

Correlation between cortisol levels in cats' claws and hair

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Summary

The accumulation of cortisol in hair and claws, which grow slowly, makes it possible to retrospectively assess chronic stress levels. The study involved 47 cats from a shelter for homeless animals. Hair from the lumbosacral region and the dead part of claws from the front paw were collected. The average cortisol levels were 0.85 ng/ml in the cats' hair and 4.56 ng/ml in the claws. There was no significant relationship between cortisol levels in hair and claws. To date, too little research has been done on the correlation between cortisol levels in the hair and claws of cats. It is insufficient to unequivocally prove the existence of such a relationship.

Keywords: feline, stress, glucocorticoids, shelter

Studies of responses to stress (both acute and chronic stress) routinely use the measurement of cortisol as an indicator of hypothalamic-pituitary-adrenal (HPA) axis activity (12). The use of hair and claws as biological material has become very popular because of their durability and non-invasive collection method (6). The advantage of measuring cortisol in hair and nails/claws is that samples can be collected quickly and with minimal stress for humans, chickens, cats, dogs, cattle, sheep, goats, and pigs, and then stored for a long time at room temperature (2). Because cortisol accumulates in hair and nails, which grow slowly, a single measurement of this hormone reflects its concentration in the body over an extended period. The hair growth rate is approximately between 1 cm (16) and 1.3 cm per month (2). Human nails grow by 3 mm per month (8). Contreras et al. (6) reported that the front claws of cats grew by 2.4 mm over a three-week period. Human nail growth rates differ significantly between hands and feet, or even between fingers of the left and right arm (3). Nails can be obtained more easily from most people, including babies and children, who often lack enough hair (17). Some animals do not even have

hair. Nails may also offer a retrospective timeline of chronic stress, since they are a keratinised matrix, similar to hair (21).

Cieszynski et al. (4) found no correlations between the cortisol concentration in hair and single-point cortisol assessment in blood, saliva, and urine in human patients with reference cortisol levels. Mohan et al. (19) studied the relationship between cortisol levels in pigs' plasma, saliva, urine and faeces. They found that the levels of cortisol in saliva reflected its plasma levels at the time of collection most closely among the biological samples studied. Correlations between cortisol levels in hair and nails were studied by Nejad et al. (20). They found significant correlations in cortisol levels between fingernails and toenails, between fingernails and facial hair, and between toenails and facial hair. So far, only Contreras et al. (6) have studied the correlation between cortisol levels in the hair and claws of cats. In their study, hair and nail cortisol concentrations were significantly correlated.

Chronic stress has a destructive effect on the body, adversely affecting health, predisposing to the development of behavioral disorders, and limiting the adapta-

tion capabilities of animals in shelters (15). Chronic stress in cats causes them to neglect the care of their coat, as well as predisposes them to disorders in the excretory, digestive and respiratory systems (24). Some of the most common consequences of long-term stress in cats are aggressive behavior and defecating in inappropriate places (23).

The present study aimed to determine whether there was a correlation between cortisol levels in hair and claws in a group of shelter cats.

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Material and methods

Approval for the study was obtained from the Animal Welfare Committee of the Faculty of Animal Sciences and Bioeconomy of the University of Life Sciences in Lublin, Poland (ZdsDZ/1/2022 of May 25, 2022).

The study involved 47 cats from a shelter for homeless animals. Among the cats, there were 17 females (3 were intact) and 30 males (two intact). They were mixed breed cats. Only non-aggressive cats adequately socialized with humans were selected for the study. In addition, those cats were not stressed by hair and claw cutting and did not require forced immobilization. The veterinarian determined the cats' approximate age and health status upon admission to the shelter. Basic morphological blood tests and urine analysis were performed. The test results were within reference values. The veterinarian described the cats as healthy. The animals had stayed at the shelter for at least one month. They had last had their claws trimmed at least three weeks earlier. Hair from the lumbosacral region and the dead part of several claws from a forelimb was collected. The hair was cut with scissors close to the skin, and the claws were trimmed with commonly available clippers. The biological material was stored in foil bags at room temperature until laboratory analysis.

