Thesis

Measuring COVID-19 related stress in medical personnel

submitted by

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Graz, 23.11.2022

Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 23.11.2022 Philipp Wöhler m.p.

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Abbreviations

HPA: Hypothalamic-pituitary-adrenal

ANS: Autonomic nervous system

SNS: Sympathetic nervous system

PNS: Parasympathetic nervous system

CNS: Central nervous system

CRH: Corticotropin-releasing hormone

CRF: Corticotropin-releasing factor

CRFR1: Corticotropin-releasing factor receptor 1

POMC: Proopiomelanocortin

PVN: Paraventricular nucleus

AVP: Arginine Vasopressin

cAMP: Cyclic adenosine monophosphate

ACTH: Adrenocorticotropic hormone

GABA: γ-aminobutyric acid

MC2R: Melanocortin 2 receptor

β-LPH: β-lipotropin

RAAS: Renin-angiotensin-aldosterone system

Epi: Epinephrine

NE: Norepinephrine

SAMS: Sympathoadrenal medullary system

GI tract: Gastrointestinal tract

HMG-CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A

HSL: Hormone-sensitive lipase

CoA: Acyl-Coenzyme A

ACAT: Cholesterol Acyltransferase

SCC: Cholesterol side-chain cleavage enzyme

CBG: Corticosteroid binding globulin

11β-HSD: 11β-hydroxysteroid dehydrogenase

PEPCK: Phosphoenolpyruvate carboxykinase

IL-2: Interleukin 2

MAO: Monoamine oxidase

COMT: Catechol-o-methyltransferase

UFC: Urinary free cortisol

HCC: Hair cortisol concentration

HTC: Hair testosterone concentration

PSS: Perceived stress scale

TICS: Trier Inventory for the Assessment of Chronic Stress

GnRH: Gonadotropin-releasing hormone

LH: Luteinizing hormone

FSH: Follicle-stimulating hormone

SARS-CoV-2: Severe acute respiratory syndrome Coronavirus 2

COVID-19: Coronavirus disease 19

SARS-CoV: Severe acute respiratory syndrome coronavirus

MERS-CoV: Middle East respiratory coronavirus

CFR: Case fatality rate

ELISA: Enzyme-linked immunosorbent assay

PBS: Phosphate-buffered saline

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Zusammenfassung

Einleitung: Im Rahmen der COVID-19 Pandemie kam es im Zeitraum vom 16.03.2020 bis zum 01.05.2020 in Österreich zu besonderen Belastungen der Bevölkerung und insbesondere des medizinischen Personals (1). Um dies zu quantifizieren, wurden in dieser Studie die Haar-Cortisol-Konzentration (HCC) und die Haar-Testosteron-Konzentration (HTC) von medizinischem Personal gemessen.

Methodik: Im Zeitraum vom 18.06.2020 bis 15.08.2020 wurden in Graz und der näheren Umgebung Haarproben von insgesamt 396 Probanden gesammelt, von denen 67 in dieser Studie untersucht wurden. Die Haarproben wurden gewaschen, getrocknet und gewogen, bevor die Hormone mit Aceton und Methanol extrahiert wurden. Daraufhin wurde ein ELISA mit anschließender Spektralphotometrie durchgeführt. Zur statistischen Analyse wurde jeweils eine ANCOVA durchgeführt und mögliche Einflussfaktoren auf HCC und HTC wurden mittels Mann-Whitney-U-Test untersucht.

Ergebnisse: Der mittlere HCC-Wert stieg von 13,698 ng/mg im ersten Monat auf 20,436 ng/mg im fünften Monat an (49,19 % Anstieg). Die Signifikanz des HCC-Anstiegs wurde durch eine ANCOVA ($F(1, 190) = 3,538$, $p=.012$, $\eta2=.059$) bestätigt, wobei sich Monat vier und fünf von den anderen Monaten unterschieden ($p<.001$). Der mittlere HTC-Wert stieg leicht von 0,988 pg/mg im ersten Monat auf 1,031 pg/mg im fünften Monat (4,35 % Anstieg), ein signifikanter Anstieg, der durch eine ANCOVA ($F(1, 190) = 3,656$, $p=0,22$, $\eta2=0,061$) bestätigt wurde, wobei sich Monat fünf von den anderen Monaten unterschied ($p<0,5$). Unter den weiteren Einflussfaktoren zeigten die Lebenssituation, Schichtarbeit und das Auftreten dramatischer Lebensereignisse einen Einfluss auf die Hormonkonzentrationen.

Schlussfolgerung: Die Ergebnisse deuten auf ein erhöhtes Stressniveau des österreichischen medizinischen Personals als Folge der COVID-19-Pandemie und der politischen Maßnahmen hin. In diesem Zusammenhang scheint die Haar-Cortisol-Konzentration als biologischer Stressmarker gut geeignet zu sein, während die Haar-Testosteron-Konzentration nur geringe Veränderungen zeigte.

Abstract

Introduction: In the context of the COVID-19 pandemic, the Austrian population and in particular the clinical personnel were confronted with several novel stressors in the period from March 16 to May 1, 2020 (1). To quantify this stress, hair cortisol concentration (HCC) and hair testosterone concentration (HTC) of medical personnel were measured in this study.

Methods: In the period from June 18 to August 15, 2020, hair samples were collected in Graz and the immediate surrounding area from a total of 396 participants, 67 of whom were examined in this study. Hair samples were washed, dried and weighed before extracting the hormones using acetone and methanol. An ELISA followed by spectrophotometry were performed. For statistical analysis, an ANCOVA was performed in each case and other factors influencing HCC and HTC were examined using a Mann-Whitney U test.

Results: Mean HCC increased from 13.698 ng/mg at month one to 20.436 ng/mg at month five (49.19% increase). The significance of the increase in HCC was confirmed by an ANCOVA ($F(1, 190) = 3.538$, $p=.012$, $\eta 2=.059$), with month four and five differing from the other months ($p<.001$). Mean HTC increased slightly from 0.988 pg/mg at month one to 1.031 pg/mg at month five (4.35% increase), a significant increase was confirmed by an ANCOVA ($F(1, 190) = 3.656$, $p=0.22$, $\eta 2=0.061$), with month five differing from the other months ($p<0.5$). Among the other influencing factors, living situation, shift work, and the occurrence of dramatic life events showed an influence on the hormone concentrations.

Conclusion: The results suggest an increased stress level of Austrian medical personnel as a consequence of the COVID-19 pandemic and the political measures. In this context, HCC seems to be well suited as a biological stress marker, whereas HTC showed only small changes.

1 Introduction

1.1 Physiology of stress

1.1.1 Definition and causes

Stress is characterized by complex physiological and behavioral responses to intrinsic or extrinsic, real or perceived adverse forces (stressors), which threaten homeostasis. These adaptive responses are depending on highly interconnected neuroendocrine, cellular and molecular mechanisms and are needed to maintain the optimal body equilibrium (eustasis). (2) On one hand, the stress response can be caused by environmental (physical) stressors like extreme temperature, noise, infectious agents or physical work and physiological stressors such as sleep deprivation, dehydration, hypotension, fever, malnutrition, hypoglycemia, illness or injury. (3-5) On the other hand, it can be caused by cognitive (psychological) stressors such as too much or too little information, ambiguity, uncertainty, isolation, time pressure, unpredictability, rules of engagement or difficulty and emotional (psychological) stressors like anxiety-producing threats, grief-producing losses, resentment, interpersonal feelings or spiritual confrontation. (3-5) Today's society is characterized by the increase of complex environmental conditions, such as social, professional, economic, and political circumstances and challenges, which lead to an increase in psychological and physical stressors. There are several key pathways belonging to the stress system, the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system (ANS) with its components, the sympathetic (SNS) and parasympathetic (PNS) nervous system, and the behavioral fight or flight response (6). Moreover, several areas of the central nervous system (CNS) play an important role in stress regulation like the prefrontal cortex, amygdala and hippocampus (7). The activation of these pathways leads to the release of distinct mediators like corticotropin-releasing-hormone (CRH), arginine vasopressin (AVP), the proopiomelanocortin- (POMC-) derived peptides ACTH, α-melanocyte-stimulating hormone and β-endorphin, the glucocorticoids and the catecholamines norepinephrine and epinephrin (8). These mediators promote the adaptation to an acute stressor by binding to specific receptors and by regulating various central and peripheral physiologic functions.

Chronic elevation of the same mediators can lead to pathophysiological changes, for example, in the cardiovascular system (7). As a result, stress can have both protecting and damaging qualities, that are determined by individually unique genetic, environmental, and developmental factors (2).

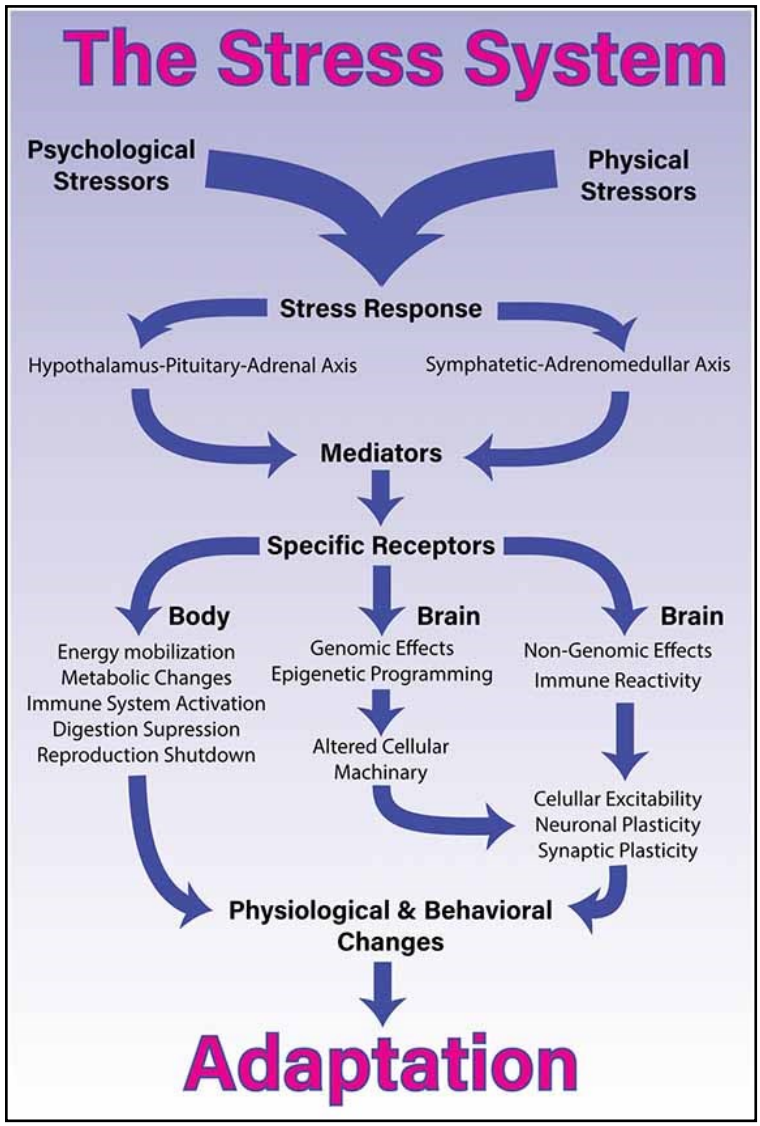


Figure 1: The stress system (9)

1.1.2 The HPA axis

The hypothalamic-pituitary-adrenal axis is a neuroendocrine pathway that represents a primary hormonal response to homeostatic challenge. Almost every stressor leads to an activation of this system, therefore it can be understood as a hallmark of the physiological stress response. (10)

Complex positive and negative feedback influences between the hypothalamus, pituitary gland and adrenal gland regulate the release of cortisol and other corticosteroids (11). These are essential for survival and regulate metabolic, immunologic, and behavioral functions as well as blood pressure and energy levels.

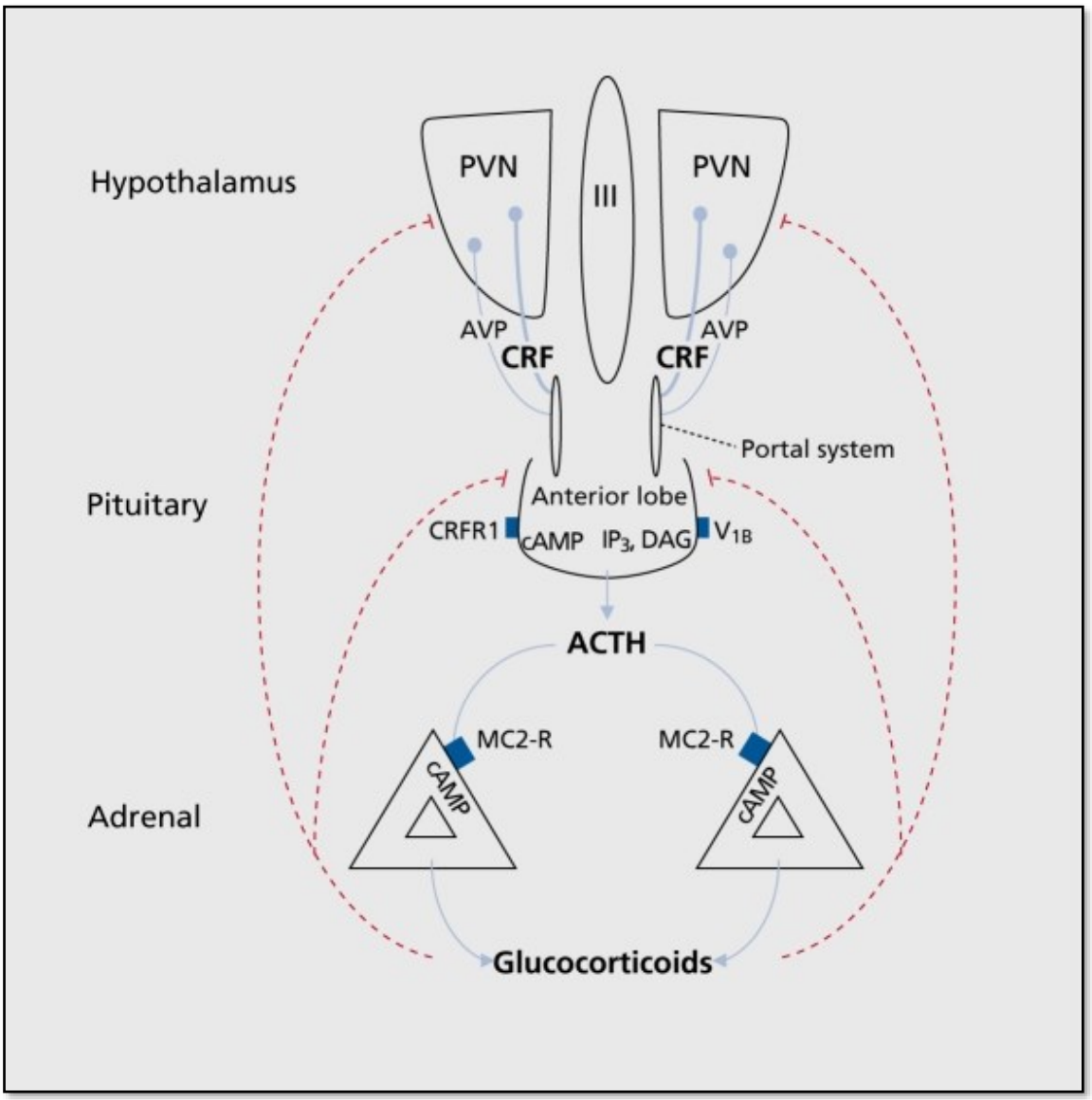


Figure 2: Hypothalamic-pituitary-adrenal control loop (12)

The paraventricular nucleus (PVN) of the hypothalamus is innervated by different afferent nerve projections in particular brain stem neurons, cell groups of the lamina terminalis, extra-PVN hypothalamic nuclei, and forebrain limbic structures. (12)

During stress, these brain regions transmit sensory information via nerve impulses to hypophysiotropic neurons of the PVN, which synthesize corticotropin-releasing hormone (CRH) and vasopressin (AVP) (12). The release is precisely coordinated by inhibitory effects (e.g., γ-aminobutyric acid [GABA] and opioids) and excitatory effects (e.g., norepinephrine and serotonin) (13). CRH is released into hypophysial portal vessels that access the anterior pituitary gland and binds to the CRF type 1 receptor (CRFR1) of hypophysial neurons. Binding activates the intracellular cyclic adenosine monophosphate (cAMP) pathway and leads to the production of proopiomelanocortin (POMC) which serves as the basis for several stress-related hormones such as adrenocorticotropic hormone (ACTH), β-lipotropin (β-LPH), and β-endorphin. (12, 13) β-LPH is a polypeptide with lipolytic functions and the precursor for β-endorphin which induces analgetic effects through opioid receptors and plays an important role in profound central effects characteristic of morphine (14). As a result of CRFR1 stimulation, ACTH is released from the anterior pituitary gland into the systemic circulation. Furthermore, AVP causes synergistic effects on ACTH release that are mediated through the vasopressin V_{1b} receptor. (12) Circulating ACTH then induces the release of glucocorticoids such as cortisol and corticosterone from the zona fasciculata of the adrenal gland by binding to the melanocortin 2 receptor (MC2R) (11). Furthermore, the release of mineralocorticoids like aldosterone from the zona glomerulosa and androgens from the zona reticularis is stimulated. Corticosteroids are the final product of the HPA axis and regulate a lot of different processes in various areas of the periphery and the central nervous system. They also play an important role in negative feedback mechanisms by inhibiting CRH neuronal activity in the PVN and secretion of ACTH in the pituitary gland. (10)

1.1.3 Physiology of glucocorticoids

The most important glucocorticoid in the human body is cortisol, a steroid hormone that is synthesized from cholesterol in the adrenal cortex (15). ACTH increases the production and availability of cholesterol by activation of HMG-CoA reductase, which is the rate limiting enzyme in cholesterol synthesis, increase of LDL-C esters uptake, activation of hormone-sensitive lipase (HSL) and inhibition of acyl-coenzyme A (CoA): cholesterol acyltransferase (ACAT). As shown below,

glucocorticoid production involves cholesterol desmolase (respectively cholesterol side-chain cleavage enzyme - SCC), 17α-hydroxylase, 3-β-hydroxysteroid dehydrogenase (3B), 21-hydroxylase and 11β-hydroxylase. (16)

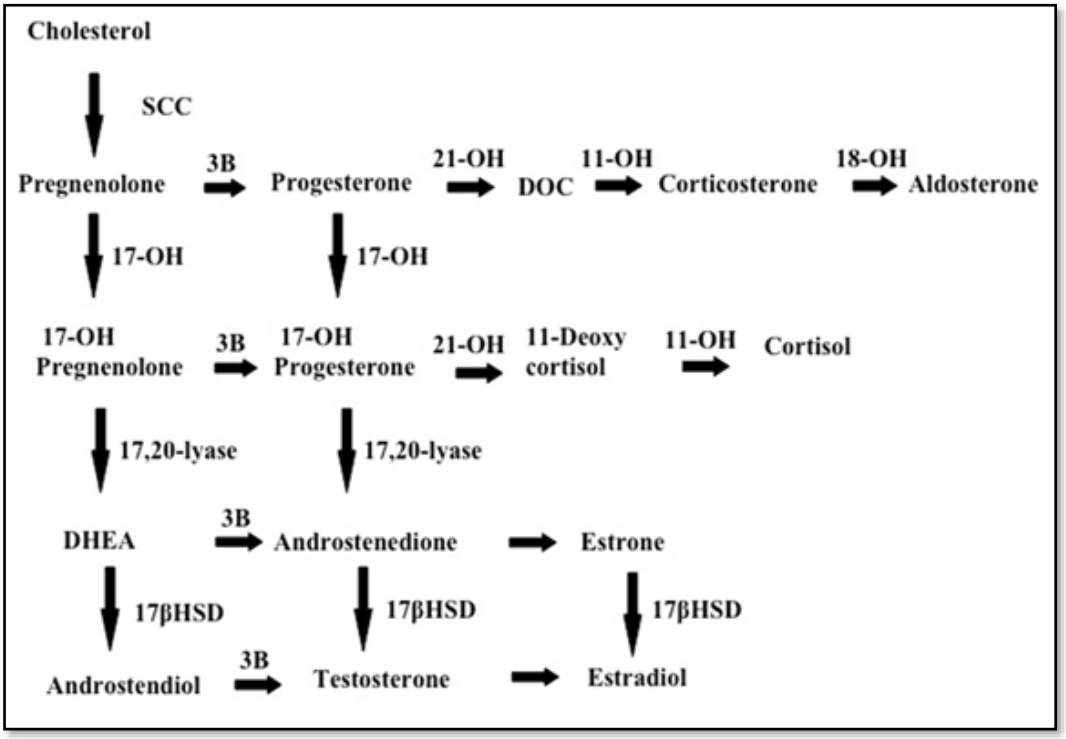


Figure 4: Main pathways of adrenal steroidogenesis (17)

