# Dynamics of Changes in Skin Lipids after Stress: Effects of Exogenous Melatonin

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Changes in the skin lipid composition induced by water-immersion stress in rats treated and untreated with melatonin were studied by thin-layer chromatography. Skin lipids showed a delayed reaction to stress. Melatonin exerted a protective effect which was manifested on the 2nd day after treatment in restoration of the level of total lipids and the absolute content of the majority of lipid fractions. The data suggest modification of the metabolic relationships between skin lipids as well as lipids of the blood and subcutaneous adipose tissue.

**Key Words:** *stress: skin; lipids; melatonin*

Recently, growing attention has been focused on the pineal hormone melatonin. Extensive studies of melatonin have been stimulated by a broad spectrum of its physiological effects: it is involved in the maintenance of circadian rhythms, regulation of physiological and biochemical properties of the skin, antioxidant defense, and other functions [1,2,7,8]. Melatonin is considered to be a universal inhibitor of the endocrine system [6]. This hormone was reported to affect the hypothalamic-pituitary-adrenal system which participates in the body's response to stress [2,9]. Hence, it can be suggested that melatonin administered *in vivo* should exert a stress-protective action.

Here we studied the changes in skin lipids after water-immersion stress in rats treated and untreated with melatonin.

## MATERIALS AND METHODS

Experiments were carried out on 72 male Wistar rats weighing 224.7±20.6 g. The rats were housed in groups of 4 under natural illumination (light period from 7:05 to 20:10), at 20-22°C, with free access to food and water. The experiments were performed in the daytime (10.00-16.00). Groups 1 and 2 animals received intraperitoneal injections of sterile saline (1 ml), groups 3 and 4 received intraperitoneal injections of melatonin (1 mg/kg in 1 ml saline). Groups 2 and 4 rats were exposed to 4-h water-immersion stress immediately postinjection. The rats were decapitated 6, 24, and 48 h postinjection and skin samples (80-270 mg) were taken from the interscapular area. Lipids were extracted by Folch's technique and fractionated by thin-layer chromatography on silica gel [4]. The contents of total lipids (TL) and individual fractions were measured as described previously [4].

We determined the concentration of TL (mg/100 ml wet tissue) and the absolute and relative concentrations of the major lipid fractions (percent of total lipids): phospholipids, diglycerides, non-esterified cholesterol, free fatty acids (FFA), triglycerides, and cholesterol esters (CE). The data were treated statistically [5].

## RESULTS

In the control group injected with saline, the tissue content of TL ranged from 1674.2 to 1795.6 mg/100 ml (Tables 1 and 2) and remained at approximately the same level throughout the observation period.

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After 6 h, the content of TL in rats subjected to stress and treated with melatonin little differed from the control, but significantly increased after 24 h. Water immersion elevated TL by 35.6%, melatonin by 46.1%, and the two factors acting together increased the TL content by 55.2%.

Diverse changes in the TL content were observed 48 h postinjections. In groups 2 and 3 rats (saline+stress and melatonin, respectively), the skin content of TL remained at the 24-h level, while in group 4 rats (melatonin+stress) it returned to the initial level.

Triglycerides, phospholipids and CE were the major lipid fractions in the skin from control rats (Table 2). The total fraction of diglycerides, cholesterol, and FFA did not exceed 5.4-10.3%. Only insignificant variations in the content of some lipid fractions occurred during the observation period.

Significant differences in the relative and absolute content of lipid fractions in experimental groups 2, 3, and 4 were observed 24 h postinjection (Tables 1 and 2), while after 6 h these indices did not differ from the controls. The highest lability was characteristic of triglycerides, phospholipids, FFA, and sometimes of cholesterol.

An increase in the absolute content of all fractions occurred in group 2 rats 24 h postinjection (Table 1). This elevation was especially pronounced with respect to phospholipids, triglycerides and CE, although their relative fractions remained almost the same (Table 2). Both the absolute and relative contents of triglycerides tended to decrease on the next day with simultaneous increase in CE.

The above-described relations between the lipid fractions can be illustrated by the following scheme:

$$\text{Triglycerides}\downarrow \rightarrow \text{FFA}\uparrow \rightarrow \text{CE}\uparrow \leftarrow \text{Cholesterol}\downarrow,$$

where $\uparrow\downarrow$ indicate elevation and decrease in lipid fractions, respectively.

