Obesity is a worldwide health problem which occurs in industrialized countries and influences the duration and quality of life. Currently few data have been reported on the impact of obesity in the kidney and its link with insulin resistance and metabolism [1].

A good translational animal model to study obesity is represented by leptin-deficient homozygous mice (ob/ob), that display hyperphagy, over-weight, hypertension and insulin resistance like human subjects. Considering that mitochondria are favourite target for excessive energy requirement in obesity [2] and that restoration of their proper structure is necessary to renal activity, we tested the efficacy of melatonin, the indoleamine of the pineal gland, in mitochondria in the ob/ob mice kidney.

This microscopic study aimed to demonstrate the anti-oxidant role of melatonin in the obese mice kidney, by focusing on proximal tubular epithelium mitochondria morphology and on the renal localization of markers of mitochondrial health and apoptotic signalling. Twenty male mice (3 weeks of age) were organized into four groups containing both C57BL6 lean, as controls, and ob/ob supplemented or not with melatonin in drinking water (100 mg/kg/day) for 8 weeks. Kidneys were extracted and processed for histopathological (H&E and PAS), immunohistochemical and ultrastructural analysis. Body and kidney weights were estimated in all groups. Mitochondria health was evaluated by immunofluorescence of mitofusin 2 (Mf2), a resident protein involved in mitochondria metabolism, and by TEM analysis, the gold technique to visualize mitochondria structure and density. Furthermore renal expression of Bax, a member of Bcl2 family associated to mitochondria-triggered apoptosis, cytochrome c and caspases were visualized at a CLSM.

Melatonin treatment did not modify body and kidney weights in lean group but significantly reduced body and kidney weights in the obese mice. Moreover melatonin did not affect renal ultrastructure mitochondria, nor stimulated apoptosis in controls, that showed intense Mf2 signal. By contrast, in ob/ob mice kidney, Mf2 fluorescence disappeared and mitochondria were round, with short peripheral cristae (Figures 1-2). Moreover Bax, cytochrome c, caspases 9 and 3 were visualized in cortical tubules. Remarkably, in ob/ob mice receiving melatonin, Mf2 staining appeared again, even if at lesser grade than in controls (Figure 3), mitochondria were elongated (Figure 4), while apoptotic markers weak. These novel observations suggest that melatonin, by restoring tubular Mf2 signal, influences mitochondria in ob/ob mice kidney and limits the onset and progression toward apoptosis.


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Fig. 1: Mitofusin 2 (Mf2) immunofluorescence in ob/ob mice kidney. Note almost complete disappearance of Mf2 green signal in tubules, in blue DAPI-positive nuclei and glomerulus (400x) CLSM ZEISS 510Meta

Fig. 2: TEM micrograph of proximal tubular mitochondria in ob/ob mice. Note hydropic appearance, round shape and short peripheral cristae bar=1µm TEM FEI Tecnai G2 Spirit

Fig. 3: Mf2 immunofluorescence in ob/ob mice kidney receiving melatonin. Note restored Mf2-green staining in cortical tubules, DAPI-positive blue nuclei and glomerulus (400x) CLSM ZEISS 510Meta

Fig. 4: TEM micrograph of proximal tubular mitochondria in ob/ob mice kidney receiving melatonin. Note elongated mitochondria in basal infoldings with regular cristae. bar=2µm TEM FEI Tecnai G2 Spirit