The extraction methodology was developed based on Koren et al. (14), Accorsi et al. (1), and Fusi et al. (9) and used by the authors of this study in their previous research (28). Twenty milligrams of hair chopped into 1-2 mm fragments was put in a glass vial. Claws were chopped into 0.5-1 mm fragments, and 10 mg was put in another glass vial. Methanol (Sigma-Aldrich, Poznań, Poland) were added, and vials were incubated at 50°C with gentle shaking for 24 h. After incubation, the supernatant was filtered to separate the liquid phase and put into disposable glass culture tubes. Next, this supernatant was evaporated to dryness under an air-stream suction hood at 37°C. The dry residue was then dissolved in 1 ml (in the case of hair) or 200 µl (in the case of claws) of phosphate-buffered saline (PBS) 0.05 M, pH 7.5. The samples were vortexed for one minute, followed by another 30 seconds until they were well mixed.

Cortisol concentrations in the samples were determined with the DRG Salivary Cortisol HS ELISA assay. The procedures followed the manufacturer's instructions. Cortisol concentrations were expressed in ng/ml.

Statistical analysis was performed with Statistica 13.1. The relationship between the levels of cortisol in the hair and claws was assessed on the basis of Pearson's correlation coefficients. Statistically significant results were those at $p < 0.05$.

Results and discussion

The average cortisol levels were 0.85 ng/ml in the cats' hair and 4.56 ng/ml in their claws. This difference was statistically significant ($p < 0.0001$). The average cortisol level was also assessed, broken down by the sex of the cats. The average cortisol level in the males' hair was 0.67 ng/ml, which was lower than 1.15 ng/ml in the females' hair. This difference, however, was not statistically significant. The average cortisol level in the males' claws, 5.15 ng/ml, was higher than that in the females' claws, 3.53 ng/ml. This difference was not statistically significant either.

When assessing the relationship between the cortisol level in the hair and the cortisol level in the claws (without distinction of sex), no significant relation-

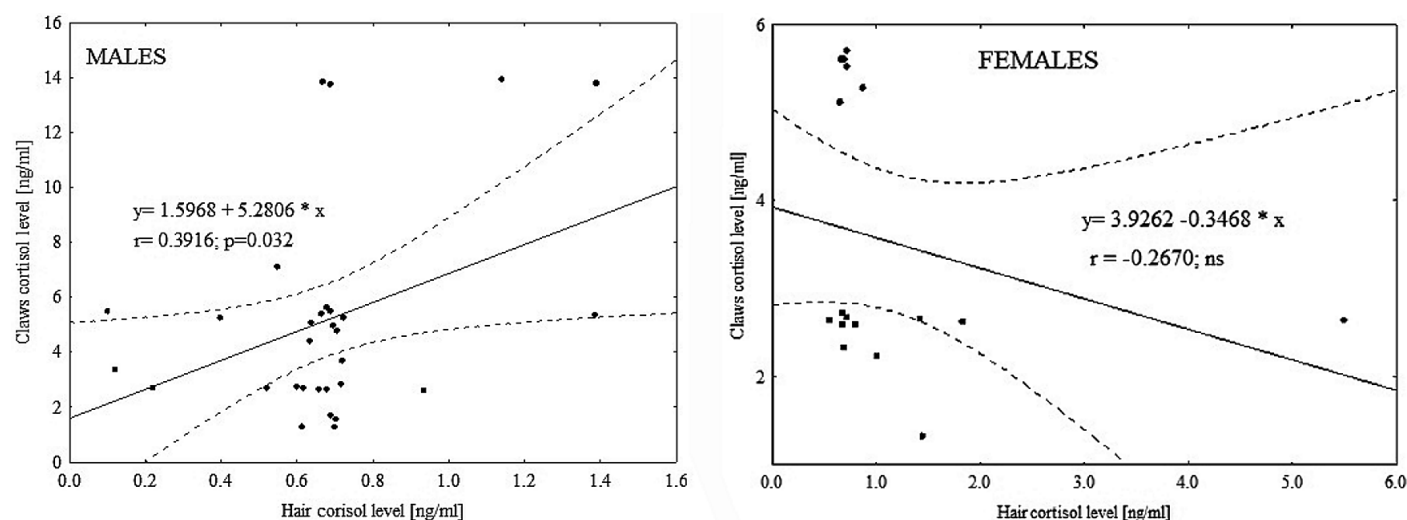


Fig. 1. Relationship between hair and claw cortisol levels in male and female cats