A 30.5-55.8% increase in the absolute content of all fractions was observed in the melatonin-treated rats (group 3) 24 h postinjection, in particular, the content of triglycerides increased by 55.8 %, FFA by 54.3%, and cholesterol by 52.9% (Table 1). However, the relative content of phospholipids and CE tended to decrease with a parallel elevation of triglycerides (Table 2). After 48 h the absolute contents of phospholipids and triglycerides returned to the control with simultaneous elevation of total cholesterol (cholesterol+CE) (Table 1). Similar changes were observed when calculating the percent ratios of the lipid fractions (Table 2).

Metabolic changes in lipid fractions by the end of day 2 can be schematized as follows:

$$\begin{array}{c}\text{Phospholipids}\downarrow \\ \text{Triglycerides}\downarrow \rightarrow \text{FFA}\uparrow \rightarrow \text{Cholesterol}\uparrow \rightarrow \text{CE}\uparrow.\end{array}$$

The dynamics of changes in the skin lipids in group 4 rats subjected to stress after melatonin injection retained some features of the individual effects of melatonin and stress. Twenty-four hours postinjection, the absolute content of all fractions was elevated, while their relative content remained at the control level, except for phospholipids. The decrease in phospho-

**TABLE 1.** Absolute Contents of Skin Lipids in Melatonin-Treated and Stressed Rats (mg/100 ml wet tissue, $M\pm m$, $n$=4)

| Groups and time after melatonin injection, h | | TL | Phospholipids | Diglycerides | Cholesterol | FFA | Triglycerides | CE |
|---|---|---|---|---|---|---|---|---|
| 1 | 6 | 1735.4±82.3 | 351.2±44.9 | 111.0±14.7 | 136.3±10.9 | 180.2±53.2 | 686.2±14.1 | 270.5±19.2 |
| | 24 | 1674.2±102.9 | 317.9±47.3 | 113.2±31.0 | 120.6±24.8 | 196.4±71.6 | 649.8±45.9 | 276.2±41.9 |
| | 48 | 1795.6±121.5 | 372.9±33.2 | 127.8±14.6 | 136.5±9.4 | 176.5±20.1 | 688.3±23.0 | 293.7±38.7 |
| 2 | 6 | 1679.1±143.5 | 353.6±45.3 | 94.3±12.2 | 120.0±9.1 | 150.6±47.8 | 663.6±14.3 | 296.8±17.8 |
| | 24 | 2276.3±93.1*$^{x}$ | 470.7±13.4* | 121.1±13.3 | 158.6±18.0 | 207.6±47.5 | 909.6±58.5* | 408.6±13.4* |
| | 48 | 2277.9±104.7* | 488.8±29.7* | 130.0±15.7 | 139.4±14.1 | 236.9±46.0 | 821.9±68.5 | 461.7±41.3* |
| 3 | 6 | 1558.6±178.9 | 305.0±44.4 | 99.1±19.1 | 104.3±17.6 | 175.7±15.2 | 620.8±67.4 | 253.7±35.8 |
| | 24 | 2277.1±136.2*$^{x}$ | 407.7±28.1 | 138.1±33.6 | 159.1±12.2$^{x}$ | 270.2±35.0$^{x}$ | 966.4±28.3*$^{x}$ | 335.5±26.5 |
| | 48 | 2107.4±88.1* | 317.9±23.4$^{x}$ | 138.8±29.3 | 192.9±17.5* | 274.3±26.9* | 708.7±29.6$^{x}$ | 474.9±27.6*$^{x}$ |
| 4 | 6 | 1691.0±179.5 | 345.1±49.6 | 101.1±18.8 | 105.5±15.5 | 168.5±19.5 | 683.0±73.1 | 287.6±42.0 |
| | 24 | 2624.8±116.0$^{x}$ | 449.6±31.6 | 145.4±45.4 | 184.2±12.9$^{+}$ | 278.5±53.9 | 1115.3±90.0* | 451.7±36.2* |
| | 48 | 1627.9±91.5$^{o+x}$ | 375.7±25.7$^{o}$ | 91.4±20.7 | 105.6±7.2*$^{x+}$ | 202.6±26.1 | 490.1±44.1*$^{o+x}$ | 362.2±37.0 |

**Note.** Here and in Table 2: $p<0.05$: *in comparison with group 1; $^{o}$in comparison with group 2; $^{+}$in comparison with group 3; $^{x}$in comparison with previous test in the same group.