In humans, 80-90% of the circulating glucocorticoids are bound to corticosteroid binding globulin (CBG) and 10-15% are bound to albumin, while only 5% are free and bioactive (18). In most tissues, glucocorticoid availability is sustained by 11β-hydroxysteroid dehydrogenases (11β-HSDs). 11β-HSD1 converts cortisone, another important glucocorticoid, to its more active form cortisol, while 11β-HSD2 facilitates the opposite conversion. Thus, glucocorticoid activity is precisely maintained on a cellular level. (19) Cortisol is a fat-soluble molecule that can cross the cytoplasmatic membrane of its target cells. After entering the cell, it binds to an intracellular glucocorticoid receptor (GR) and the cortisol-GR complex is translocated into the nucleus. The various hormonal effects are induced by affecting the expression in a variety of genes. Therefore, the cortisol-GR complex is a transcription factor and directly regulates DNA expression. (15, 20) Because glucocorticoids are essential for survival, they are secreted in a pulsatile pattern that follows a circadian rhythm. The highest levels are typically reached at around 8 AM, while the lowest levels can be measured between midnight and 4 AM. (16)

Nearly every tissue in the human body expresses glucocorticoid receptors. Thus, cortisol is able to affect almost every organ system, including the cardiovascular, respiratory, nervous, immune, reproductive, musculoskeletal and integumentary system. (15) Firstly, cortisol is involved in maintaining glucose homeostasis and inducing hyperglycemia at times of stress. This is achieved by upregulation and activation of enzymes (e.g., PEPCK) involved in gluconeogenesis and glycogenolysis, as well as antagonization of the actions of insulin, such as glycogen synthesis and glucose uptake by GLUT-4 transporters. Furthermore, glucagon secretion from the pancreas is increased. Secondly, glucocorticoids are causing a catabolic state in skeletal muscles, inducing peripheral muscle breakdown and mobilizing amino acids towards the liver to be used in gluconeogenesis. Thirdly, hormone-sensitive lipase (HSL) in the adipose tissue is activated and more fatty acids are available for β-oxidation. All these metabolic functions lead to a higher availability of glucose for the brain, skeletal muscles and red blood cells. (16, 21) Another important function of cortisol is immune system modulation. These effects are very extensive and complex but can be generally summarized as anti-inflammatory and immunosuppressive (22). For example, neutrophile migration in tissues is decreased, apoptosis and sequestration of eosinophils in the periphery is induced and interleukin-2 (IL-2) signaling is inhibited which causes an inhibition of T-cell proliferation. Moreover, degranulation of mast cells is reduced, a decrease of cytokine gene expression is caused by inhibition of the transcription factor NF-kB and B-cell antibody production is decreased. (22, 23) In the cardiovascular system, cortisol and other glucocorticoids take several effects like an increased glomerular filtration rate and glomerular hypertension, as well as the synthesis of angiotensinogen and atrial natriuretic peptide (ANP). Prostaglandin synthesis and thereby vasodilation is decreased, while responsiveness to vasopressors is increased. (24) Another function of glucocorticoids can be found in the GI tract, where the secretion of gastric acid is promoted and blood flow in the gastric mucosa is increased (24). Moreover, they reduce bone remodeling by directly affecting osteoblast, osteoclast, and osteocyte function. Renal calcium excretion is increased, while calcium absorption in the GI tract is decreased. This can lead to a lowered serum calcium concentration and a reactive elevation of parathormone. (24)

A lot of other bodily processes are influenced by glucocorticoids, such as fetal organ development, gonadal function, epithelial integrity, wound healing and distinct functions of the nervous system (23).

1.1.4 Physiology of mineralocorticoids

During activation of the HPA axis, ACTH also stimulates the secretion of mineralocorticoids, the most important being aldosterone. Other mechanisms of regulation are the renin-angiotensin-aldosterone system (RAAS) and concentration of plasma ions (especially K^+). (25) The most important function of aldosterone is the regulation of blood pressure by regulating electrolyte and fluid homeostasis. In the epithelia of the distal colon and renal nephron, Na^+ (re)absorption and K^+ secretion are stimulated and water follows the Na^+ movement via osmosis, increasing blood volume and therefore blood pressure. (26) Although, Aldosterone is not known for being a stress hormone, its secretion is partly regulated by the same mechanism as cortisol. Acute and chronic stressors may cause an alteration in mineralocorticoid regulation that could lead to stress related pathological changes, especially in the cardiovascular system (27).

1.1.5 The autonomic nervous system

Following the exposure to an acute stressor, the autonomic nervous system (ANS) acts as the most immediate response via its sympathetic (SNS) and parasympathetic (PNS) arms that induce rapid alterations in physiological states through neural innervation of end organs (28). Therefore, the slow acting stress response of the HPA axis is complemented by this faster acting system. Activity of the ANS is partly regulated by autonomic reflexes which are generated in major homeostatic control centers in the hypothalamus and brainstem after the transmission of sensory information from cranial and peripheral nerves. This information is integrated and a response is carried out by the transmission of nerve signals that modify the activity of preganglionic autonomic neurons. (28) In addition, these control centers are also affected by higher brain areas such as the cerebral cortex and the limbic system (28).

In this way, psychological and emotional stressors can lead to an activation of the ANS. The SNS promotes behavioral activation in response to an acute stressor to enhance physical ability (fight or flight response) by direct innervation of end organs (29).

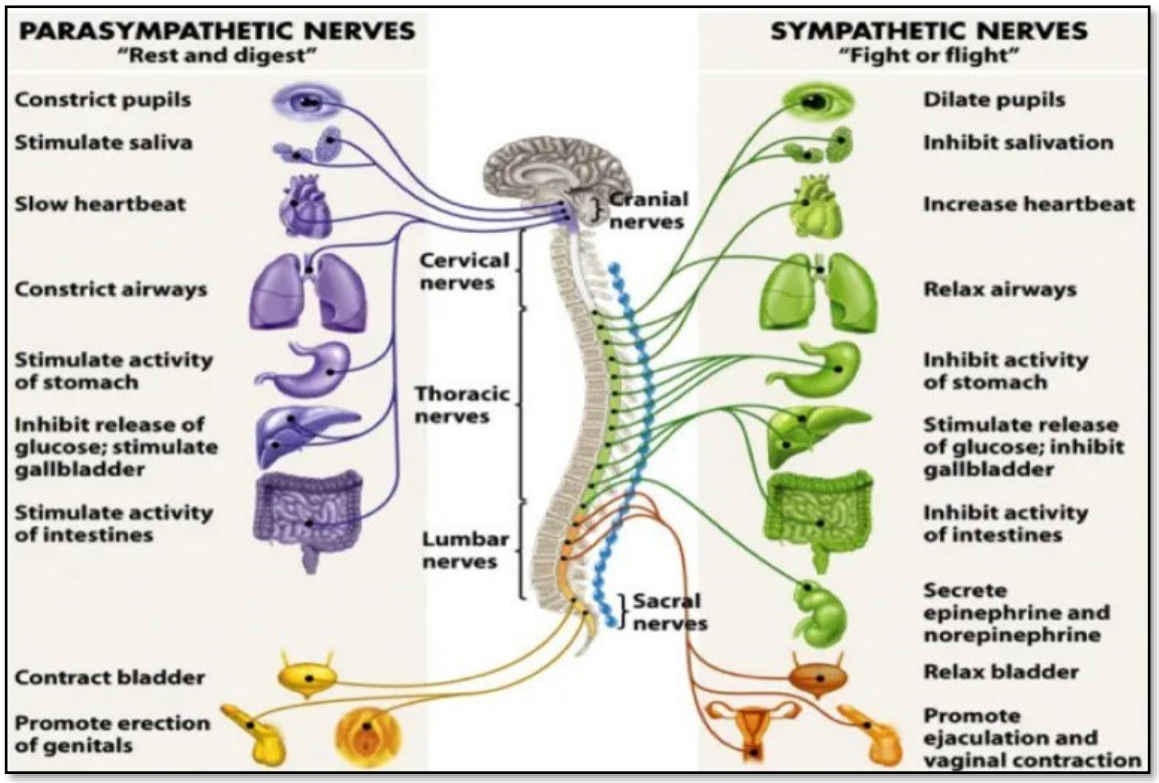


Figure 4: Sympathetic and parasympathetic nervous system (Taken from: https://fourdirectionswellness.com/2017/02/27/lets-talk-stress-kids/)

Moreover, SNS activity stimulates the biosynthesis and endocrine secretion of the catecholamines, epinephrine (Epi) and norepinephrine (NE) from chromaffin cells of the adrenal medulla which is innervated by preganglionic sympathetic fibers of the splanchnic nerve (Sympathoadrenal medullary system - SAMS) (30). Epi and NE are functioning as neurotransmitters in the central and peripheral nervous system as well as hormones, therefore they are called neurohormones. As circulating hormones, they are taking effect through binding to G Protein-coupled adrenergic α_1, α_2, β_1, β_2 and β_3 receptors (ARs). ARs are expressed in the heart, vascular system, lungs, GI tract, eyes, kidneys, liver, pancreas, endocrine glands, CNS, skeletal muscle and fat tissue. (31) Both Epi and NE are acting as powerful cardiac stimulants by increasing the heart rate and the force of myocardial

contraction (β-ARs). Redistribution of blood flow from organs like the kidneys, GI tract and skin is achieved by vasoconstriction in these organs and dilation of blood vessels in skeletal muscles. Vasoconstriction is mediated by α-ARs, whereas vasodilation is mediated by β_2-ARs. (31) Other effects of the catecholamines include the contraction of the pupillary dilator (α_1-ARs), piloerection (α_1-ARs) and relaxation of smooth muscles in the GI tract, urinary tract, and bronchioles (β_2-ARs). Furthermore, blood glucose levels are increased by stimulating glycogenolysis in the liver (β_2-ARs), increased glucagon secretion (β_2-ARs) and decreased insulin secretion (α_2-ARs) from the pancreas, and lipolysis in fat tissue (β_3-ARs). (32) These changes lead to a quick increase in the delivery of well-oxygenated, nutrient-rich blood to the working skeletal muscles. (33) Even though catecholamines do not cross the blood brain barrier, there is considerable evidence indicating that circulating Epi and NE bind to β-ARs at the intermedial spinal column of the spinal cord to stimulate the vagus nerve and evoke the release of NE from vagal afferents projecting to the nucleus tractus solitarii and the locus coeruleus (33-35). Activation of these brain areas further stimulates the release of NE from efferent fibers to stimulate neurons of the basolateral amygdala, increasing their firing rates and initiating signaling cascades to activate many other brain regions, including the prefrontal cortex, hippocampus, caudate nucleus, and nucleus accumbens (31, 36). As a result, circulating catecholamines can have an effect on several functions of the central nervous system, such as learning, memory, attention, mood, arousal and general human behavior (31). Epi and NE both have a rapid onset of action, but a short duration of action with a half-life of less than 5 minutes. They are quickly degraded into their inactive metabolites by monoamine oxidase (MAO) and catechol-o-methyltransferase (COMT). Metabolism is primarily in the liver, but also the kidneys, skeletal muscle and mesenteric organs. (37, 38) The PNS, on the other hand, is activated when the stressful situation is alleviated because the PNS and SNS are highly coordinated to maintain homeostasis (30). Parasympathetic actions are generally opposite to those of the sympathetic nervous system (28). Therefore, the PNS mediates a decrease in heart rate, constriction of the airways, stimulation of gastric motility and secretion, exocrine and endocrine secretion from the pancreas, contraction of the urinary bladder and contraction of the pupils (rest and digest) (39).

1.1.6 Acute vs. chronic stress

Under the right circumstances, the stress response is highly adaptive and improves the chances of survival (2). A common example is the typical fight or flight response of an animal when it is directly threatened by a predator. All stress induced changes like the dilation of bronchioles, increase in heart rate, better working muscles, dilation of pupils, etc. mobilize reserves of the prey animal and possibly are the crucial factor for escaping death. As soon as the animal is save again, the stress response is coming to an end. Because these events usually do not last very long and happen very unfrequently in the animal kingdom, there is enough time to recover from the situation. Humans in the western society are typically not threatened with death by any predators, but they still possess very similar physiologic mechanisms regarding the stress system. Modern stressors like job loss, divorce, death of a loved one or extreme working conditions can trigger the same reaction as in our animal ancestors. If the stressors, which can be of physiological or psychological nature, are only temporary and can be coped with effectively, this can be classified as acute stress. In case the stressor can be controlled constructively, it can even be a rewarding, pleasant or even exciting experience and lead to emotional and intellectual growth and development (2). If the stressful stimulus is perceived as too intense, or too long, an individual may fail coping with it and maladaptation can arise (40). The result is the development of chronic stress that can be detrimental to physiological functions and lead to several clinical manifestations. These include, but are not limited to, the development or exacerbation of mental illness such as depression and anxiety (41), increased risk of cardiovascular conditions such as hypertension (42, 43), obesity (44), type 2 diabetes (45), exacerbation of chronic obstructive pulmonary disease or asthma (46, 47), exacerbation of skin conditions such as psoriasis (48), increased risk of ulcerative colitis (49), reduced fertility (50), and poor pregnancy outcomes (51, 52). As the potency and duration of the stressor increases, the specificity of the adaptive responses decreases in order to eventually present the relatively nonspecific stress syndrome phenomenology. (2) Therefore, the efficiency and duration of the stress response is largely defined by the capability of the individual to anticipate and control stressful events (40).

1.1.7 Allostasis and allostatic load

The stress induced changes in homeostasis that ideally facilitate adaptation to the environment can be referred to as allostasis (53). The term was first mentioned by Sterling and Eyer in 1988. It is described as a critical principle of physiology in which an organism must vary the parameters of its internal milieu and match them appropriately to environmental demands to maintain stability. Sterling and Eyer refer to this principle as allostasis, meaning “stability through change”. (54) An example for an allostatic state is the elevation of catecholamines and glucocorticoids during physical activity to mobilize and replenish energy stores needed for brain and body function under challenge (55). Allostatic load describes the cumulative effect of the allostatic changes over time. In case of chronic stress, new set points for future adaptations are established, and the body does not return to the original set point for homeostasis (53). Within limits, allostatic load can be understood as an adaptive response to temporary and other demands. Although, if additional loads of unpredictable events such as disease, human disturbance, and social interactions are added, then allostatic load can increase dramatically. This phenomenon is called allostatic overload. (55)

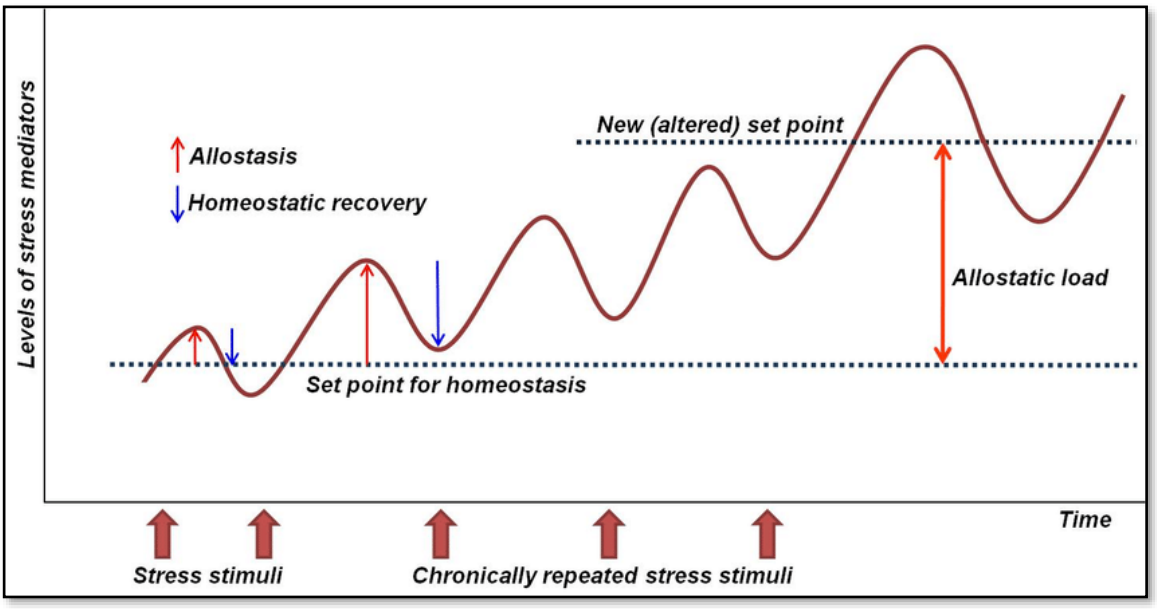


Figure 5: Stress, allostasis and allostatic load (53)

A good example for allostatic overload is the development of diabetes mellitus type 2 resulting from repetitive chronic stress. In this case, insulin resistance occurs, because the baseline fasting glucose level has been set to a higher level than before (53). Therefore, biomarkers of allostatic load such as glucocorticoids could be measured for predicting cumulative biological risks of imminent diseases and their pre-stages (53, 56-58). Figure 6 shows different physiologic responses to stress over time. The first diagram illustrates the normal allostatic response that is triggered by a stressor and sustained for a certain period before it is turned off. The other diagrams show different circumstances which lead to allostatic load: repeated hits from multiple stressors, lack of adaptation, prolonged response, and an inadequate response that leads to the compensatory release of other mediators. (59)

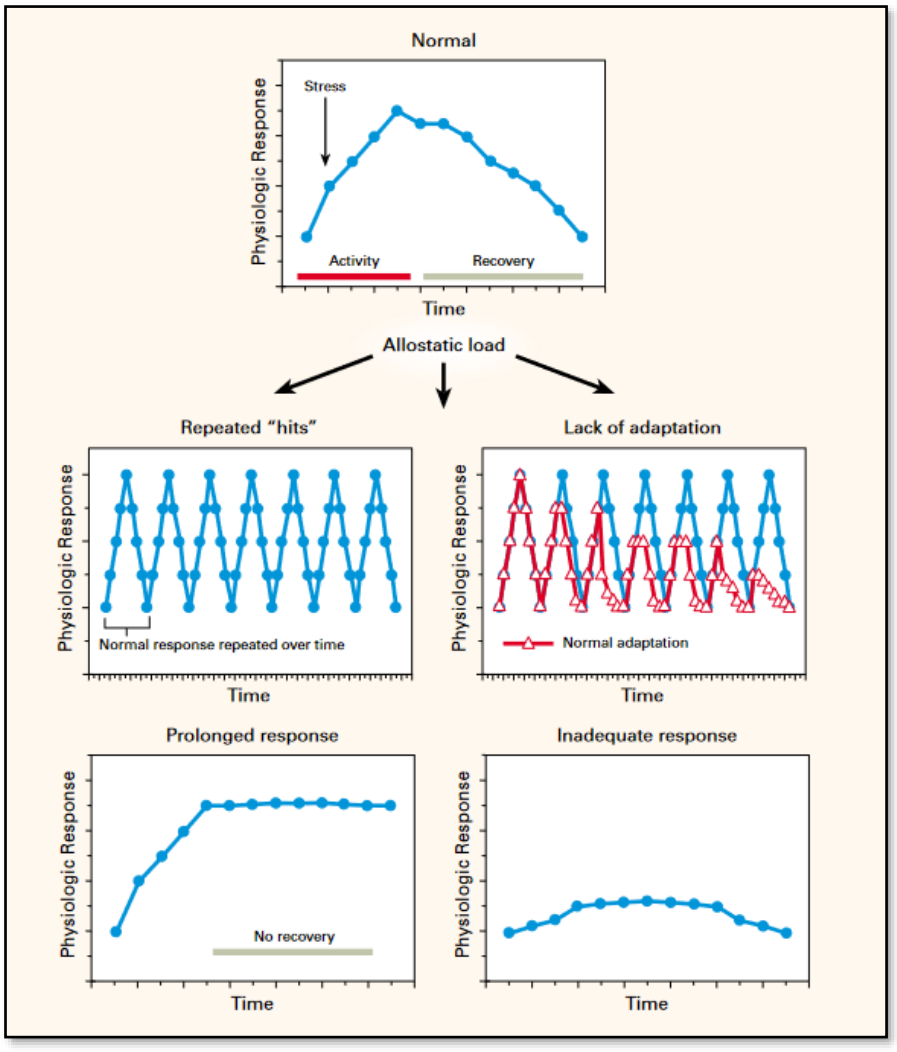


Figure 6: Different types of allostatic load (59)

1.2 Possibilities of stress measurement

Since stress plays a major role in the development of various diseases, the question arises as to how this stress can be measured in humans. Such measurements could be used to identify stressors to reduce their damaging effects and prevent disease. Because the quality of treatment in medical facilities highly depends on the mental and physical health of the staff, the recognition of stressors is of particular importance in this context. Psychological questionnaires are one way to measure stress. These can be used to determine both the frequency of occurrence and type of stressors, as well as the resulting subjective perception or impairment. Studies have shown that it is not the mere presence of a stressor that is decisive for the damaging effect, but the extent of the psychological burden experienced by the individual. (60) Therefore, these questionnaires should capture the types of stressor exposure, a broad range of psychological, cognitive, behavioral, and physiological responses to exposure, as well as contextual and individual-level factors that moderate the effects of exposure and response (60). Possibilities for evaluation include self-measurement, measurement by an interviewer, or objective assessment based on proximity to a stressful event (60). In 2019, a Danish study concluded that daily smartphone-based self-assessed stress seems to be a valid measure of perceived stress (61). However, because stress is perceived subjectively, psychological questionnaires may inaccurately reflect actual stress. This could be especially true for chronic stress if the questionnaire is completed to evaluate a longer time period in the past. Moreover, questionnaires primarily reflect perceptions of psychological stress. Physiological effects on bodily functions of e.g., lack of sleep, noise or physical work can often only be assessed indirectly. Therefore, another possibility for measurement is to examine biomarkers of the physiological stress response. Such a method could provide a more objective solution to this problem. Because activation of the HPA axis is a more prolonged response compared with the autonomic stress response, measurement of the primary product, cortisol, may prove useful. Based on the concept of ‘glucocorticoid cascade hypothesis’, in a study by Sapolsky et al, cortisol received considerable attention in relation to pathophysiological changes caused by allostatic overload (53, 62).

