SUMMARY

Introduction: Increasing number of reports reflect that mitochondrial dysfunction can be induced by some of the commonly used drugs and can play a key role in the development of their adverse effects. One of these drugs is a phenol derivative propofol. Propofol is an intravenous, fast and short-acting hypnotic agent, routinely used either for induction and maintenance of anaesthesia during surgery, or for sedation in intensive care units. Propofol infusion syndrome (PRIS) is a rare, but serious adverse effect of the drug with a very high mortality. Typical features of the syndrome include metabolic acidosis, arrhythmias, ECG changes that are similar to those of Brugada syndrome, hypertriglyceridemia, fever, hepatomegaly, rhabdomyolysis, cardiac and/or renal failure. The risk of the syndrome increases with raising dose and duration of propofol administration (>48 hours). The mechanism of the syndrome is still unknown: pilot studies performed on animal models are suggestive of its mitochondrial origin. In the first part of the study, we performed the analysis of 153 published case reports and all experimental studies related to PRIS. Another aim of the study was to test hypothesis of propofol-induced mitochondrial damage by in vitro exposure of human skeletal muscle-derived cells to a range of propofol concentrations and then assessment of mitochondrial functions by Extracellular Flux Analysis.

Methods: We searched for all case reports describing PRIS (published between 1990 and 2014) and we analysed both the relationship between signs of PRIS and the rate and duration of propofol infusion, and risk factors for mortality. In the in vitro study, we used human skeletal muscle cells that were isolated from skeletal muscle (m. vastus lateralis) obtained from patients (n=30) undergoing hip replacement surgery. Cells were differentiated into myotubes and exposed to a range of 4 propofol concentrations resembling its levels in human plasma during propofol infusion at sedation or anaesthesia (1, 2.5, 5 a 10 µg/mL). After 96 hours of exposure, energy metabolism was assessed using XF-24 Extracellular Flux Analyzer. During experiment, we measured oxygen consumption rate (OCR) at baseline and after addition of three agents: inhibitor of ATPase, uncoupler and inhibitor of respiratory chain. The measurement enabled us to determine basal OCR, ATP production, proton leak and maximal respiratory capacity of the respiratory chain. The capacity of fatty acid oxidation was measured using both Extracellular Flux Analyzer (by etomoxir-induced inhibition of OCR after addition of palmitate and uncoupler) and radioactively labelled [1-\(^{14}\)C] palmitate. In addition, we measured respiration after addition of specific substrates and
inhibitors for individual complexes of the respiratory chain (complex I, II, III and IV). Activities of individual complexes were also measured spectrophotometrically.

**Results:** Meta-analysis of 153 case reports about PRIS showed more than >51% mortality. Risk factors associated with high mortality were dose and duration of propofol infusion, fever and craniotrauma. Cardiac failure and metabolic acidosis occurred early after initiation of propofol infusion and are dose-dependent, whilst arrhythmias, ECG changes and rhabdomyolysis appeared more frequently after prolonged propofol infusion, irrespective of dose. In the *in vitro* study, we compared effect of propofol on myotubes with control cells incubated in fresh medium or exposed to either lipid vehicle (Intralipid) or non-esterified fatty acids (NEFA) mixture resembling Intralipid composition (55% of linoleic acid, 27% of oleic acid and 10.5% of palmitic acid). Cell viability and basal oxygen consumption were influenced by only the highest propofol concentration (10 µg/mL), whilst the lowest concentration already caused decrease of maximal respiratory capacity. Exposure to propofol caused a mild uncoupling of inner mitochondrial membrane, irrespective of propofol dose. Individual complexes of the respiratory chain were not inhibited. The most significant propofol-induced abnormality was inhibition of fatty acid oxidation to 36% and 33% of baseline values (exposure to 2.5 and 10 µg/mL of propofol, respectively).

**Conclusion:** Diagnosis of PRIS may be challenging as some of its typical features are often (>95%) missing (hypertriglyceridemia, fever, hepatomegaly, cardiac failure) and others (e.g. arrhythmias) appear later. Clinical features of PRIS are suggestive of its mitochondrial origin. Our *in vitro* study showed that propofol decreases maximal respiratory capacity and causes profound inhibition of fatty acid oxidation in human skeletal muscle.