**TABLE 2.** Relative Contents of Skin Lipids in Melatonin-Treated and Stressed Rats (% of Total Lipid Fractions, $M\pm m$, $n$=4)

| Group | Time, h | Phospholipids | Diglycerides | Cholesterol | FFA | Triglycerides | CE |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 21.8±2.8 | 5.4±0.7 | 6.9±0.6 | 9.1±2.7 | 40.8±0.8 | 16.1±1.1 |
| | 24 | 20.4±3.1 | 5.7±1.6 | 6.3±1.3 | 10.3±3.8 | 40.2±2.8 | 17.0±2.6 |
| | 48 | 22.3±2.0 | 6.0±0.7 | 6.7±0.5 | 8.6±1.0 | 39.5±1.3 | 16.9±2.2 |
| 2 | 6 | 21.1±2.8 | 5.6±0.7 | 7.2±0.6 | 9.0±2.7 | 39.5±0.8 | 17.6±1.1 |
| | 24 | 20.7±0.6 | 5.3±0.6 | 7.0±0.8 | 9.1±2.0 | 40.0±1.2 | 17.9±0.7 |
| | 48 | 21.5±1.3 | 5.7±0.6 | 6.1±0.6 | 10.4±1.9 | 36.0±1.1$^{x}$ | 20.3±0.6$^{**x}$ |
| 3 | 6 | 21.0±3.0 | 5.4±1.0 | 5.9±1.0 | 9.9±10.5 | 41.0±4.5 | 16.8±2.4 |
| | 24 | 19.3±1.8 | 5.1±1.3 | 6.1±0.5 | 10.4±1.7 | 43.9±1.3 | 15.2±1.2 |
| | 48 | 16.2±1.5* | 5.6±1.2 | 8.0±0.7$^{x}$ | 12.3±1.1* | 34.7±1.5$^{**x}$ | 23.2±1.4$^{**x}$ |
| 4 | 6 | 20.4±0.6 | 6.0±1.0 | 6.2±1.0 | 10.0±1.1 | 40.4±4.4 | 17.01±2.4 |
| | 24 | 17.1±1.2$^{ox}$ | 5.5±1.6 | 7.0±0.5 | 10.6±2.1 | 42.5±3.1 | 17.21±1.2 |
| | 48 | 23.1±1.7$^{x}$ | 5.6±1.2 | 6.5±0.5 | 12.5±1.2* | 30.1±2.5$^{**x}$ | 22.25±1.7$^{x}$ |

lipid content, which manifested itself as a tendency in group 3 (Table 2), became significant in group 4: 16.1% ($p$<0.05) compared with the initial level. The absolute content of TL dropped by the end of the observation period (Table 1). The content of triglycerides, cholesterol, and FFA underwent the most dramatic changes. The level of triglycerides was far below the control (by 28.8%). Complex changes were observed in the relative content of the lipid fractions (Table 2): a more pronounced decrease (compared to groups 2 and 3) in the triglyceride level (26.8%) was accompanied by a 29.6% increase in the CE fraction. In contrast to group 3 where phospholipid content was slightly lowered, group 4 revealed a 22.1% increase in this lipid fraction.

Thus, pretreatment with melatonin resulted in more complex perturbations in skin lipids after water immersion stress: TL content was sharply reduced, while the absolute and, to a lesser extent, relative content of the major lipid fractions was normalized. These changes can be considered as manifestations of the protective effect of melatonin on skin lipids under conditions of water-immersion stress and can probably be explained by switching between the transacylase and hydrolase mechanisms [3] responsible for metabolic relationships between skin lipid fractions. It seems likely that stress-induced modifications of skin lipids are intimately related to lipid metabolism in other tissues, in particular blood and subcutaneous fat.

Our data showed that pretreatment with melatonin significantly affected the dynamics of post-stress changes in skin lipids. The protective effect of melatonin became evident after 2 days and manifested itself in restoration of the TL level and the absolute content of the majority of lipid fractions. At the same time, their relative content in this period significantly differed from the controls and this should be taken into consideration when assessing the changes in biochemical parameters under the influence of extreme factors.

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