Even though the region of Bratislava is considered to be heavily polluted by chemical industry, the results of our previous study showed an increased content of lead and mercury in the placental samples from the region of Stará Ľubovňa (Reichrtová *et al.* 1995). In addition, histochemical analyses revealed accumulation of particles of the heavy metals lead and nickel in the syncytiotrophoblast layer in the placentas from this region (Reichrtová *et al.* 1998). However, placentas from Bratislava had significantly higher concentrations of organochlorine pollutants compared to placentas from Stará Ľubovňa (Reichrtová *et al.* 1999). We hypothesize that fine metal particles deposited in the placental tissue might be phagocytozed by the syncytiotrophoblast, contributing to the decreased oxygen level in placental tissue, since phagocytic activity is associated with a high oxygen demand. Decreased oxygen levels in the tissue may in turn affect placental glucose metabolism leading to increased LDH activity. This is in accordance with the results of Kay *et al.* (1997) who found that lower oxygen tensions are associated with higher lactate dehydrogenase activity in placental villous explants. The high placental content of heavy metals in the region of Stará Ľubovňa is possibly due to the high traffic density at the border between Slovakia and Poland and the direction of prevailing wind from the heavily industrialized areas around the Polish coal basin (Ciezsyn, Katowice). A high content of lead and cadmium has been reported in human placentas as well as maternal and neonatal blood in the Polish coal basin area (Baranowska 1995). The impact of this pollution on the natural flora of the High Tatras (situated close to the Stará Ľubovňa region) is also suggested by a study dealing with the genotoxic effect of xenobiotics on plant pollen in selected areas of Slovakia (Mičieta and Murín 1999).

The activity of LDH was evaluated separately also for the pooled groups of smokers (n = 46) and non-smokers (n = 43). Significantly increased LDH activity was found in the placentas of smokers (209.0±6.6) in comparison with those who did not smoke (189.5±6.8). Cigarette smoking has long been associated with poor reproductive outcomes. The main toxic compounds in tobacco are carbon monoxide, nicotine, and tars. Carbon monoxide binds to hemoglobin to form carboxyhemoglobin, a major factor in hypoxia and vascular accidents. Moreover, experiments on rabbits have shown that cigarette smoke is associated with the development of mitochondrial cardiomyopathy, deterioration of the mitochondrial respiratory chain and oxidative phosphorylation (Gvozdjáková *et al.* 1999). Deterioration of oxidative phosphorylation may lead to a shift towards anaerobic glycolysis, linked to increased LDH activity (Prinz and Schuhmann 1980, Kay *et al.* 1997).

The results of our study suggest that the type of environmental pollution at the place of residence of pregnant women and their smoking habits affect LDH activity in the placenta. Increased LDH activity might reflect a shift from oxidative phosphorylation to anaerobic glycolysis as an adaptation to hypoxic conditions in placental tissue resulting from accumulation of heavy metals and toxic compounds of tobacco smoke.

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