Because blood cortisol levels increase with long-term exposure to chronic stressors over an extended period of time, cortisol is widely accepted as a biomarker of allostatic load at the moment (53, 58). Therefore, the question arises which possibilities there are to determine cortisol and which significance these methods have in each case.

1.3 Traditional sample matrices to measure cortisol

1.3.1 Blood serum

One possibility for cortisol measurement is the use of automated immunoassays and liquid chromatography on serum or plasma samples. Since cortisol is secreted in a pulsatile pattern and the concentration is strongly dependent on the time of day and the current physical condition, there is a strong fluctuation in the measured levels. In addition, 90 percent of cortisol in the blood is bound to proteins, so that false readings can occur in people with disturbed serum protein concentrations. (63) When measurements are taken from blood, total cortisol is measured rather than the free, bioactive one. Another shortcoming is that taking blood samples from the vein and the fear of needles can cause a stress reaction, which could lead to falsified values. (64, 65) For these reasons, it is not possible to make valid statements about a chronic increase in cortisol with just a few measurements using blood samples.

1.3.2 Urine

Measurement of urinary cortisol is mainly used for the diagnosis and therapy monitoring of diseases associated with clinically relevant hypo- or hypercortisolism such as Cushing’s disease or adrenal insufficiency. This is done by assessing 24-hour urinary free cortisol (UFC). (66) Different studies have shown that there is a high inter-individual variability in urinary cortisol excretion rates and that renal secretion of cortisol is depending on glomerular and tubular functions. (64, 67) For these reasons, although urinary cortisol measurement is useful in individual cases to draw conclusions about endocrinological function, it is not useful for measuring chronic stress because there is little comparability between different individuals and the readings do not provide information over longer periods of time.

1.3.3 Saliva

Due to the disadvantages of cortisol measurement from serum and urine, more attention has been paid to measurement from saliva in recent years. This is since this method is simple, standardized, non-invasive, less stressful, easy to repeat, does not require special training or equipment and reflects free (unbound) cortisol. Moreover, it can be acquired in a non-laboratory und non-clinical setting, can be stored at room temperature and may be kept for up to 4 weeks without significant loss of cortisol from the samples. (64) Salivary cortisol, like serum cortisol, reflects acute changes at a given time point but is not well suited to evaluate longer-term systemic cortisol exposure because of circadian variations and its protein-binding capacity. (53)

1.4 Hair cortisol as a marker of chronic stress

1.4.1 Relevance of hair cortisol measurement

In the search for a sample matrix from which cortisol can be obtained to estimate chronic stress, measurement from hair has emerged as a new and promising method. Hair analysis already is an established method used to detect exposure to recreational drugs, anabolic steroids and environmental toxins. (52, 68) More recently, the question has been raised as to whether endogenous hormones can also be detected by measurements from hair (69). The crucial difference compared to the other sample matrices is that changes in exposure to the hormones or drugs can be determined over a longer period of time. This can be explained by the fact that hair predictably grows about 1 cm per month on average and cortisol is therefore stored in the hair depending on the time (70). The rate of scalp hair growth shows slight variations between individuals depending on factors such as sex, age, and race. However, a rate of 1cm per month is generally accepted and shows the most uniform growth rates at the posterior vertex region of the scalp. (70, 71) Therefore, a 6 cm hair sample can be used to measure cortisol levels for the last six months. For this reason, it is possible to retrospectively examine cortisol production at a time when the stressor was most salient without having to sample at that exact time. These readings can then be compared to those reflecting a period when the stressor was not yet present or

was less severe. Hair cortisol analysis has many other advantages, which are mainly justified by the ease of collection, good possibility of storage, low invasiveness and the fact that the measurements do not need to be repeated often (69). Table 1 shows the advantages compared to the other sample matrices in detail. These advantages could be especially useful to answer epidemiological and etiological questions where many participants are needed.

Table 1: Comparison of hair with other matrices of cortisol (68, 72, 73)

Characteristics	**Hair**	**Other Matrices (saliva, blood and urine)**
Length of cortisol information	Chronic	Acute
Storage	Can be stored at room temperature	Require specific storage (e.g., refrigeration or freezing)
Situational and intra-individual variability	Cortisol in hair is not easily influenced by acute situational and individual factors—although evidence suggests that it might be influenced by hair hygiene and cosmetic related behavior (e.g., hair treatment and hair dyeing)	Measurements are vulnerable to factors such as time of the day, cigarette smoking, and acute stress
Repeated Measurements	Less need for repeated measurements—although some individuals might be unwilling to give hair	Need for repeated measurements—expensive, burdensome for study participants and likely to increase incomplete sample collection and loss to follow-up
Invasiveness	Small amount of hair is needed; not invasive	Although saliva and urine are less invasive, obtainment of blood can be invasive and painful for participants.

1.4.2. Incorporation of cortisol into hair

Hair growth begins in the hair follicles, which are located 3-4 mm below the skin surface and are in close contact with capillaries, sebaceous glands, and sweat glands. These glands discharge their secretions into the hair follicles, while eccrine sweat glands discharge their products onto the skin surface. (74-76) The most common hypothesis for the uptake of cortisol into hair is the multi-compartment model, which states that drugs and hormones such as cortisol enter the base of hair follicles by passive diffusion from the bloodstream (figure 7). During keratogenesis, they are then incorporated into the interior of the hair shaft, depending on the respective concentration in the blood. (77) In this scenario, it is assumed that hair cortisol mainly reflects free, unbound cortisol, but not total cortisol, which is measurable in serum (52). Furthermore, cortisol from the secretions of the sebaceous, sweat, and eccrine glands could accumulate on the outer hair layer (71). However, there are no studies that confirm that sebum or sweat contains significant amounts of cortisol (52). Studies have shown that small amounts of cortisol are also synthesized and secreted in the hair follicles themselves, as these can be considered a functional unit of the HPA axis (78, 79).

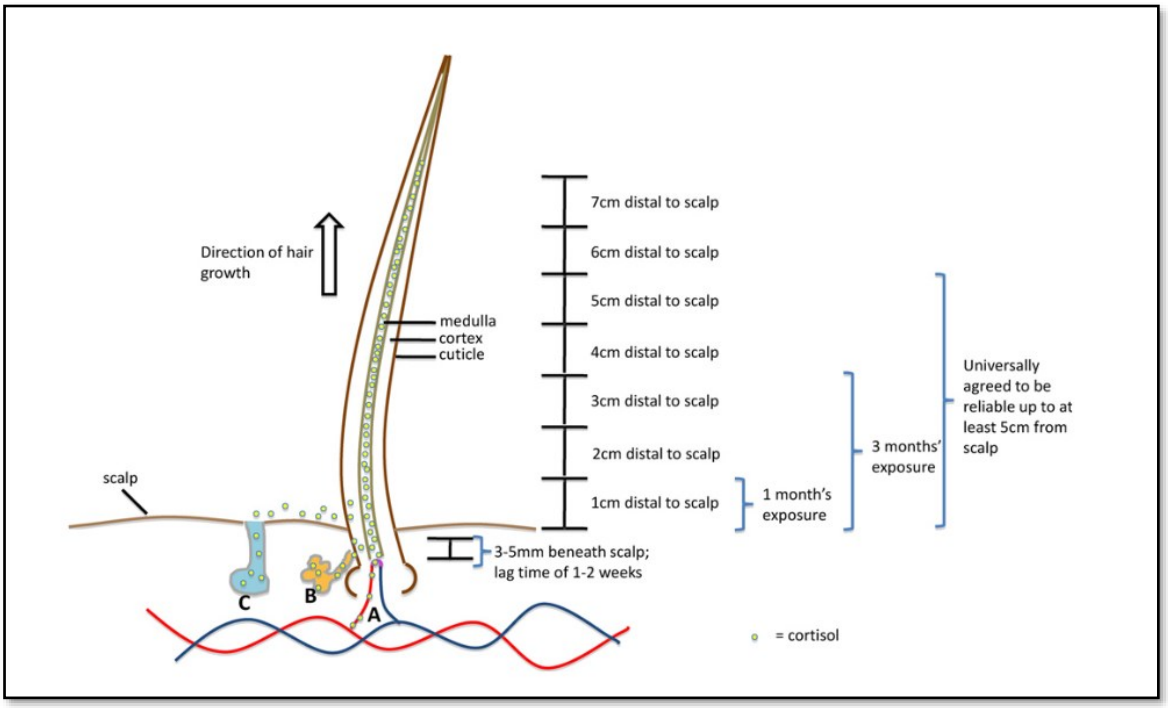


Figure 7: Proposed mechanisms for incorporation of cortisol into hair via blood (A), sebum (B), and sweat (C) (52)

Finally, it is possible for cortisol to be applied externally to the hair through the use of cortisol-containing creams or ointments (80). This can happen, for example, if the person scratches his/her head after using such a preparation (69). It is also possible that measurements may be falsified by contamination of the hair by environmental substances such as smoke, pollution, physical contact, chemicals, etc. (75, 76).

1.4.3 Correlation of hair cortisol and other matrices

When assessing the validity of hair cortisol concentration (HCC) measurement the question arises whether these values reflect those of traditional methods such as measurements from serum, saliva, and urine. Examining a group of non-obese participants, a 2007 study by Sauvé et al. found a significant correlation between HCC and 24-h free cortisol in urine, but not in serum or saliva, taken at a single time point. In addition, this study showed that natural hair color had no influence on HCC, but that dyeing the hair shortly before testing lowered the values. (81) Another study by Van Holland et al published in 2011 showed that mean salivary cortisol concentrations taken six times a day for 3 consecutive days were moderately correlated with HCC ($r=0.41$, $p=0.03$) (82). Results of a 2012 study by Vanaelst et al confirmed this and showed a significant correlation between HCC and the area under the curve for salivary cortisol taken three times a day for 2 consecutive days (83). Overall, the studies suggest that HCC allows adequate conclusions to be drawn about the concentration of systemically active cortisol.

1.4.4 Correlation of hair cortisol and chronic stress

There are many studies that have investigated the relationship of HCC and chronic stress. The main question is whether participants who are under chronic stress, as for example evaluated by questionnaires, have an increased hair cortisol concentration compared to the participants not exposed to the stressors. In a 2007 study, a significant relationship was found in healthy women during pregnancy between the HCC of the previous month and the stress level, which was evaluated with a perceived stress scale (TICS; $P<0.05$) (84). Another study by van Uum et al from 2008 has shown that in chronic pain patients taking opioid painkillers, HCC

was significantly higher than in the comparison population not suffering from chronic pain (83.1 vs. 46.1pg/mg). In addition, the scores of the PSS were also significantly higher than in the control group (p<0,001). (85) To examine the effects of unemployment, a major psychological stressor, on HCC, Dettenborn et al compared hair samples from individuals who had been unemployed for at least one year with those from a control group without unemployment. In addition, chronic stress levels in both groups were determined using the Trier Inventory for the Assessment of Chronic Stress (TICS) and PSS. The HCC values of the last 3 months (P<0.05) as well as the values of the last 3-6 months (P<0.05) were significantly higher in the unemployed group. In addition, participants from the unemployed group reported significantly higher levels of worry in the TICS (P<0.01) and had higher scores in the PSS (P<0.01). (86) Similar results were shown in a 2011 study by Steudte et al, in which 3cm hair samples from survivors of the civil war in Uganda were examined. Participants who had suffered trauma from this period were tested for PTSD using the Clinician-Administered Posttraumatic Stress Disorder Scale. It was shown that the participants with PTSD had significantly higher HCC scores than the traumatized participants who did not have PTSD (p=0,03). In addition, there was a positive correlation between the total number of traumatic events and HCC (r=0,41, p<0,05). (87) Consideration of these studies suggests that there is a relationship between chronic stress and HCC and that the level of stress in recent months can be assessed by HCC.

1.5 Relationship between serum cortisol and serum testosterone

Testosterone is the primary male sex hormone and plays a central role in the regulation of sex differentiation, producing male sex characteristics, spermatogenesis and fertility (88). As an end product of the hypothalamic-pituitary-gonadal axis, following the secretion of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), testosterone is produced primarily in the testes in males and in smaller quantities in the adrenal medulla in women (88). Figure 8 shows the hypothalamic-pituitary-gonadal axis and its negative feedback mechanisms. There is much evidence in the literature of the negative correlation of serum cortisol and serum testosterone in men. For example, in a study by Cumming et al, it was shown that both an endogenous and

an exogenous increase in serum cortisol led to a decrease in serum testosterone (89). Because serum testosterone is much lower in women and has less importance physiologically, there are few sources that address the relationship of cortisol and testosterone in women. A study by Pletzer et al describes a significant decrease in serum testosterone in women and men after a stressful event (90). Therefore, it can be assumed that when serum cortisol increases, there tends to be a decrease in serum testosterone and this trend is independent of sex, although absolute serum testosterone levels are different in women and men.

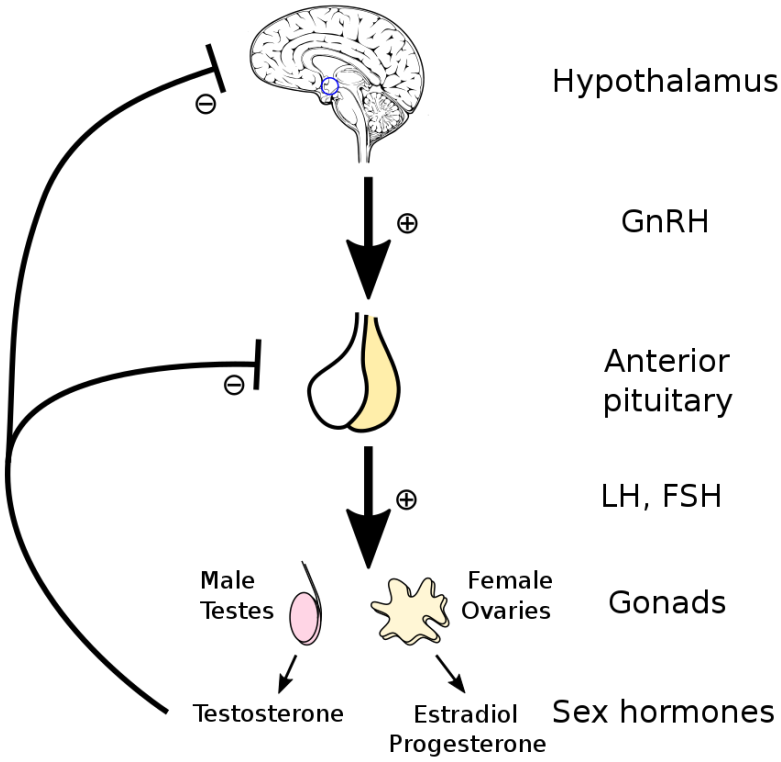


Figure 8: The hypothalamic-pituitary-gonadal axis (Taken from: https://en.wikipedia.org/wiki/Hypothalamic%E2%80%93pituitary%E2%80%93gonadal_axis)

1.6 COVID-19

1.6.1 Origin

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus that was first detected in Wuhan, Hubei Province, in China in December 2019. It is believed that the pathogen originated from the consumption of wildlife and therefore may stem from an animal reservoir, likely that of bat. The disease, which is caused by infection with the virus, was named COVID-19 (coronavirus disease 19) by the World Health Organization (WHO) and declared to be a public health emergency of international concern on March 11, 2020. (91) SARS-COV-2 belongs to the same Betacoronavirus group as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory coronavirus (MERS-CoV). MERS-CoV originated in 2012, while SARS-CoV is known since 2002. (91)

1.6.2 Epidemiology

A cluster of 27 pneumonia cases with unknown etiology was reported by the Wuhan Municipal Health Committee on December 31, 2019, and 7 severe cases were found to be linked to the Huanan Seafood Wholesale Market in Wuhan, while other patients showed no epidemiological link with this market (91). After the disease first appeared in China, it spread rapidly worldwide and the number of cases increased exponentially. The first known case in Austria was detected between Jan. 24-26, 2020, and in Germany, the first case was detected on Jan. 27, 2020 (92, 93). As of 29 February 2020, COVID-19 had affected patients in 57 countries and 85,403 cases were confirmed globally, while 79,394 (92,9%) of these were reported in China and only 6,009 (7,3%) in other countries (94). An overview of documented cases and deaths in April 2020, immediately following the first period after the outbreak, is shown in table 2. It is noticeable that the number of cases and deaths in Europe and the USA has risen dramatically.