ship was found between the parameters compared ($r = -0.035$; $p = 0.812$). However, considering the sexes separately, we found a positive relationship between the level of cortisol in the hair and claws of male cats ($r = 0.3916$), and this relationship was statistically significant ($p = 0.032$) (Fig. 1).

The results of studies on cortisol levels in hair and nails in both humans and animals vary. In our study, the average cortisol level in the cats' hair was 0.85 ng/ml. In our previous studies (27), in which the research group consisted of client-owned cats, their average hair cortisol level was 0.45 ng/ml, i.e. almost half as much as it was in the shelter cats. However, in the aforementioned group of client-owned cats, animals behaving aggressively towards household members had high levels of cortisol, as did the shelter cats (27).

Values of the average cortisol level in the hair of cats can also be found in studies by other authors. For example, in those by Galuppi et al. (11), the concentration of cortisol in cats' hair ranged from 0.02 to 76.27 pg/mg (median = 2.39, range = 76.27). In studies by Finkler & Terkel (7), cortisol levels were 1.997 ± 0.637 pg/mg in the hair of neutered female cats and 10.956 ± 2.894 pg/mg in the hair of intact female cats. With regard to humans, for example, in studies by Cieszyński et al. (5), hair cortisol levels ranged from 2 pg/mg up to 51.63 pg/mg.

In their research on dogs, Fusi et al. (10) found a significant positive correlation ($r = 0.277$; $p < 0.05$) between coat and claw cortisol concentrations. Veronesi et al. (25) found a highly significant positive correlation between puppy coat and claw cortisol levels ($r = 0.68$, $p < 0.0001$). However, nail cortisol concentrations were not significantly correlated with hair cortisol concentrations in dogs ($r = 0.366$, $p = 0.15$, $n = 19$) in a study by Mack and Fokidis (18). In our studies, there was no significant relationship between cortisol levels in the hair and claws of cats. We collected hair and claws from cats at the same time. Some researchers have observed differences in cortisol levels during material collection. For example, cortisol levels in hair significantly correlated with those in fingernails that were collected only three months after the hair collection ($r = 0.48$, $p < 0.05$) (13).

With regard to human hair and nails, Voegel et al. (26) found no significant correlation between cortisol concentrations in hair and nails ($r = 0.12$, $p > 0.05$). However, in studies on stress in humans, Nejad et al. (20) found significant correlations between cortisol levels in fingernails and facial hair ($p = 0.01$, $r = 0.54$) and between those in toenails and facial hair ($p = 0.001$, $r = 0.73$).

To our knowledge, cortisol levels in the claws of cats have been evaluated only by Contreras et al. (6). They found a significant positive association between cortisol levels in hair and claws ($r_s = 0.70$; $p < 0.001$). However, our study found such a relationship only in male cats. We suppose that the statistically significant

correlation between cortisol levels in the hair and claws of male cats was due to the greater number of male cats than female cats in our study.

In our studies, cortisol levels in claws were higher than they were in hair. For comparison, cortisol concentrations in wolf claw samples were lower than in hair ($p = 0.001$) and undercoat ($p = 0.001$) in research by Roffler et al. (22). In a study by Voegel et al. (26), statistical analysis revealed higher concentrations of cortisol ($p < 0.0001$) in human hair compared to nails.

The results of studies conducted so far on the relationship between cortisol levels in hair and nails or claws, both in humans and animals, are inconclusive. The present study confirms this relationship only in male cats. More research is needed in this direction, especially regarding cats.

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