Table 2: COVID-19 outbreak, WHO statistics (April 12, 2020) (95)

Region	Total (new) cases in last 24 hr	Total (new) death in last 24 hr
Globally	1,773,084 confirmed (76,498)	111,652 deaths (5,702)
Western Pacific Region	121,426 confirmed (1,310)	4,125 deaths (67)
European Region	913,349 confirmed (33,243)	77,419 deaths (3,183)
South-East Asia Region	16,883 confirmed (842)	766 deaths (38)
Eastern Mediterranean Region	99,713 confirmed (3,768)	5,107 deaths (164)
American Region	610,742 confirmed (36,804)	23,759 deaths (2,228)
African Region	10,259 confirmed (531)	464 deaths (21)

Due to this rapid development, different preventive and control strategies such as exit restrictions, mandatory wearing of face masks in public places, restriction of international traffic and closure of stores, restaurants, and other facilities such as schools and universities were introduced in many countries. Despite these measures, a total of 200,840,180 confirmed COVID-19 cases and 4,265,903 deaths globally were reported to WHO as of 6 August 2021 (96). From these numbers, a death rate of 2.1% can be calculated. The actual case fatality rate (CFR) is estimated to be between 0.3 and 3%, and there is solid evidence that the CFR is highly dependent on other factors, such as age and territory (96-99). In Austria, there were 658,748 confirmed cases and 10,548 deaths as of 9 August 2021 (100). As shown in figure 8, the 7-day incidence, which is calculated from new infections in the last seven days per 100,000 population, varied widely, reaching values of up to 562 in November 2020. During other periods, however, the 7-day incidence also declined sharply or was consistently lower. The periods with high 7-day incidences were therefore called COVID waves in the media. In the chart below, newly identified cases of SARS-CoV2 infection are shown in yellow and the 7-day incidence is shown in blue. (101)

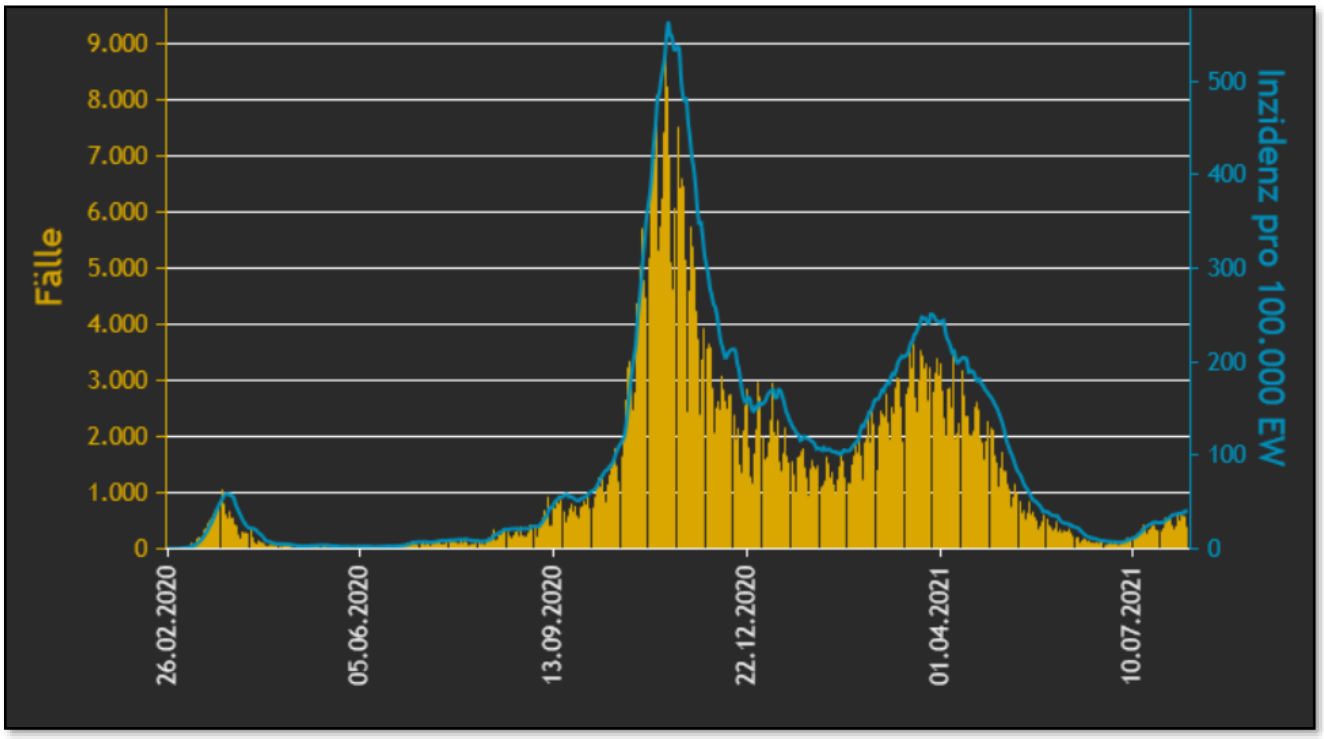


Figure 9: New COVID-19 infections and 7-day incidence in Austria (101)

1.6.3 Transmission and clinical characteristics

After the initial transmission to humans, the virus spread at a high rate, so it can be assumed that it is passed on via human-to-human transmission. Therefore, transmission via airborne droplets, large or small, and direct contact with an infected person or contaminated surfaces is the most likely route of infection. (102) A preliminary reproduction number (R_0) of 2.24 to 3.58 has been estimated in this context (103). Typically, the disease manifests in the respiratory system and is associated with symptoms such as fever, dyspnea, cough, expectoration, headache, myalgia and fatigue (102). Less common signs can include diarrhea, hemoptysis, and shortness of breath (104). It can be assumed that all age groups are susceptible to COVID-19 infection and that the median age at infection is around 50 years (105, 106). However, the clinical manifestations differ with age. It was observed that older men (>60 years old) with co-morbidities suffer more severe courses of respiratory disease, more often requiring hospitalization or leading to death, while most young people and children only have mild courses or are asymptomatic (105, 107, 108). In severe cases, renal failure, myocardial damage, liver dysfunction, leukocyte abnormalities, septic shock, and

disseminated intravascular coagulation may also occur (109). Therefore, treatment in an intensive care unit is often necessary in such cases.

1.6.4 Treatment options

Since COVID-19 is a viral infectious disease, the treatment of patients has been challenging. Therefore, the available therapy for the disease is primarily symptomatic and dependent on the severity of the disease. (109) In addition to supportive care measures such as artificial respiration, fluid therapy, and the use of anticoagulants to prevent thrombosis and embolism, there have been clinical studies regarding the use of antiviral drugs such as remdesivir, lopinavir, or oseltamivir, antiparasitic drugs such as chloroquine, hydroxychloroquine, and Ivermectin, and the glucocorticoid dexamethasone. (109, 110) Due to the fact that there is no clear scientific evidence regarding these pharmacological therapy options and in some cases only a very small benefit for the outcome or no benefit at all could be shown, there are no universal guidelines and the treatment of COVID-19 patients, especially those with severe respiratory disease, remains demanding and without adequate causal therapy options (111).

1.6.5 COVID-19 as a stressor

Because it was an unknown pathogen and that the disease spread rapidly, the COVID-19 pandemic triggered a worldwide crisis with significant consequences for the economic system, the respective health care systems of the individual countries, education at schools and universities, and the private lives of citizens (1). During the crisis, there were several so-called lockdowns, which were accompanied by curfews, the closure of restaurants and bars, stores and workplaces. As a result, many people began developing existential fear, as further developments were not foreseeable and job losses or bankruptcy were imminent. In addition, schools and universities were temporarily closed and teaching was switched to distance learning. For that reason, parents had to watch over their children around the clock and support them with their school work, which was an additional burden. For many university students, the crisis meant a loss of daily structure and increased problems based on loneliness and uncertainties about the

future (112). The pandemic also put a particularly heavy burden on medical personnel, as the consequences for their private lives were compounded by the need to care for COVID-19 sufferers or other patients who were at particularly high risk for a severe course in the case of infection. On the one hand, this was extremely frustrating, as many patients had to be treated in intensive care units and the treatment options were very limited, so that many patients died of the disease during treatment. On the other hand, medical staff had to implement intricate hygiene measures, such as wearing protective suits, in order not to infect oneself, other patients or family members. In addition, many employees in medical facilities were required to work long shifts and care for many patients at any given time, as staff shortage was a common problem in many hospital wards. (113) The personal and professional burdens of the crisis therefore suggest that medical personnel were exposed to severe and exceptional stress during this period, composed of both psychological stressors, such as uncertainty, time pressure, anxiety-provoking threats or resentments, and physiological stressors, such as sleep deprivation and physical strain.

2. Aims and Objectives

2.1 Aim

Since in the modern world there are more and more stressors, and these also occur in the form of international conflicts as for example in the COVID-19 crisis, it is important to be able to recognize these stressors and to be able to assess the magnitude of their influence on the population. This is especially important since many pathological conditions, such as cardiovascular diseases, metabolic diseases such as diabetes and psychiatric disorders can be a result of these stressors. On the one hand, this contributes to a high level of personal suffering for those affected, and on the other hand, it can be a cause for leaving the respective profession. Since there is already a shortage of personnel in the health care system in parts of Austria, too high a stress load on medical staff would be counterproductive for a functioning health care system. The measurement of steroid hormones such as cortisol and testosterone from hair for the assessment of stressors over an extended period is not yet a procedure that is routinely used. Therefore, this method has the potential to measure stressors independently of questionnaires, and thus to make stressors quantitatively measurable and to be able to avert illness or job loss.

The aim of this thesis is to find out, if in the period of the first COVID-19 lockdown from 16.03.2020 to 01.05.2020 more stress was put on the investigated medical staff and if this can be quantified by the analysis of hair cortisol and hair testosterone.

2.2 Hypotheses

The first hypothesis is that during the Covid-19 lockdown there was an increase in the cortisol concentration in the hair of the participants reflecting a higher stress load in their professional and private lives.

The second hypothesis is that the concentration of testosterone in the hair either behaves in the opposite way to HCC, i.e., decreases, or that it increases slightly, but not as much as that of HCC.

3. Materials and Methods

3.1 Ethics approval

An application regarding the study was submitted to the Ethics Committee of the Medical University of Graz (EK: 32-433 ex 19/20). This was dealt with in an expedited review on 19.05.2020 and it was decided that there was no objection to conducting this study. On the basis of the declaration of Helsinki, informed consent was obtained from each participant prior to the start of the study and those without informed consent were excluded. An important part of informed consent was the ability to withdraw from the study at any time and the strict anonymization of the collected data. In addition, participants had the right to report any violation of data protection. All research members handling the data were subject to the Austrian Data Protection Act and the General Data Protection Regulation (GDPR).

3.2 Recruitment and participants

The study was conducted in Graz, Styria, Austria at the Institute of Physiology of the Medical University of Graz. Hair samples were collected between 18.06.2020 – 15.08.2020 from a total of 396 participants, 67 of whom worked in a medical profession. The participants were recruited from various clinics and from the municipal health department in Graz and the surrounding area. 15 of the participants were employed in a medical profession, which was not further defined. This included physicians, nurses, nursing facility staff, and laboratory personnel responsible for Covid-19 testing. 9 participants worked in the emergency department, and another 11 worked in a COVID-19 ward. From the local health department, 15 employees participated in the measurements, from the dental clinic there were 12 and another 5 came from endocrinology and orthopedics. A total of 12 of the participants also had contact with COVID-19 patients during this period. These primarily included COVID-19 station staff.

3.3 Questionnaire

Before sampling, each participant received a questionnaire to collect socio-demographic data such as age, gender, education, living situation, etc. These data were primarily used to record the personal background of the participants to draw conclusions about the causes of stress and stress level or possible confounders of HCC and HTC. In addition, the questionnaire included specific questions regarding the professional situation during the time of the COVID-19 crisis. The questionnaire asked whether the participants had a job, what kind of job they had, whether they worked in shifts, whether they worked from home, whether they lost their job, and whether they suffered financial losses. These questions could reveal reasons whether and why medical personnel might have been under a particular stress load due to work-specific stressors such as shift work and the added stressors of the COVID-19 crisis such as job insecurity. Another component of the questionnaire was the collection of a brief past medical history and a medication history. This included diabetes type 1 and 2, hypertension, bronchial asthma, heart failure, myocardial infarction, Cushing's disease, chronic pain and cancer, especially with chemotherapy within the last 6 months, as well as a psychotherapeutic or psychiatric treatment. In the case of medication intake, a specific question was asked regarding the use of topical cortisone preparations, as these could lead to a falsification of the measured values. They were also asked about a change in diet and weight loss, as well as a dramatic life event within the past 6 months.

3.4 Sample collection

As shown in figure 9, the hair was collected by cutting it off at the posterior vertex of each participant as close to the scalp as possible. It was made sure to remove a strand of hair of approximately the same size from each subject to ensure equal conditions. At least 6cm strands were cut off, so that at least five 1cm long segments could be cut during the process. The hair was then bundled into strands using two strings, the beginning of each strand was marked, each strand was attached to a cardboard piece and given an ID so that they could be assigned to the participants. After collection, the samples were stored in envelopes in a dry and dark container at room temperature in the laboratory.

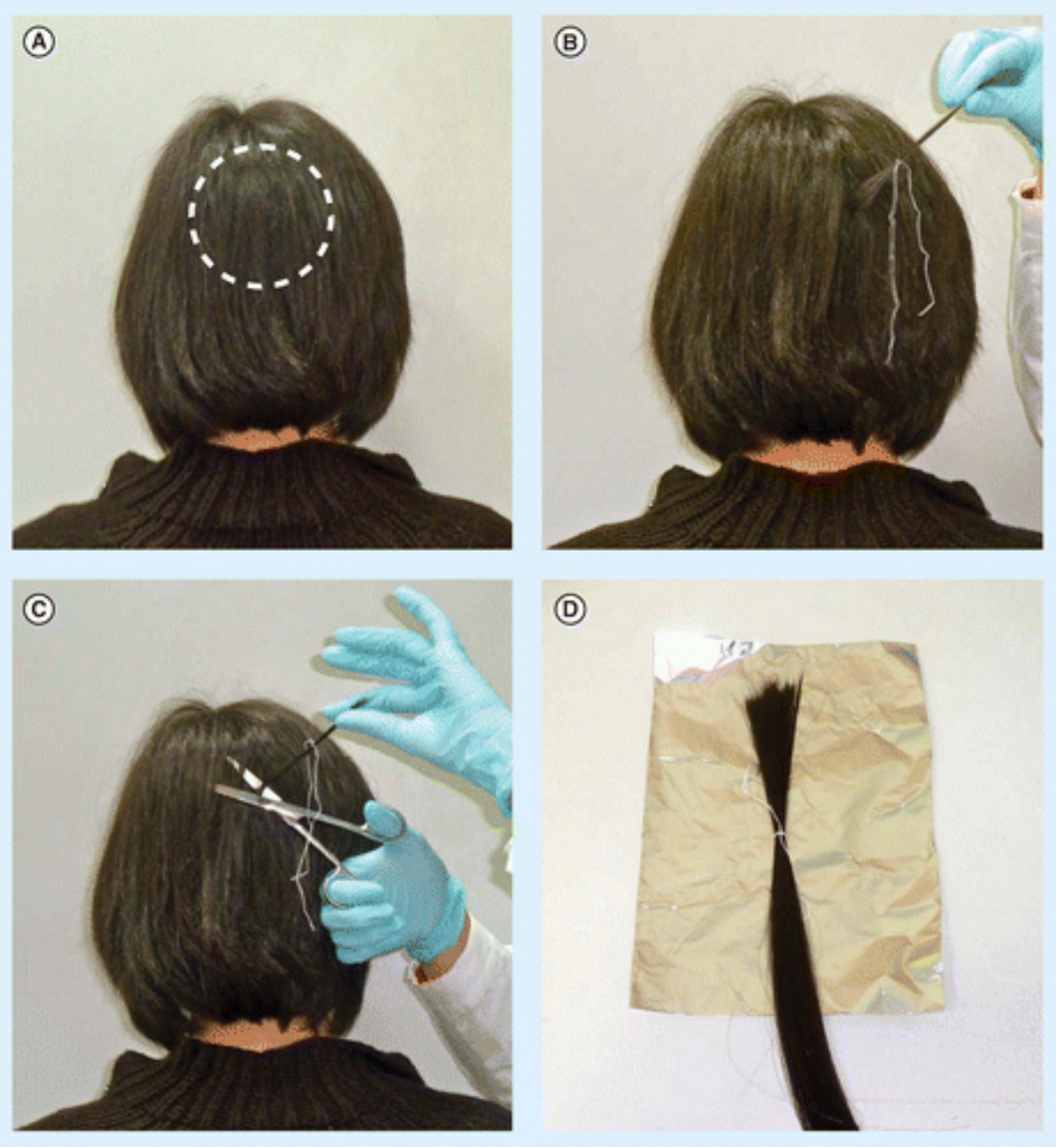


Figure 10: Method of taking hair samples from the posterior vertex (114)

3.5 Washing and drying

First, each strand of hair was washed in a bath of distilled water and then in a bath of laboratory grade acetone (VWR Chemicals) for one minute each. In this step any remaining dirt, sebum and sweat were removed, as these could lead to falsified readings. Then, the washed samples were stored in a drying oven for half an hour at a temperature of 37°C to achieve equal humidity of the samples.

3.6 Cutting

To cut the hair strands into 1cm long samples, a custom-made plate with slits 1cm apart and a laboratory knife were used (figure 10). Thus, for each strand of hair six consecutive samples were created, each of which was one centimeter long. To ensure that none of the hair was lost and to enable precise cutting, the strands were rolled up in cigarette paper and fixed in place before cutting. The remaining hair was further stored in the envelope.

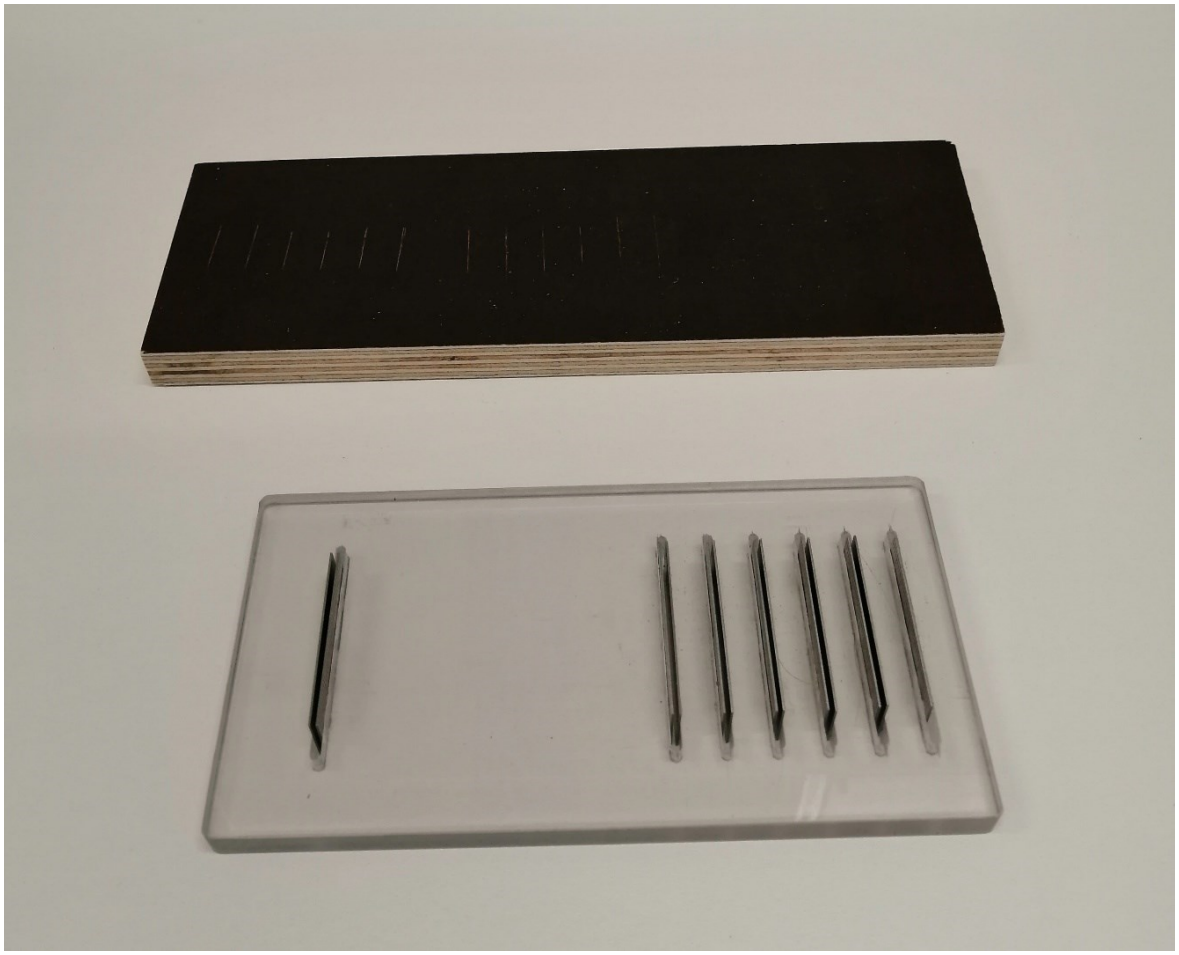


Figure 11: Cutting tool with 1 cm spacing (Photo: Philipp Wöhler – 2021)

3.7 Weighing

In the next step, the individual samples were each transferred into test tubes using tweezers while removing the remaining cigarette paper. Weighing was done with a laboratory balance model Mettler AE163 (figure 11), care was taken to calibrate the balance before each sample. Samples with a lower weight than 10 mg/cm were excluded from the further process.

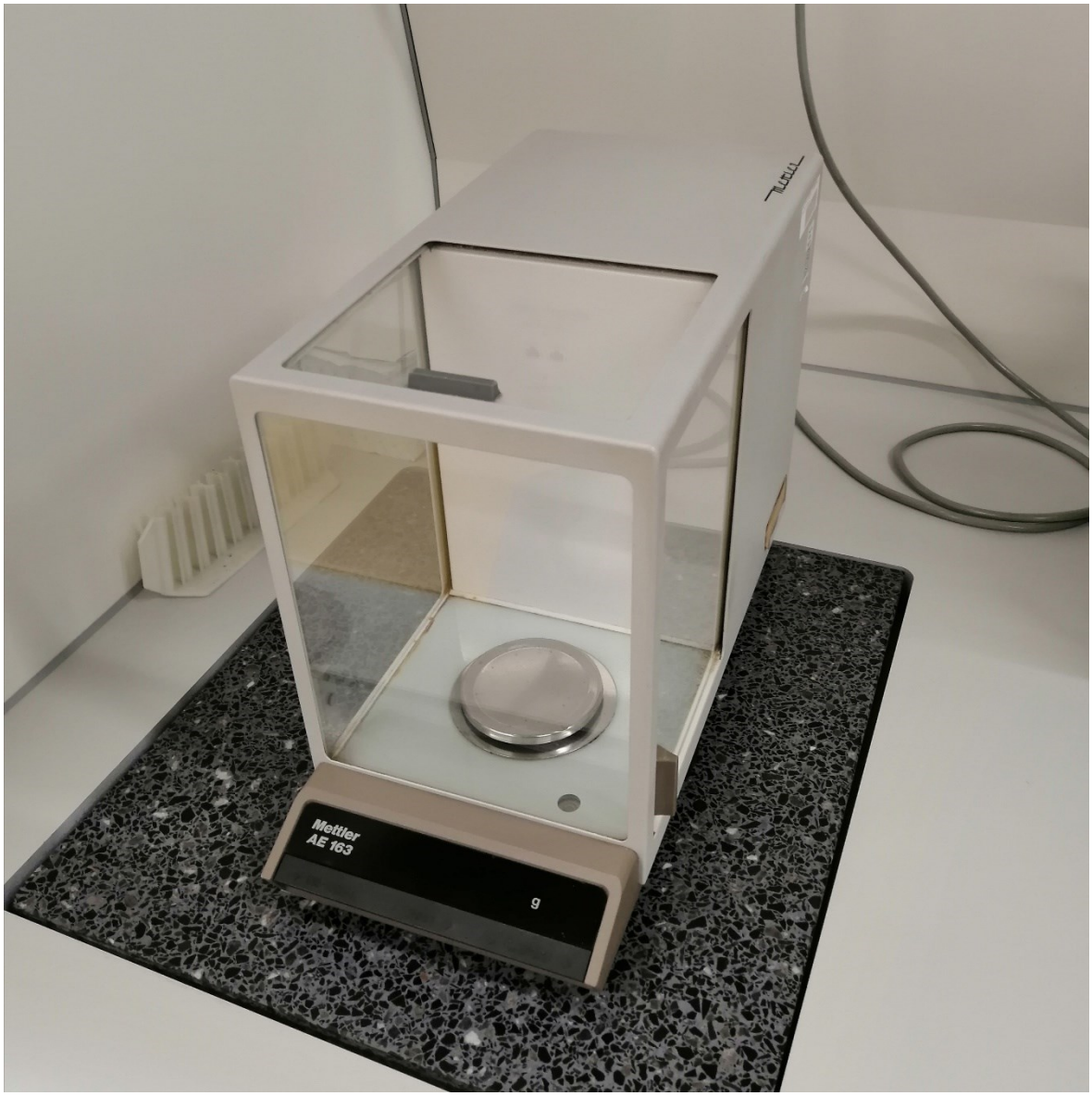


Figure 12: Mettler AE163 precision scale (Photo: Philipp Wöhler – 2021)

3.8 Hormone extraction

The extraction of steroid hormones was performed in several steps. First, 1 mL of laboratory grade methanol (VWR Chemicals) was added to each sample, then the test tubes were sealed and vortexed for one minute using a Heidolph Reax 2000 vortex mixer. Afterwards they were incubated in a drying oven for 18 hours at a temperature of 52 °C. The next day, all samples were placed in an ultrasonic bath (Elma Transsonic T 6060/H) for 60 minutes to loosen adherent particles from the hair fragments. The temperature of the bath did not exceed 40 °C at any time. Then the samples were centrifuged for 10 minutes at a speed of 5000 rpm in a Model Eppendorf 5804R centrifuge before the supernatants from the test tubes were transferred to new tubes. 1 mL of acetone was added to the hair samples, they were vortexed and centrifuged again before adding the respective supernatants to the previous ones. The new tubes with the collected supernatants were then stored in a fridge and the previously described extraction steps were performed a second time. Then, the collected supernatants were completely dried under nitrogen gas (VWR Chemicals) using a Stuart SBH130/D block heater as a stand for the test tubes.

3.9 Measuring hormone concentrations using ELISA

To determine the HCC and HTC of each hair sample, an enzyme-linked immunosorbent assay (ELISA) was performed. For this purpose, the dried samples were first transferred to 100 µl of phosphate-buffered saline (PBS). The test kit used for measuring HCC was a cortisol saliva ELISA kit (NT-DSNOV20) from NovaTec. A Salimetrics test kit was used for measuring HTC. The method works by binding of specific antibodies to the hormones and allows a quantitative determination of the hormone content in ng/ml. This is possible due to an enzyme coupled to the antibodies, which causes a color change of a substrate that is added later in the process. For HCC the lowest measurable concentration was specified with 0.12 ng/ml at 95% confidence limit, for testosterone it was <1.0 pg/ml. Samples with values below this limit were excluded from further analysis. Since the individual hair samples had different weights, the measurements had to be divided by the respective weight of the sample to obtain comparable values in pg/mg.

3.10 Spectrophotometry

After ELISA, a spectrophotometry was performed using a Perkin Elmer Wallac 1420 VICTOR² multilabel counter. The photometric measurement, using light of a specific wavelength, is proportional to the enzyme reaction of the ELISA and therefore also to the amount of steroid hormones in the sample.

3.11 Time scale of the samples

The measurement results of the 1cm pieces were each assigned to a period of four weeks, i.e., one month. As shown in figure 12, the most distal segment represents month 1 (calendar weeks 9-12), the next segments represent month 2 (calendar weeks 13-16), month 3 (calendar weeks 17-20), month 4 (calendar weeks 21-24) and finally the most proximal segment represents month 5 (calendar weeks 25-28). This time scale was established individually for each sample based on the date of sampling to ensure the most accurate assignment to months. Since the 1st COVID-19 lockdown with exit restrictions in Austria lasted from calendar week 12-18, months 2 and 3 best represent the lockdown period. Month 1 describes the time before the lockdown and months 4 and 5 describe the time after the lockdown, but at that time there were other restrictions such as keeping a minimum distance and wearing a mouth-nose mask. (115, 116)

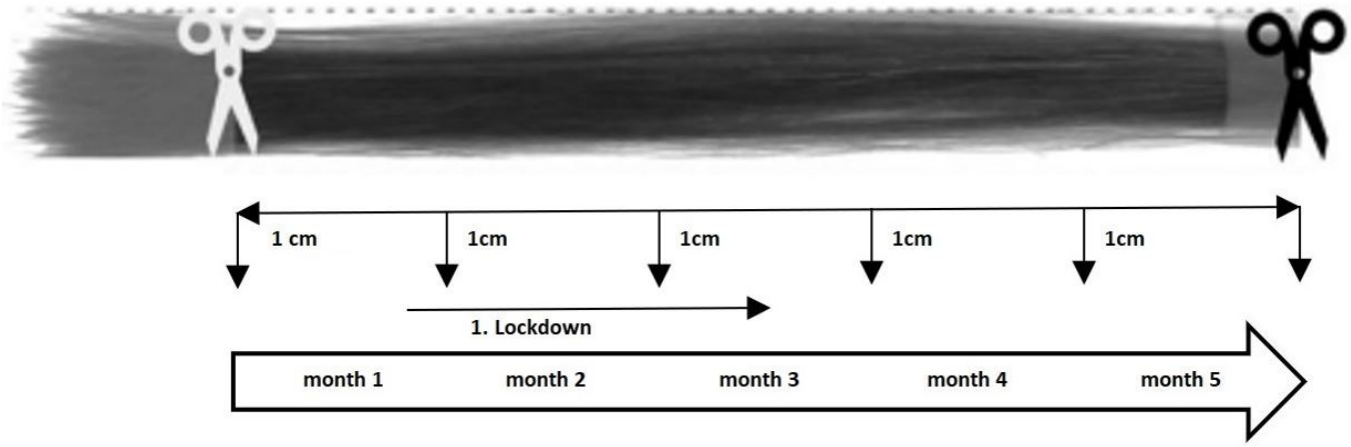


Figure 13: Timeline of months in relation to the associated hair segments (Partly taken from: (117))

3.12 Statistical analysis

Overall, date from 67 participants was analyzed. Of these, 6 were excluded due to partially missing values. Among the 61 remaining participants, 59 were female and 2 were male. Since hormone levels, especially testosterone, differ between males and females, it was decided to analyze only data from the female participants and to exclude the remaining 2 males. Since skewed distributions of the data were found in the exploratory data analysis for HCC and HTC (table 3), a $\log_{(10)}$-transformation was carried out prior to metric statistics. After transformation, a normal distribution was confirmed according to Shapiro-Wilk's test (table 4).

Table 3: Test of Normality (original data)

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HCC_1	,189	59	<,001	,840	59	<,001
HCC_2	,155	59	,001	,817	59	<,001
HCC_3	,210	59	<,001	,818	59	<,001
HCC_4	,197	59	<,001	,809	59	<,001
HCC_5	,161	59	<,001	,878	59	<,001
HTC_1	,237	59	<,001	,677	59	<,001
HTC_2	,205	59	<,001	,719	59	<,001
HTC_3	,158	59	<,001	,845	59	<,001
HTC_4	,168	59	<,001	,896	59	<,001
HTC_5	,108	59	,082	,870	59	<,001

a. Lilliefors Significance Correction

***Table 4:* Test of Normality (log data)**

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Log(HCC_1)	,095	59	,200*	,970	59	,146
Log(HCC_2)	,061	59	,200*	,994	59	,991
Log(HCC_3)	,084	59	,200*	,958	59	,040
Log(HCC_4)	,075	59	,200*	,969	59	,140
Log(HCC_5)	,054	59	,200*	,991	59	,945
Log(HTC_1)	,113	59	,060	,974	59	,233
Log(HTC_2)	,114	59	,055	,965	59	,091
Log(HTC_3)	,112	59	,064	,954	59	,026
Log(HTC_4)	,079	59	,200*	,965	59	,090
Log(HTC_5)	,100	59	,200*	,954	59	,025

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Data are presented as means of HCC and HTC in pg/mg in all tables and figures. Because of large differences between $_{log}$HCC and $_{log}$HTC, the data were analyzed by two separate repeated measures ANCOVA, applying month as the within-subjects factor. To screen for confounders, the HCC of months 4 and 5 were correlated with the sociodemographic data of the participants by Pearson's correlation (r). Months 4 and 5 were used as a reference, as these were the most recent measurements. Neither height, nor weight, nor BMI showed a significant correlation to HCC at months 4 and 5. Both age and hair color showed a significant correlation with HCC measurements. Age showed a correlation only to the HCC from month 4 ($r=0.323$, $p=0.013$). Hair color was correlated significantly to HCC from month 4 ($r=0.288$, $p=0.027$) as well as month 5 ($r=0.342$, $p=0.008$). Therefore, age and hair color of the participants were used as covariates for the repeated measures ANCOVA to control for confounding. Beyond that, no other significant correlations could be found. Degrees of freedom were corrected in case of violating sphericity assumption by Greenhouse-Geisser epsilon (ε). Post-hoc tests with Bonferroni adjusted critical α-level were applied. To analyze possible associations between sociodemographic characteristics and HCC and HTC, two different approaches were used. On the one hand, the mean values of the entire period were compared between the respective groups. On the other hand, the mean differences between month 5 and month 1 were calculated and then compared between the groups. In this way, differences in absolute HCC and HTC as well as differences in changes over the test period could be assessed. Because the different groups were too small for some characteristics (e.g., 54 cases of no weight loss and only 1 case of weight loss), it was chosen to further examine the relationship between the characteristics living situation, school children, shift work, medication, dramatic life events and HCC, respectively HTC. For the assessment of significant differences between the groups a Mann-Whitney-U-test was used.

4. Results

4.1 Sociodemographics

As shown in table 5, the average age of the participants was 38.66 years, with a minimum of 18 years and a maximum of 64 years. Height ranged from 1.54m to 1.83m with an average height of about 1.68m. The average body weight was 68kg, with a range from 45kg to 110kg. This resulted in a minimum BMI of 18.12 kg/m^2, a maximum BMI of 35.92 kg/m^2 and an average of 24.13 kg/m^2.

Table 5: Age, height, weight and BMI

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Age	59	46	18	64	38,66	12,610	159,021
Height	59	29,00	154,00	183,00	167,6780	5,63071	31,705
Weight	59	65,00	45,00	110,00	68,1525	13,91825	193,718
BMI	59	18,12	17,80	35,92	24,1288	4,18559	17,519
Valid N (listwise)	59						

Among the hair colors, brown was the most common with a total of 42, followed by blonde with a total of 9, white/grey 5, black 2 and red hair occurred only once (table 6).

Table 6: Hair color

		Frequency	Percent	Valid Percent	Cumulative Percent
Hair color	Blonde	9	15,3	15,3	15,3
	Red	1	1,7	1,7	16,9
	Brown	42	71,2	71,2	88,1
	Black	2	3,4	3,4	91,5
	White/Grey	5	8,5	8,5	100,0
	Total	59	100,0	100,0	

As shown in table 7, most participants came from the city of Graz (39; 66.1%), while a total of 18 (30.5%) lived in a rural region. 46 (76%) lived in a household with more than one person, while 11 (18,8%) lived alone. There were no pregnancies and while 43 (72.9%) reported having a school-age child, 15 (25.4%) did not. Mandatory school was stated only 3 times (5.1%), Apprenticeship 13 times. Much more frequently, the participants had a high school diploma (14; 23.7%) or a university education (27; 45.8%). Almost all participants (55; 93.2%) said they had roughly adhered to the government's policies on the COVID-19 crisis. 5 (8.5%) of the participants reported playing competitive sports, and this was not true for 50 (84.7%) others. There were 7 (11.90%) cases of dramatic life events with emotional stress such as death, serious illness of a relative, separation or violence). Except for 1 person (1.7%), no one lost weight or went on a diet.

Table 7: Sociodemographic and personal status

		Total	Percent
Population	<1.000	1	1,70%
	1.000-5.000	10	16,90%
	5.000-10.000	6	10,20%
	10.000-100.000	1	1,70%
	>100.000	39	66,10%
	Missing	2	3,40%
Living situation	Living together with others	46	78%
	Living alone	11	18,60%
	Missing	2	3,40%
Pregnancy	No pregnancy	57	96,60%
	Missing	2	3,40%
Schoolchildren	No schoolchildren	43	72,90%
	Schoolchildren	15	25,40%
	Missing	1	1,70%
Educational level	Mandatory school	3	5,10%
	Apprenticeship	13	22%
	High school diploma (Matura)	14	23,70%
	University	27	45,80%
	Missing	2	3,40%
Compliance	Compliance with policy measures	55	93,20%
	Missing	4	6,80%
Endurance sport	No endurance sport	50	84,70%
	Endurance sport	5	8,50%
	Missing	4	6,80%
Dramatic life event	No dramatic life event	48	81,40%
	Dramatic life event	7	11,90%
	Missing	4	6,80%
Weight loss or diet	No weight loss or diet	54	91,50%
	Weight loss or diet	1	1,70%
	Missing	4	6,80%

In table 8, the professional and financial status of the participants is shown. Overall, 55 (93.2%) of the participants had a job, while 4 (6.8%) were unemployed. The most common occupational groups included nurses (21; 35.6%) and physicians (10; 16.9%). Also, employees (13, 22%) and others (11; 18.6%) whose jobs were not further specified. There was one (1.7%) housewife and two (3.4%) university teachers. Most reported working full time (46; 78%), only 8 (13.6%) worked part time. Shift work and no shift work were reported equally often, 19 times each (32.20%). An above-average number of participants abstained from this question. As work from home increased during the lockdown, 12 (20,30%) participants reported working from home, while 43 (72.9%) did not. Two (3.4%) of the participants were in short-time work. No one reported losing their job during the study and financial losses occurred in only 5 (8.5%) cases.

Table 8: Professional and financial status

		Total	Percent
Employment	Not employed	4	6,80%
	Employed	55	93,20%
Profession	Housewife	1	1,70%
	Employee	13	22%
	University teacher	2	3,40%
	Nurse	21	35,60%
	Physician	10	16,90%
	Other	11	18,60%
	Missing	1	1,70%
Full time/Part time	Full time	46	78%
	Part time	8	13,60%
	Missing	5	8,50%
Shift work	No shift work	19	32,20%
	Shift work	19	32,20%
	Missing	21	35,60%
Working style	Not working from home	43	72,90%
	Working from home	12	20,30%
	Short-time work	2	3,40%
	Missing	2	3,40%
Loss of employment	No loss of employment	55	93,20%
	Missing	4	6,80%
Financial losses	No financial losses	47	79,70%
	Financial losses	5	8,50%
	Missing	7	11,90%

During the study, there were only two (3,4%) cases of an institutionalization in a hospital, nursing home or rehabilitation clinic. A total of 20 (33,9%) participants reported taking a medication. These included antihypertensives, thyroid hormones, statins, oral contraceptives, antidepressants and antipsychotics. The respective drugs were represented with approximately equal frequency, so that the influence of a single drug can be considered low. Corticosteroid-containing cream was used by only one participant (1,7%). However, since the measured values for HCC and HTC were neither conspicuously high nor conspicuously low, exclusion from the study was not necessary. There were no cases of chemotherapy and 6 (10,2%) cases of psychotherapy during the study. There were no cases of type 1 or type 2 diabetes, no morbus Cushing, 3 (5,1%) cases of cancer, 3 (5,1%) cases of hypertension, 1 (1,7%) case of heart failure, no myocardial infarction and 1 (1,7%) case of chronic pain.

Table 9: Medical status

		Total	Percent
Institutionalized	Not institutionalized	53	89,80%
	Institutionalized	2	3,40%
	Missing	4	6,80%
Medication	No medication	36	61%
	Medication	20	33,90%
Corticosteroid medication	No corticosteroid medication	52	88,10%
	Corticosteroid medication	1	1,70%
Chemotherapy	No chemotherapy	55	93,20%
	Missing	4	6,80%
Psychotherapy	No Psychotherapy	49	83,10%
	Psychotherapy	6	10,20%
	Missing	4	6,80%
Type 1 diabetes	No Type 1 diabetes	59	100%
Type 2 diabetes	No type 2 diabetes	59	100%
Morbus cushing	No morbus cushing	59	100%
Cancer	No cancer	56	94,90%
	Cancer	3	5,10%
Hypertension	No hypertension	56	94,90%
	Hypertension	3	5,10%
Heart failure	No heart failure	58	98,30%
	Heart failure	1	1,70%
Heart attack	No heart attack	59	100%
Chronic Pain	No chronic pain	58	98,30%
	Chronic pain	1	1,70%

4.3 Changes in hair cortisol concentration

Mean HCC values ranged from 13.698 ng/mg in month 1 to 20.436 ng/mg in month 5. The percentage change from month 1 to month 5 was 49.19%, or just under 50%. In figure 13, the progression of HCC is shown using the original data.

Table 10: Mean HCC in pg/mg

			95% Confidence Interval	
Months	Mean	Std.-Error	Lower Bound	Upper Bound
1	13,698[a]	1,301	11,091	16,305
2	15,060[a]	1,468	12,119	18,002
3	15,251[a]	1,602	12,042	18,459
4	18,892[a]	1,657	15,573	22,211
5	20,436[a]	1,637	17,158	23,715

a. Covariates appearing in the model are evaluated at the following values: hair color = 2,88, age 38,66

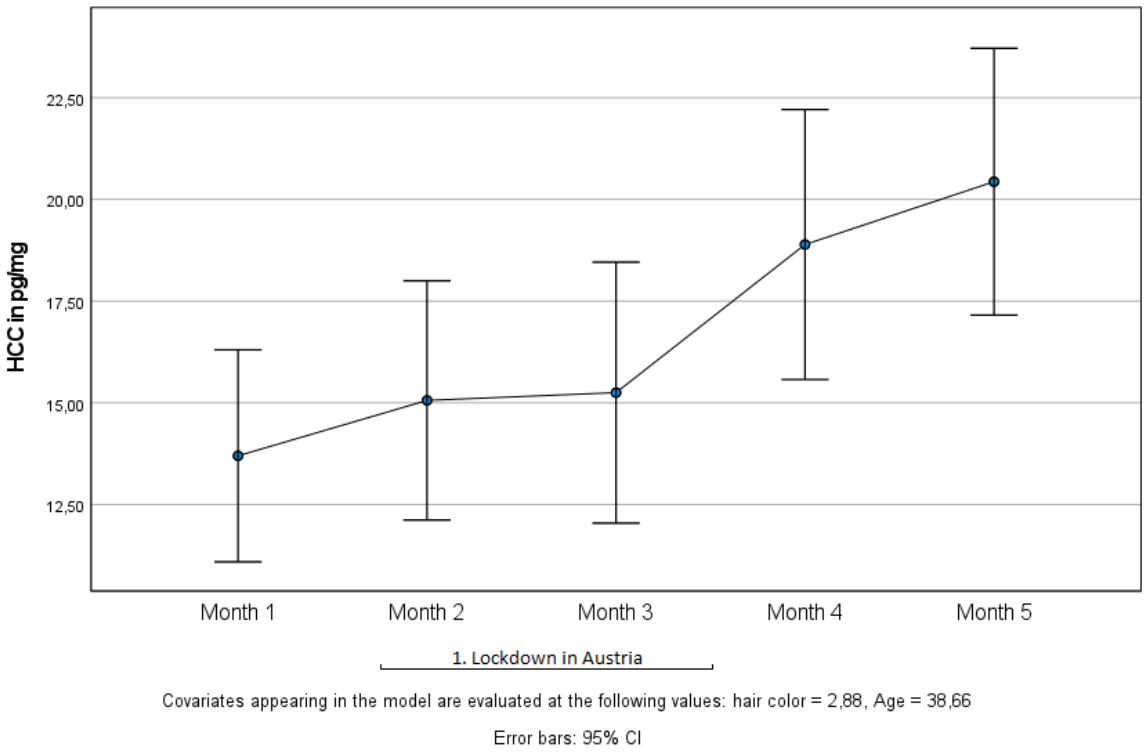


Figure 14: Mean HCC in pg/mg over the study period of 5 months. Note: Month 1: Calendar weeks 9-12, 2020; Month 2: Calendar weeks 13-16, 2020; Month 3: Calendar weeks 17-20, 2020; Month 4: Calendar weeks 21-24, 2020; Month 5: Calendar weeks 25-28, 2020; 1. Lockdown in Austria: Calendar weeks 12-18, 2020.

Repeated measures ANCOVA, controlling for age and hair colour, revealed a significant main effect for the factor 'months' ($F(1, 190) = 3.538$, $p=.012$, $\eta^2=.059$), which is shown in table 11. Neither hair colour nor age showed a significant interaction. Respective post-hoc tests indicated a significant increase of HCC at month four and five compared to the other months ($p<.001$), thus a little more than two months after the first lock-down.

Table 11: ANCOVA HCC Pairwise Comparisons

					95% Confidence Interval for Difference[b]	
(I) months	(J) months	Mean Difference (I-J)	Std.-Error	Sig.[b]	Lower Bound	Upper Bound
1	2	-,065	,036	,721	-,169	,039
	3	-,044	,037	1,000	-,154	,065
	4	-,180*	,034	,000	-,278	-,082
	5	-,222*	,038	,000	-,335	-,110
2	1	,065	,036	,721	-,039	,169
	3	,021	,030	1,000	-,066	,108
	4	-,115*	,029	,002	-,199	-,032
	5	-,157*	,031	,000	-,248	-,067
3	1	,044	,037	1,000	-,065	,154
	2	-,021	,030	1,000	-,108	,066
	4	-,136*	,031	,000	-,226	-,046
	5	-,178*	,039	,000	-,292	-,064
4	1	,180*	,034	,000	,082	,278
	2	,115*	,029	,002	,032	,199
	3	,136*	,031	,000	,046	,226
	5	-,042	,025	1,000	-,116	,032
5	1	,222*	,038	,000	,110	,335
	2	,157*	,031	,000	,067	,248
	3	,178*	,039	,000	,064	,292
	4	,042	,025	1,000	-,032	,116

Based on estimated marginal means

*. The mean difference is significant at the 0,5 level.

b. Adjustment for multiple comparisons: Bonferroni.

4.4 Changes in hair testosterone concentration

The mean values of HTC ranged from 0.988 pg/mg in month 1 to 1.031 pg/mg in month 5, which is shown in table 12. The percentage change from month 1 to month 5 was 4.35%, or just under 5%. The changes in HTC are shown in figure 14.

Table 12: Mean HTC in pg/mg

			95% Confidence Interval	
Month	Mean	Std.-Error	Lower Bound	Upper Bound
1	,988[a]	,132	,723	1,252
2	1,003[a]	,126	,750	1,256
3	,984[a]	,103	,779	1,190
4	,990[a]	,093	,804	1,176
5	1,031[a]	,090	,850	1,211

a. Covariates appearing in the model are evaluated at the following values: hair color = 2,88, age 38,66

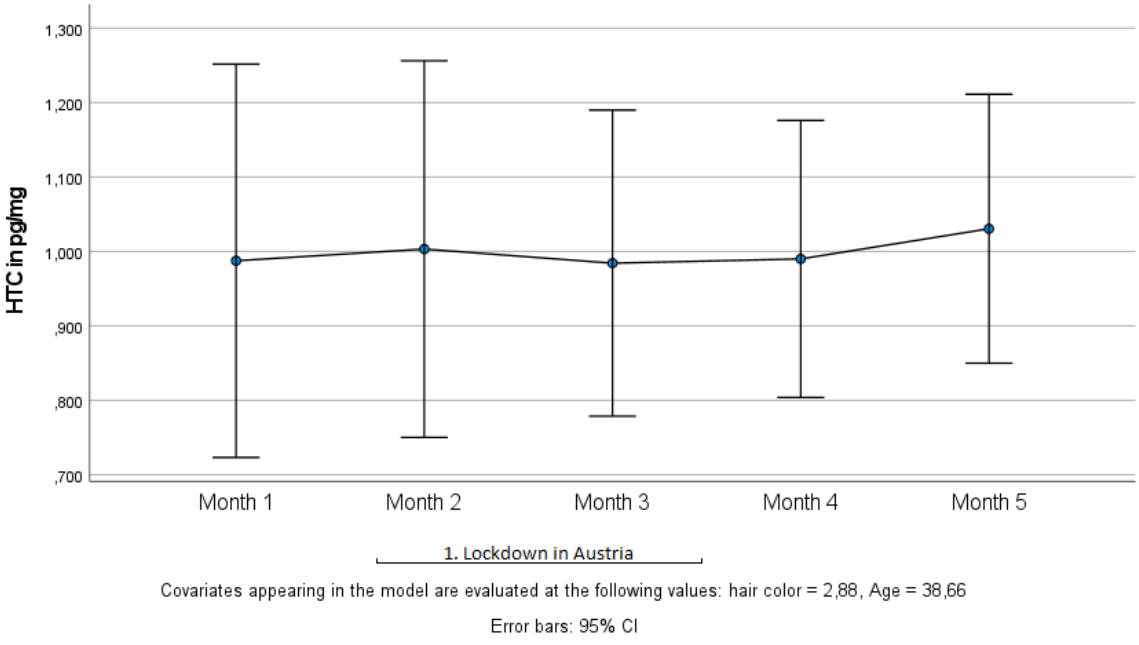


Figure 15: Mean HTC in pg/mg over the study period of 5 months. Note: Month 1: Calendar weeks 9-12, 2020; Month 2: Calendar weeks 13-16, 2020; Month 3: Calendar weeks 17-20, 2020; Month 4: Calendar weeks 21-24, 2020; Month 5: Calendar weeks 25-28, 2020; 1. Lockdown in Austria: Calendar weeks 12-18, 2020.

Repeated measures ANCOVA, controlling for age and hair colour, revealed a significant main effect for the factor ‘months’ ($F(1, 190) = 3.656$, $p=0.22$, $\eta^2=0.061$), which is shown in table 13. Neither hair colour nor age showed a significant interaction. Respective post-hoc tests indicated a significant increase of HTC at month five compared to the other months ($p<.05$).

Table 13: ANCOVA HTC Pairwise Comparisons

(I) months	(J) months	Mean Difference (I-J)	Std.-Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-,011	,020	1,000	-,070	,047
	3	-,017	,022	1,000	-,083	,049
	4	-,044	,029	1,000	-,128	,039
	5	-,091*	,030	,040	-,179	-,003
2	1	,011	,020	1,000	-,047	,070
	3	-,006	,012	1,000	-,041	,030
	4	-,033	,020	1,000	-,093	,027
	5	-,079*	,023	,013	-,148	-,011
3	1	,017	,022	1,000	-,049	,083
	2	,006	,012	1,000	-,030	,041
	4	-,027	,018	1,000	-,079	,024
	5	-,074*	,021	,010	-,136	-,012
4	1	,044	,029	1,000	-,039	,128
	2	,033	,020	1,000	-,027	,093
	3	,027	,018	1,000	-,024	,079
	5	-,046*	,016	,046	-,092	,000
5	1	,091*	,030	,040	,003	,179
	2	,079*	,023	,013	,011	,148
	3	,074*	,021	,010	,012	,136
	4	,046*	,016	,046	,000	,092

Based on estimated marginal means

*. The mean difference is significant at the 0,5 level.

b. Adjustment for multiple comparisons: Bonferroni.

4.5 Possible influencing factors on hair cortisol and hair testosterone

To examine whether there were associations between individual sociodemographic as well as individual characteristics and HCC and HTC, further analyses were conducted. Because the different groups regarding some characteristics were too small (e.g., 54 cases of no weight loss and only 1 case of weight loss), it was chosen to further examine the relationship between the characteristics living situation, school children, shift work, medication, dramatic life events and HCC, respectively HTC.

4.5.1 Living situation

There was a significantly higher level of mean HCC (p=.019) and HTC (p=.005) in participants living in multi-person households (n=46) compared to single households (n=11), but no significant difference in the increase of HCC or HTC (tables 14, 15, 16). In figures 15 and 16, the changes in HCC and HTC of both groups are shown in diagrams.

Table 14: Living situation - mean HCC and HTC

		N	Mean	Std. Deviation	Std. Error Mean
HCC_mean	Multi-person household	46	18,3741	11,46210	1,68999
	Living alone	11	11,1322	6,77720	2,04340
HTC_mean	Multi-person household	46	1,1432	,82285	,12132
	Living alone	11	,5235	,46549	,14035

Table 15: Living situation - mean change in HCC and HTC month 1-5

		N	Mean	Std. Deviation	Std. Error Mean
Diff_HCC5_HCC1	Multi-person household	46	7,4428	10,40156	1,53363
	Living alone	11	4,1609	10,80135	3,25673
Diff_HTC5_HTC1	Multi-person household	46	,0369	,94235	,13894
	Living alone	11	,0391	,18232	,05497

Table 16: Living situation - nonparametric Mann-Whitney-U-test

	HCC_mean	HTC_mean	Diff_HCC5_HCC1	Diff_HTC5_HTC1
Mann-Whitney U	137,000	115,000	184,000	212,000
Wilcoxon W	203,000	181,000	250,000	278,000
Z	-2,346	-2,790	-1,395	-,829
Asymp. Sig. (2-tailed)	,019	,005	,163	,407

a. Grouping Variable: living situation

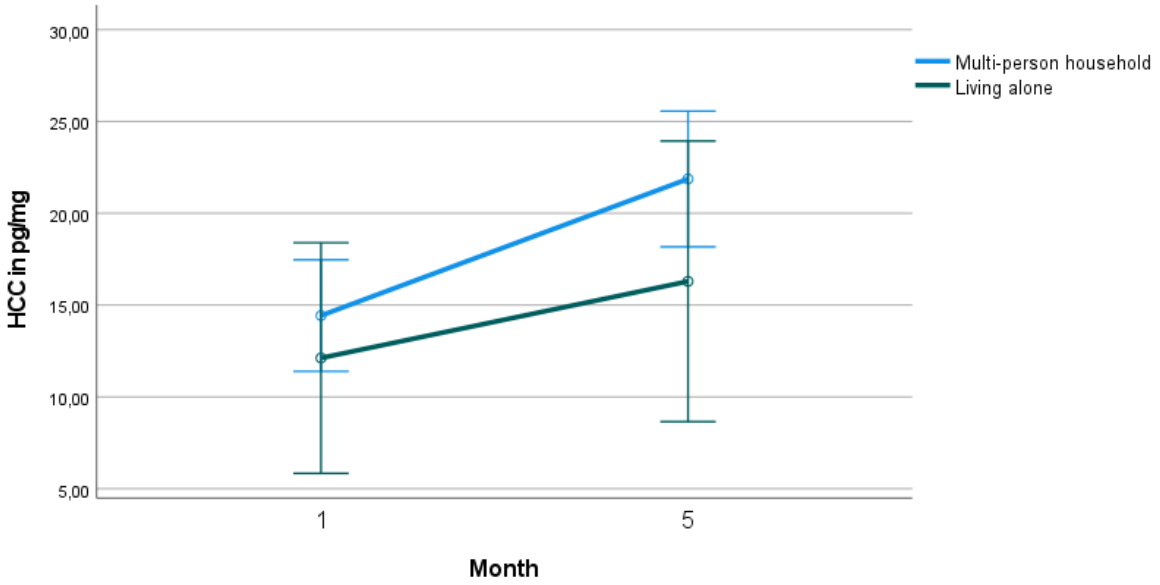


Figure 16: Living situation – changes in HCC from month 1 to month 5

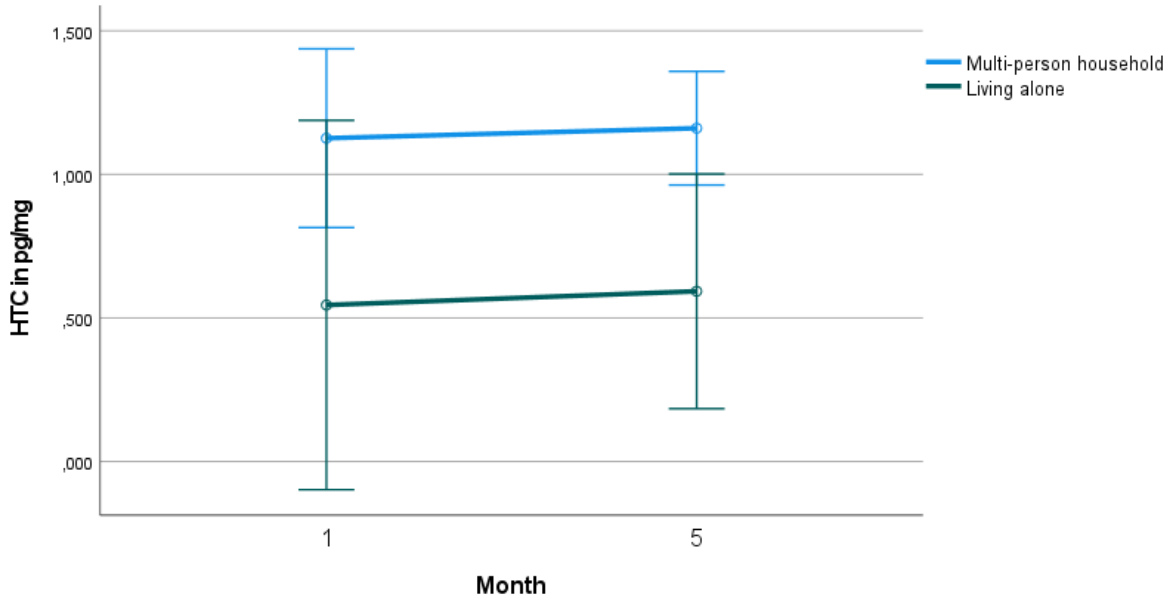


Figure 17: Living situation – changes in HTC from month 1 to month 5

4.5.2 School children

As shown in tables 17, 18 and 19, in participants with school children (n=15), the nonparametric test did not show any significant differences in mean HCC and HTC or in the changes over time compared to those without school children (n=43). In figures 17 and 18, the changes in HCC and HTC of both groups are shown in diagrams.

Table 17: School children – mean HCC and HTC

		N	Mean	Std. Deviation	Std. Error Mean
HCC_mean	No school children	43	17,9214	12,30889	1,87709
	School children	15	13,5640	5,15163	1,33015
HTC_mean	No school children	43	1,0384	,83000	,12657
	School children	15	,7366	,37468	,09674

Table 18: School children – mean change in HCC and HTC month 1-5

		N	Mean	Std. Deviation	Std. Error Mean
Diff_HCC5_HCC1	No school children	43	6,8642	11,32017	1,72631
	School children	15	6,4767	7,42643	1,91750
Diff_HTC5_HTC1	No school children	43	-,0138	,92624	,14125
	School children	15	,1140	,39756	,10265

Table 19: School children – nonparametric Mann-Whitney-U-test

	HCC_mean	HTC_mean	Diff_HCC5_HCC1	Diff_HTC5_HTC1
Mann-Whitney U	277,000	262,000	318,000	272,500
Wilcoxon W	397,000	382,000	438,000	392,500
Z	-,808	-1,074	-,080	-,888
Asymp. Sig. (2-tailed)	,419	,283	,936	,375

a. Grouping Variable: school children

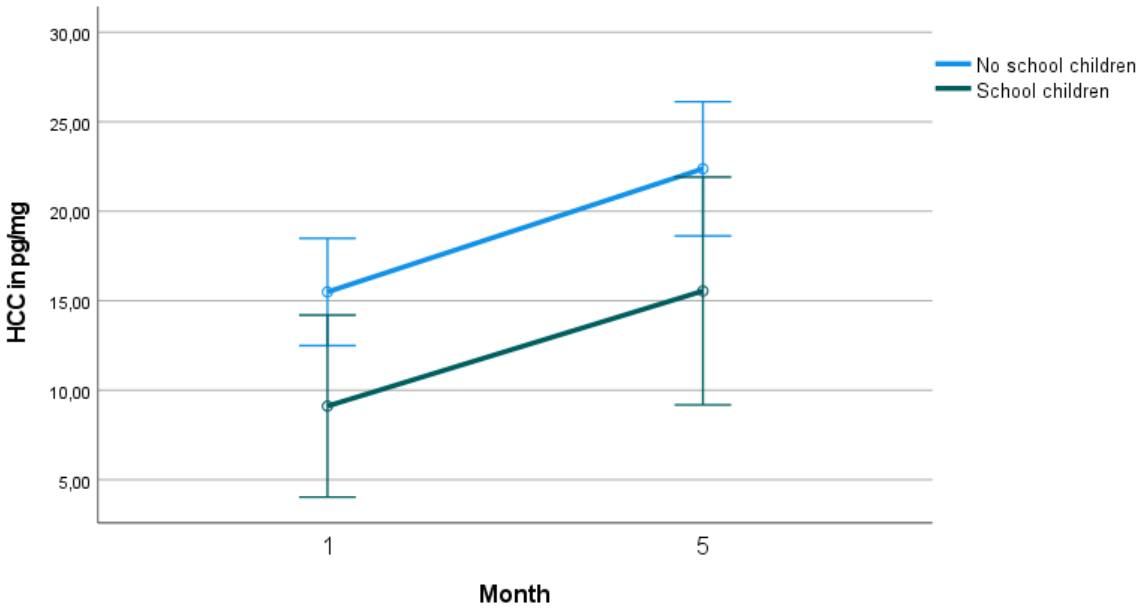


Figure 18: School children – changes in HCC from month 1 to month 5

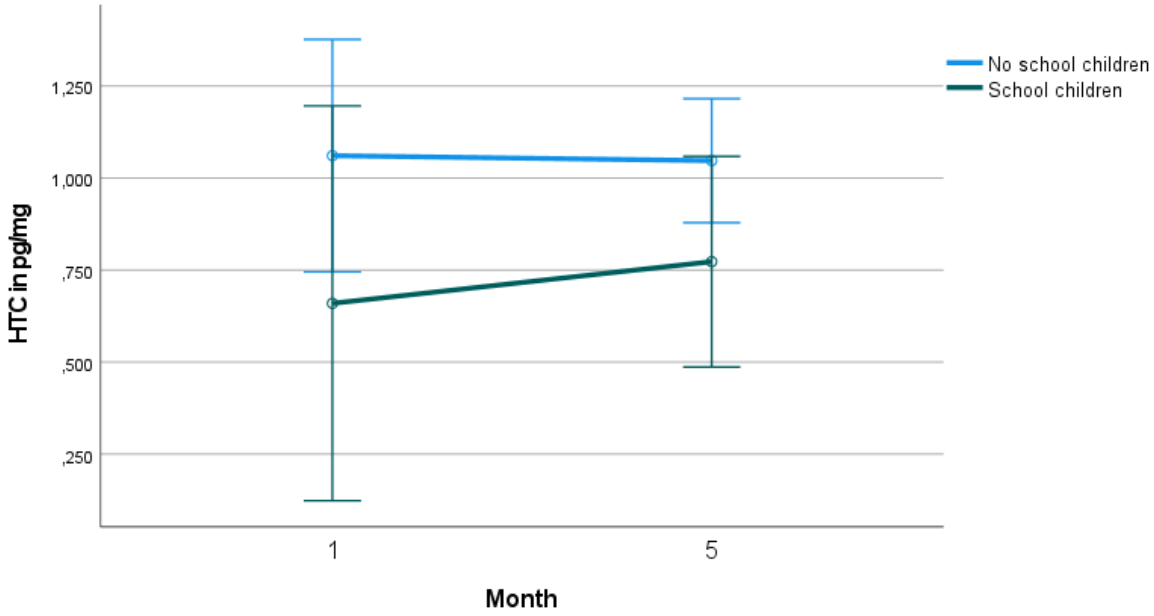


Figure 19: School children – changes in HCC from month 1 to month 5

4.5.3 Shift work

As shown in tables 20, 21 and 22, there were no significant differences in mean HCC and HTC between the participants with shift work (N=19) and those without (n=19). There was a tendency for a larger increase of HCC in participants with shift work compared to those without, but overall, no significant differences in the changes over time could be shown. In figures 19 and 20, the changes in HCC and HTC of both groups are shown in diagrams.

Table 20: Shift work – mean HCC and HTC

		N	Mean	Std. Deviation	Std. Error Mean
HCC_mean	No shift work	19	17,5312	10,10054	2,31722
	Shift work	19	13,4464	6,93186	1,59028
HTC_mean	No shift work	19	1,0384	1,07297	,24616
	Shift work	19	1,0248	,69476	,15939

Table 21: Shift work – mean changes in HCC and HTC month 1-5

		N	Mean	Std. Deviation	Std. Error Mean
Diff_HCC5_HCC1	No shift work	19	3,4926	8,75645	2,00887
	Shift work	19	8,6395	9,94277	2,28103
Diff_HTC5_HTC1	No shift work	19	-,0836	1,29582	,29728
	Shift work	19	,0679	,60306	,13835

Table 22: Shift work – nonparametric Mann-Whitney-U-test

	HCC_mean	HTC_mean	Diff_HCC5_HCC1	Diff_HTC5_HTC1
Mann-Whitney U	139,000	161,000	128,000	172,000
Wilcoxon W	329,000	351,000	318,000	362,000
Z	-1,212	-,569	-1,533	-,248
Asymp. Sig. (2-tailed)	,226	,569	,125	,804
Exact Sig. [2*(1-tailed Sig.)]	,234[b]	,583[b]	,130[b]	,817[b]

a. Grouping Variable: shift work

b. Not corrected for ties.

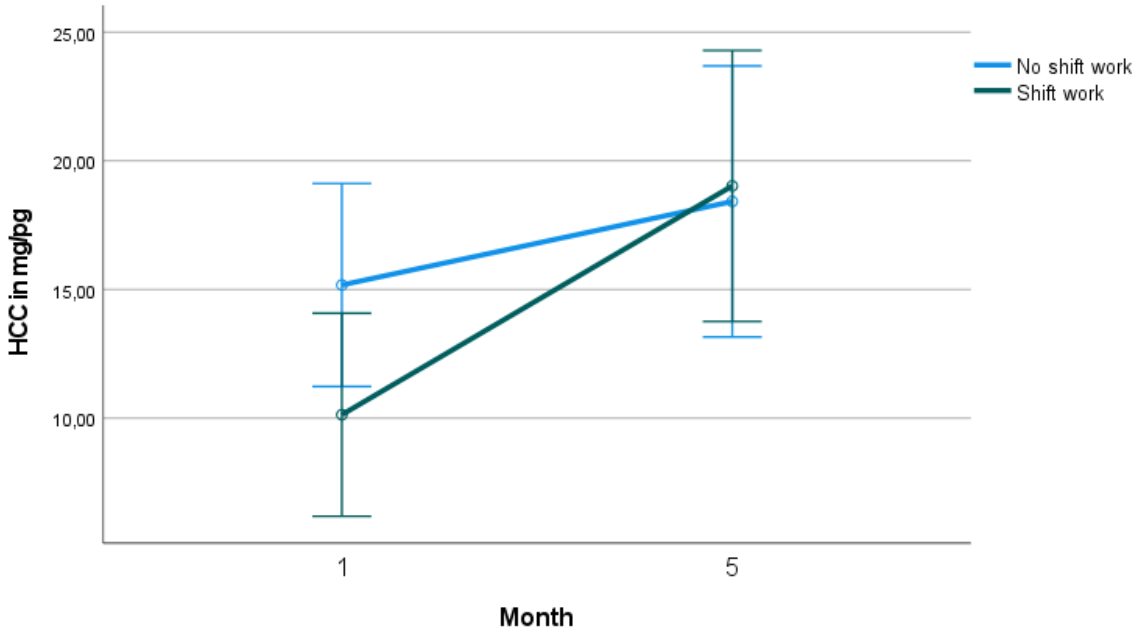


Figure 20: Shift work – changes in HCC from month 1 to month 5

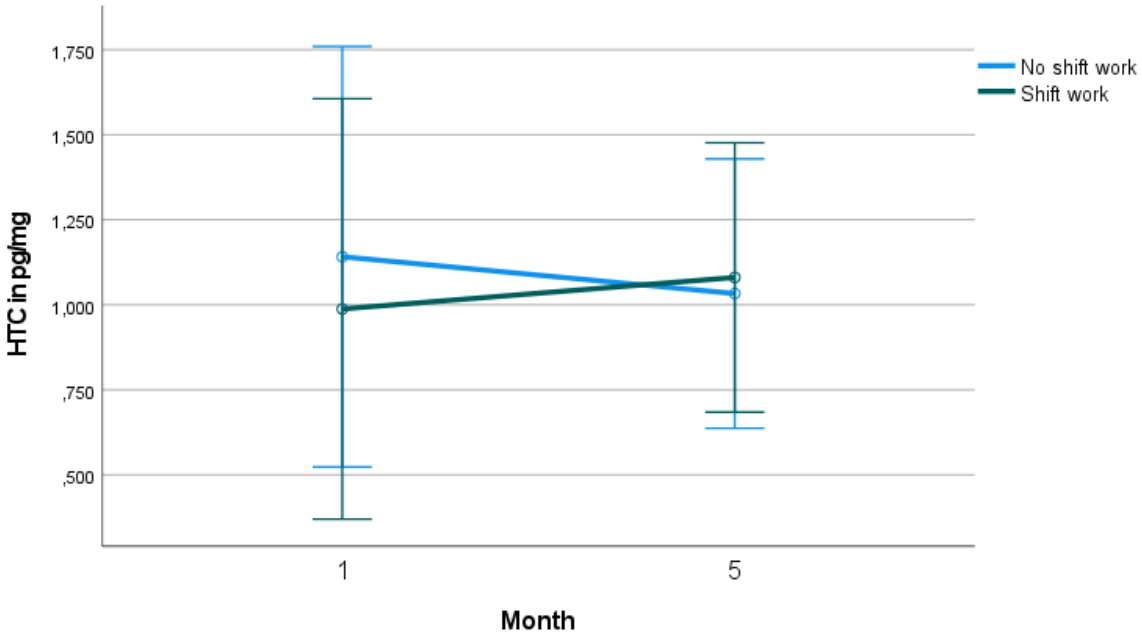


Figure 21: Shift work – changes in HTC from month 1 to month 5

4.5.4 Medication

As shown in tables 23, 24 and 25, no significant differences could be shown between the groups with medication (n=20) and without medication (n=36). In figures 21 and 22, the changes in HCC and HTC of both groups are shown in diagrams.

Table 23: Medication – mean HCC and HTC

		N	Mean	Std. Deviation	Std. Error Mean
HCC_mean	No medication	36	16,7429	11,22943	1,87157
	Medication	20	16,3067	11,01257	2,46248
HTC_mean	No medication	36	,9275	,59650	,09942
	Medication	20	1,1222	1,11880	,25017

Table 24: Medication – mean change in HCC and HTC month 1-5

		N	Mean	Std. Deviation	Std. Error Mean
Diff_HCC5_HCC1	No medication	36	6,8881	10,20925	1,70154
	Medication	20	6,5985	11,47369	2,56560
Diff_HTC5_HTC1	No medication	36	,0813	,48189	,08032
	Medication	20	-,0628	1,28403	,28712

Table 25: Medication – nonparametric Mann-Whitney-U-test

	HCC_mean	HTC_mean	Diff_HCC5_HCC1	Diff_HTC5_HTC1
Mann-Whitney U	338,000	341,000	334,000	351,000
Wilcoxon W	548,000	551,000	544,000	1017,000
Z	-,376	-,325	-,445	-,154
Asymp. Sig. (2-tailed)	,707	,745	,657	,878

a. Grouping Variable: medication

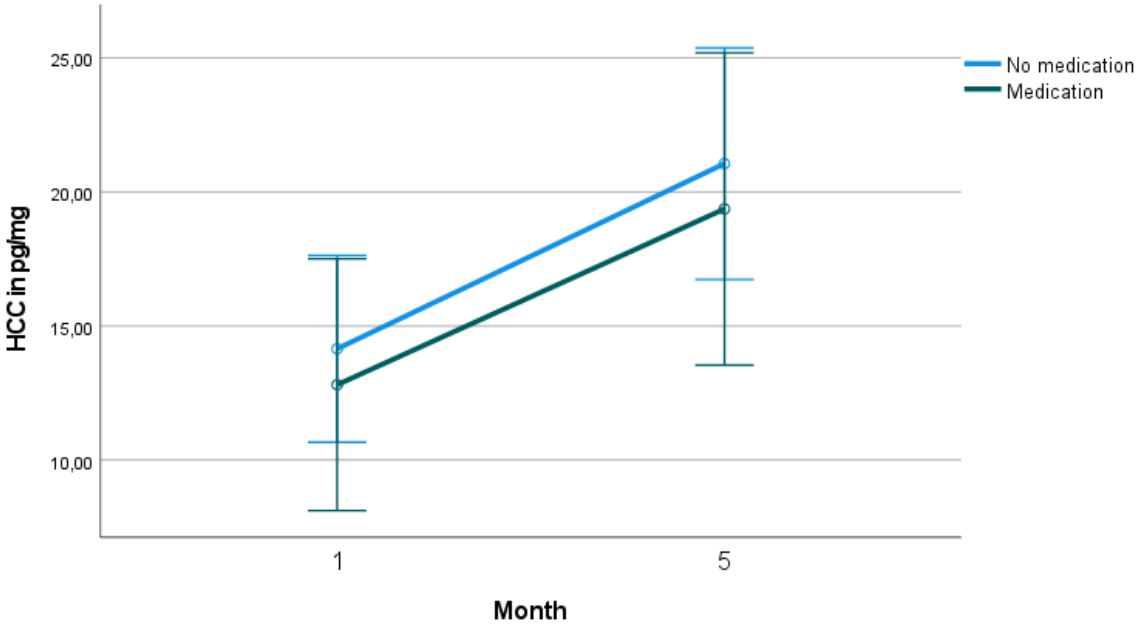


Figure 22: Medication – changes in HCC from month 1 to month 5

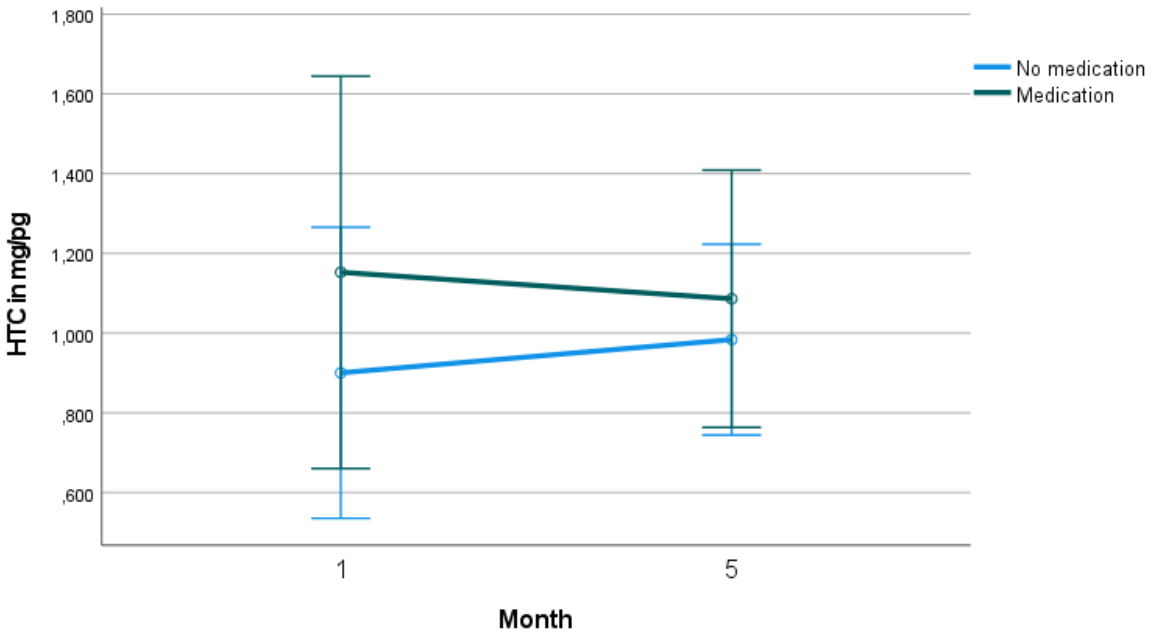


Figure 23: Medication – changes in HTC from month 1 to month 5

4.5.5 Dramatic life events

A tendency towards a higher mean HCC in the group with dramatic life events (n=7) compared to those without dramatic life events (=48) could be shown, but overall, there were no significant differences between the mean HCC and HTC. A difference in the changes of HTC, but not HCC over time could be shown (p=.025), however, due to the small size of the group, this cannot be interpreted as a significant result. In figures 23 and 24, the changes in HCC and HTC of both groups are shown in diagrams.

Table 26: Dramatic life events – mean HCC and HTC

		N	Mean	Std. Deviation	Std. Error Mean
HCC_mean	No dramatic life events	48	15,9168	11,14670	1,60889
	At least one dramatic life event	7	22,2380	9,84025	3,71927
HTC_mean	No dramatic life events	48	1,0458	,86322	,12459
	At least one dramatic life event	7	,7509	,36139	,13659

Table 27: Dramatic life events – mean change HCC and HTC month 1-5

		N	Mean	Std. Deviation	Std. Error Mean
Diff_HCC5_HCC1	No dramatic life events	48	6,5967	9,58458	1,38342
	At least one dramatic life event	7	7,9314	17,42049	6,58433
Diff_HTC5_HTC1	No dramatic life events	48	-,0200	,90583	,13075
	At least one dramatic life event	7	,3334	,23107	,08734

Table 28: Dramatic life events – nonparametric Mann-Whitney-U-test

	HCC_mean	HTC_mean	Diff_HCC5_HCC1	Diff_HTC5_HTC1
Mann-Whitney U	97,000	153,000	151,000	79,000
Wilcoxon W	1273,000	181,000	1327,000	1255,000
Z	-1,793	-,379	-,429	-2,248
Asymp. Sig. (2-tailed)	,073	,705	,668	,025
Exact Sig. [2*(1-tailed Sig.)]	,075[b]	,720[b]	,683[b]	,023[b]

a. Grouping Variable: life events

b. Not corrected for ties.

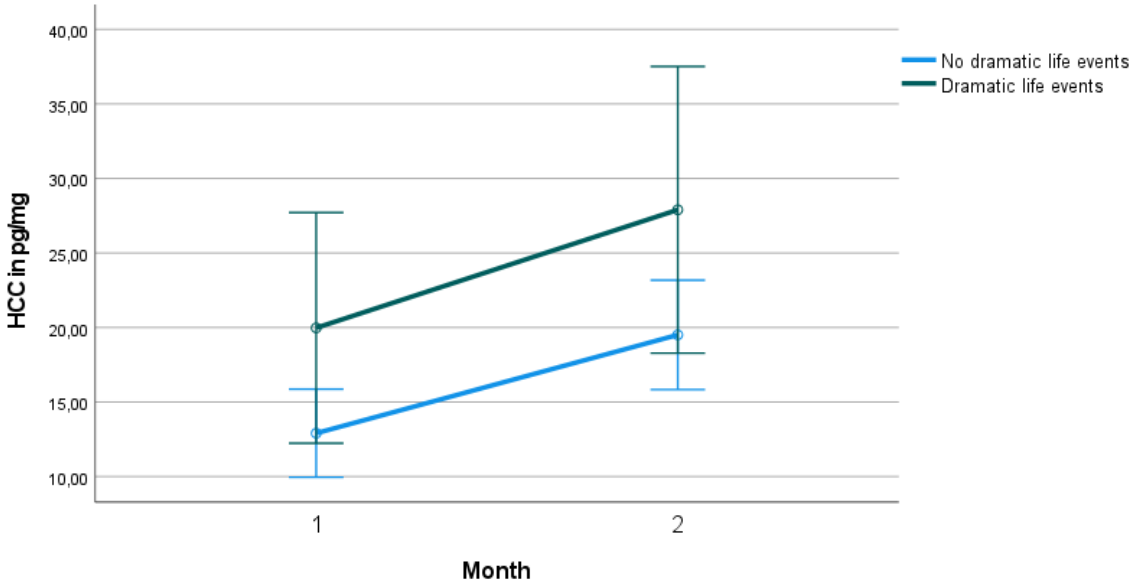


Figure 24: Dramatic life events – changes in HCC from month 1 to month 5

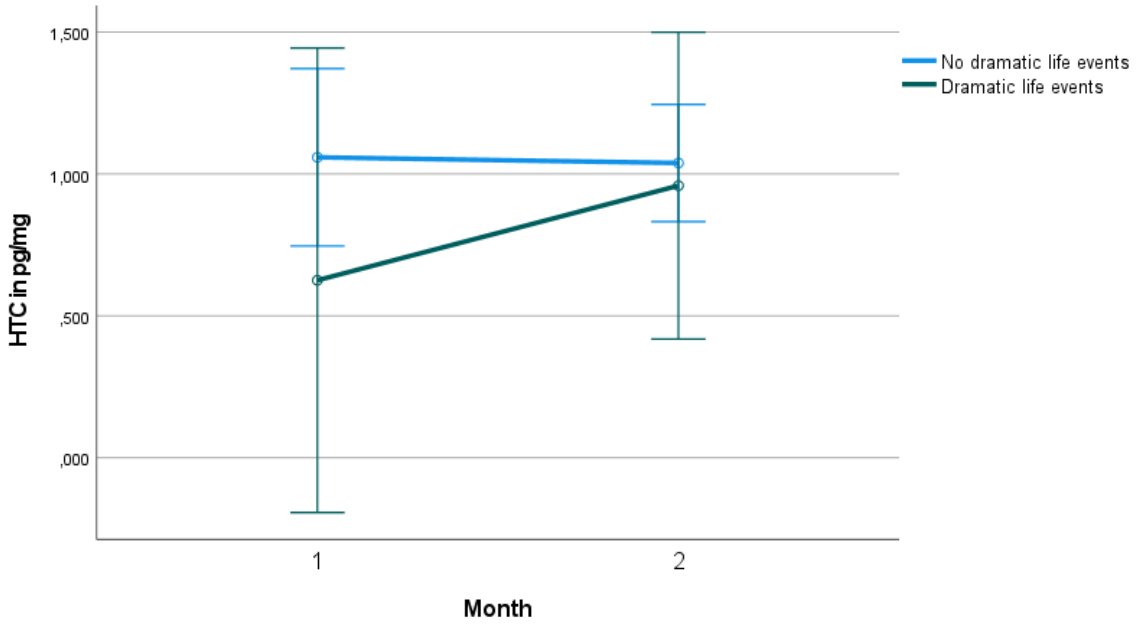


Figure 25: Dramatic life events – changes in HTC from month 1 to month 5

5. Discussion

5.1 Interpretation

Our study showed that during the first COVID-19 lockdown in Austria, there was a significant increase in the hair cortisol concentration in the studied female medical personnel from Graz and the surrounding area. The increase was progressive and continued to rise even after the lockdown period. This result confirms the first hypothesis and suggests that stress increased in the context of lockdown, nationwide policies, higher workload and uncertainty. On the other hand, the hair testosterone concentration remained comparatively stable during the observation period and only increased slightly in month 5. This suggests that an increase in cortisol over a five-month period has little to no effect on testosterone levels in women. An effect may also occur with a delay, so that it has not yet become apparent in the observation period. In addition, the result suggests that the lockdown and the other consequences of the COVID-19 crisis did not have a strong immediate impact on the testosterone levels of female medical personnel. Considering changes in HCC during COVID-19, the results are largely consistent with those of the current literature. For example, a study conducted in Slovakia found that HCC significantly increased in female nurses during the first 3 months of the first wave of the COVID-19 pandemic in 2020. At the same time, 75% of these nurses reported in an online questionnaire that they had experienced significantly more work-related stress during the period. (118) A follow-up study of the same participants came to the same result by showing that HCC increased when the pandemic worsened (119). Furthermore, in the same study, there was no significant interaction between self-report measures of perceived stress, sleep quality, social support and the changes in HCC (118). Another study from the university of Buenos Aires found a correlation between perceived stress and HCC in health workers during the COVID-19 pandemic. In this context, altered hair cortisol levels were associated with the risk of burnout as well. (120) The results of this study and the other studies suggest that HCC is suitable as a marker of increased stress. The concentration of hair cortisol seems to increase over a longer period of time, so that chronic stress can be assumed as the cause. Furthermore, the results confirm that increased chronic stress must have occurred among medical personnel in the context of the COVID-19 pandemic. Among the

potentially influencing factors, participants living in single households had an absolute lower mean HCC and HTC. Moreover, a tendency towards a higher increase in HCC over time was shown in participants doing shift work. Participants who reported dramatic life events showed a tendency for higher mean HCC and a difference in the changes in HTC over time. These results are interesting, since there seem to be protective factors as well as risk factors in the context of chronic stress. In particular, participants who experienced additional stressors during the COVID-19 pandemic, such as the death of a loved one, may also have been at greatest risk of suffering the consequences of chronic stress, such as hypertension or depression. Other factors like having school children or taking medication didn't seem to influence the levels of HCC and HTC. These results remain slightly inaccurate due to the partially small sample sizes, but leave room for speculation that there are damaging and protective factors in relation to stress and that these could also be objectified by appropriate measurements.

5.2 Limitations

There are some limitations of the study, one of which was that the samples were all taken at the same time and not continuously. Thus, a wash out effect could have resulted in the older segments of the hair samples containing less of the steroid hormones than the younger segments. According to recent studies, the use of samples up to 6 months old is reasonable, but a wash-out effect, especially in the distal hair segments, has already been observed and should not be neglected (121, 122). The fact that HTC remained stable over the course of five months makes a strong wash-out effect unlikely. The remaining uncertainty could be solved by repeated hair sampling, whereby this would mean a considerably higher organizational and financial effort. Moreover, it would be difficult to take 1cm long strands each time and to do it at the same position on the scalp. Furthermore, dynamic changes in HCC over the course of a year have been described in various studies, with HCC being lower in winter than in summer (122, 123). Because the samples in this study reflect the period just in between winter and summer, seasonal effects may have contributed to an increase in HCC. Another limiting factor was that males had to be completely excluded from the study because very few of the participants were male. Since the absolute concentration

of testosterone is much higher in males then in females and plays an important role in the difference between the sexes (88), it would have been very interesting to compare changes of HTC in males and females after chronic stress. Another limitation was that some of the subgroups studied for the influence of their sociodemographic characteristics on HCC and HTC were too small to draw reliable conclusions. For example, in the subgroup of participants with dramatic life events, there were only 7 participants, and in the group of participants living in a single household, only 11 participants. If the total number of participants had been higher, subgrouping would have worked better.

5.3 Conclusion and outlook

Our results confirm that during the course of the worldwide COVID-19 pandemic, which was very stressful for the medical personnel and families, our medical staff in Graz and the surrounding areas were exposed to acute as well as chronic stress. This was shown as a continual increase in the concentration (increase of 49.19% within the test period) of the glucocorticoid cortisol in the hair of the participants. Thus, a correlation between chronic stress and an increased cortisol concentration in hair samples was seen. Furthermore, our results suggest that hair cortisol is a reliable marker of chronic stress as the concentration of cortisol in the hair was elevated during the COVID-19 pandemic. At the same time, it was found that there was only a small increase (4.35%) in testosterone concentration in the hair of the participants during the period. Thus, it can be suggested that chronic stress in women in general and female medical personnel as shown in this study does not lead to a great difference in the concentration of testosterone, at least not in the short term. The significance of various sociodemographic and individual characteristics on the concentration of HCC and HTC showed interesting results. These suggested that people who lived together with others during the pandemic, people who worked in shifts, and those with dramatic life events had an overall higher stress load. Overall, our results lead to the conclusion that such unpredictable stressors make it all the more important to take appropriate measures to counteract the disease-promoting effects of chronic stress in the sense of allostatic overload. This could be ensured, for example, through compensatory offers such as sports and leisure activities or the provision of

psychological care for medical staff. In this way, it would be possible to prevent staff from having to give up their jobs temporarily due to stress-related illnesses. For further studies, the inclusion of standardized psychological questionnaires as well as the recording of socio-demographic and individual characteristics in sufficiently large study populations would be useful in order to identify risk factors that make people in general and medical personnel in particular more susceptible to stressors. In addition, it would be useful to further develop methodological options for measuring HCC and, further, HTC, in order to be able to perform measurements more quickly and at lower cost. For example, it would be interesting to carry out measurements with less organizational effort over longer periods of time in order to improve the in order to better answer the research questions in stress related studies. In the future, hair cortisol could eventually be used as a screening marker for chronic stress in routine diagnostics in order to be able to prevent stress-related illnesses.

6. References

1. Traunmüller C, Stefitz R, Gaisbachgrabner K, Schwerdtfeger A. Psychological correlates of COVID-19 pandemic in the Austrian population. BMC Public Health. 2020;20(1):1395.
2. Tsigos C, Kyrou I, Kassi E, Chrousos GP. Stress: Endocrine Physiology and Pathophysiology. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc. ; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278995/.
3. Karl JP, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, et al. Effects of Psychological, Environmental and Physical Stressors on the Gut Microbiota. Frontiers in Microbiology. 2018;9:2013.
4. Schneiderman N, Ironson G, Siegel SD. Stress and health: psychological, behavioral, and biological determinants. Annual Review of Clinical Psychology. 2005;1:607-28.
5. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: A review. EXCLI Journal. 2017;16:1057-72.
6. Mifsud KR, Reul JMHM. Mineralocorticoid and glucocorticoid receptor-mediated control of genomic responses to stress in the brain. Stress. 2018;21(5):389-402.
7. McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, et al. Mechanisms of stress in the brain. Nature Neuroscience. 2015;18(10):1353-63.
8. Charmandari E, Tsigos C, Chrousos G. Endocrinology of the stress response. Annual Review of Physiology. 2004;67(1):259-84.
9. Godoy LD, Rossignoli MT, Delfino-Pereira P, Garcia-Cairasco N, de Lima Umeoka EH. A Comprehensive Overview on Stress Neurobiology: Basic Concepts and Clinical Implications. Frontiers in Behavioral Neuroscience. 2018;12:127.
10. Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, et al. Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. Comprehensive Physiology. 2016;6(2):603-21.
11. DeMorrow S. Role of the Hypothalamic-Pituitary-Adrenal Axis in Health and Disease. International Journal of Molecular Sciences. 2018;19(4):986.
12. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues in Clinical Neuroscience. 2006;8(4):383-95.
13. Stephens MA, Wand G. Stress and the HPA axis: role of glucocorticoids in alcohol dependence. Alcohol Research. 2012;34(4):468-83.
14. Smyth DG. 60 YEARS OF POMC: Lipotropin and beta-endorphin: a perspective. Journal of Molecular Endocrinology. 2016;56(4):T13-T25.
15. Thau L, Gandhi J, Sharma S. Physiology, Cortisol In: StatPearls [Internet]. Treasure Island (FL): State Pearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538239/2022.
16. Chourpiliadis C, Aeddula NR. Physiology, Glucocorticoids. In: StatPearls [Internet]. Treasure Island (FL): State Pearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560897/2022.
17. Ginnard O, Nella A. Adrenal disorders (cortex and medulla) [Internet]. University of Texas Medical Branch 2019 [cited 2021 Mar 22]. Available from: https://www.utmb.edu/pedi_ed/CoreV2/Endocrine/Endocrine6.html.

18. Breuner CW, Orchinik M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. Journal of Endocrinology. 2002;175(1):99-112.
19. Ramamoorthy S, Cidlowski JA. Corticosteroids: Mechanisms of Action in Health and Disease. Rheumatic Disease Clinics Of North America. 2016;42(1):15-31.
20. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine Reviews. 2011;32(1):81-151.
21. Exton JH. Regulation of gluconeogenesis by glucocorticoids. Monographs On Endocrinology. 1979;12:535-46.
22. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Molecular Cell Endocrinology. 2011;335(1):2-13.
23. Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends In Pharmacological Sciences. 2013;34(9):518-30.
24. McKay LI, Cidlowski JA. Physiologic and Pharmacologic Effects of Corticosteroids. 2021. In: Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; 2003. Available from: https://www.ncbi.nlm.nih.gov/books/NBK13780/.
25. Agarwal MK, Mirshahi M. General overview of mineralocorticoid hormone action. Pharmacology & Therapeutics. 1999;84(3):273-326.
26. Booth RE, Johnson JP, Stockand JD. Aldosterone. Advances in Physiology Education. 2002;26(1):8-20.
27. Kubzansky LD, Adler GK. Aldosterone: a forgotten mediator of the relationship between psychological stress and heart disease. Neuroscience & Biobehavioral Reviews. 2010;34(1):80-6.
28. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. Nature Reviews Neuroscience. 2009;10(6):397-409.
29. Andreassi JL. Human Behavior & Physiological Response [4th edition] Mahwah, New Jersey: Lawrence Erlbaum Associates; 2000. Available from: https://books.google.at/books?hl=de&lr=&id=4VcnAAAAQBAJ&oi=fnd&pg=PP1&ots=7WFn9VLfd2&sig=EW4a2Z_T95Sa_kQPwDuLqe-Zx3A&redir_esc=y#v=onepage&q&f=false [
30. Won E, Kim YK. Stress, the Autonomic Nervous System, and the Immune-kynurenine Pathway in the Etiology of Depression. Current Neuropharmacology. 2016;14(7):665-73.
31. Tank AW, Lee Wong D. Peripheral and central effects of circulating catecholamines. Comprehensive Physiology. 2015;5(1):1-15.
32. Paravati S, Rosani A, Warrington SJ. Physiology, Catecholamines. In: StatPearls [Internet]. Treasure Island (FL): Statpearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507716/2022.
33. Liang KC, McGaugh JL, Yao HY. Involvement of amygdala pathways in the influence of post-training intra-amygdala norepinephrine and peripheral epinephrine on memory storage. Brain Research. 1990;508(2):225-33.
34. Miyashita T, Williams CL. Epinephrine administration increases neural impulses propagated along the vagus nerve: Role of peripheral β-adrenergic receptors. Neurobiology of Learning and Memory. 2006;85(2):116-24.
35. Williams CL, Men D, Clayton EC, Gold PE. Norepinephrine release in the amygdala after systemic injection of epinephrine or escapable footshock:

Contribution of the nucleus of the solitary tract. Behavioral Neuroscience. 1998;112:1414-22.
36. Roozendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. Nature Reviews Neuroscience. 2009;10(6):423-33.
37. Dalal R, Grujic D. Epinephrine. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482160/2022.
38. Smith MD, Maani CV. Norepinephrine. In: StatPearls [Internet]. Treasure Island (FL): State Pearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537259/2022.
39. McCorry LK. Physiology of the autonomic nervous system. American Journal of Pharmaceutical Education. 2007;71(4):78.
40. Mariotti A. The effects of chronic stress on health: new insights into the molecular mechanisms of brain-body communication. Future Science OA. 2015;1(3):FSO23.
41. Kessler RC, Price RH, Wortman CB. Social factors in psychopathology: stress, social support, and coping processes. Annual Review of Psychology. 1985;36:531-72.
42. Esler M, Eikelis N, Schlaich M, Lambert G, Alvarenga M, Dawood T, et al. Chronic mental stress is a cause of essential hypertension: presence of biological markers of stress. Clinical and Experimental Pharmacology and Physiology. 2008;35(4):498-502.
43. Dimsdale JE. Psychological stress and cardiovascular disease. Journal of the American College of Cardiology. 2008;51(13):1237-46.
44. Vicennati V, Pasqui F, Cavazza C, Pagotto U, Pasquali R. Stress-related development of obesity and cortisol in women. Obesity (Silver Spring). 2009;17(9):1678-83.
45. Pouwer F, Kupper N, Adriaanse MC. Does emotional stress cause type 2 diabetes mellitus? A review from the European Depression in Diabetes (EDID) Research Consortium. Discovery Medicine. 2010;9(45):112-18.
46. Andenaes R, Kalfoss MH, Wahl A. Psychological distress and quality of life in hospitalized patients with chronic obstructive pulmonary disease. Journal of Advanced Nursing. 2004;46(5):523-30.
47. Sandberg S, Järvenpää S, Penttinen A, Paton JY, McCann DC. Asthma exacerbations in children immediately following stressful life events: a Cox's hierarchical regression. Thorax. 2004;59(12):1046-51.
48. Malhotra SK, Mehta V. Role of stressful life events in induction or exacerbation of psoriasis and chronic urticaria. Indian Journal of Dermatology Venereology Leprology. 2008;74(6):594-99.
49. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. Brain, Behavior, and Immunity. 2017;64:208-19.
50. Ebbesen SM, Zachariae R, Mehlsen MY, Thomsen D, Højgaard A, Ottosen L, et al. Stressful life events are associated with a poor in-vitro fertilization (IVF) outcome: a prospective study. Human Reproduction. 2009;24(9):2173-82.
51. Shapiro GD, Fraser WD, Frasch MG, Séguin JR. Psychosocial stress in pregnancy and preterm birth: associations and mechanisms. Journal of Perinatal Medicine. 2013;41(6):631-45.

52. Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology. 2012;37(5):589-601.
53. Lee D, Kim E, Choi M-H. Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. BMB reports. 2014;48(4):275.
54. Sterling P, Eyer J. Allostasis: A New Paradigm to Explain Arousal Pathology. Handbook of of Life Stress, Cognition and Health 1988.
55. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Hormones and Behavior. 2003;43(1):2-15.
56. Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology. 2013;38(8):1220-35.
57. Seeman TE, McEwen BS, Rowe JW, Singer BH. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. Proceedings of the National Acadamy of Sciences USA. 2001;98(8):4770-75.
58. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neuroscience & Biobehavioral Reviews. 2010;35(1):2-16.
59. McEwen BS. Protective and damaging effects of stress mediators. New England Journal of Medicine. 1998;338(3):171-79.
60. Crosswell AD, Lockwood KG. Best practices for stress measurement: How to measure psychological stress in health research. Health Psychology Open. 2020;7(2):2055102920933072.
61. Þórarinsdóttir H, Faurholt-Jepsen M, Ullum H, Frost M, Bardram JE, Kessing LV. The Validity of Daily Self-Assessed Perceived Stress Measured Using Smartphones in Healthy Individuals: Cohort Study. JMIR Mhealth and Uhealth. 2019;7(8):e13418.
62. Sapolsky RM, Krey LC, McEwen BS. The Neuroendocrinology of Stress and Aging: The Glucocorticoid Cascade Hypothesis. Endocrine Reviews. 1986;7(3):284-301.
63. El-Farhan N, Rees DA, Evans C. Measuring cortisol in serum, urine and saliva - are our assays good enough? Annals of Clinical Biochemistry. 2017;54(3):308-22.
64. Bozovic D, Racic M, Ivkovic N. Salivary cortisol levels as a biological marker of stress reaction. Medical Archives. 2013;67(5):374-77.
65. Aardal-Eriksson E, Karlberg BE, Holm AC. Salivary cortisol--an alternative to serum cortisol determinations in dynamic function tests. Clinical Chemistry and Laboratory Medicine. 1998;36(4):215-22.
66. Odeniyi IA, Fasanmade OA. Urinary free cortisol in the diagnosis of Cushing's syndrome: how useful? Nigererian Journal of Clinical Practice. 2013;16(3):269-72.
67. Haas CS, Rahvar AH, Danneberg S, Lehnert H, Moenig H, Harbeck B. Low Impact of Urinary Cortisol in the Assessment of Hydrocortisone Replacement Therapy. Hormone and Metabolic Research. 2016;48(9):571-74.
68. Wosu AC, Valdimarsdóttir U, Shields AE, Williams DR, Williams MA. Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress. Annals of Epidemiology. 2013;23(12):797-811.
69. Gow R, Thomson S, Rieder M, Van Uum S, Koren G. An assessment of cortisol analysis in hair and its clinical applications. Forensic Science International. 2010;196(1-3):32-7

70. Wennig R. Potential problems with the interpretation of hair analysis results. Forensic Science International. 2000;107(1-3):5-12.
71. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. Clinical Chimica Acta. 2006;370(1-2):17-49.
72. Zarković M, Stefanova E, Cirić J, Penezić Z, Kostić V, Sumarac-Dumanović M, et al. Prolonged psychological stress suppresses cortisol secretion. Clinical Endocrinology (Oxf). 2003;59(6):811-6.
73. Kobelt AJ, Hemsworth PH, Barnett JL, Butler KL. Sources of sampling variation in saliva cortisol in dogs. Research in Veterenary Science. 2003;75(2):157-61.
74. Harkey MR. Anatomy and physiology of hair. Forensic Science International. 1993;63(1-3):9-18.
75. Cone EJ. Mechanisms of drug incorporation into hair. Therapeutic Drug Monitoring. 1996;18(4):438-43.
76. Henderson GL. Mechanisms of drug incorporation into hair. Forensic Science International. 1993;63(1):19-29.
77. Boumba VA, Ziavrou KS, Vougiouklakis T. Hair as a biological indicator of drug use, drug abuse or chronic exposure to environmental toxicants. International Journal of Toxicology. 2006;25(3):143-63.
78. Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, et al. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. FASEB Journal. 2005;19(10):1332-34.
79. Slominski A, Wortsman J, Tuckey RC, Paus R. Differential expression of HPA axis homolog in the skin. Molecular and Cellular Endocrinology. 2007;265-266:143-49.
80. Thomson S, Koren G, Fraser LA, Rieder M, Friedman TC, Van Uum SH. Hair analysis provides a historical record of cortisol levels in Cushing's syndrome. Experimental and Clinical Endocrinology & Diabetes. 2010;118(2):133-38.
81. Sauvé B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clinical and Investive Medicine. 2007;30(5):183-91.
82. van Holland BJ, Frings-Dresen MH, Sluiter JK. Measuring short-term and long-term physiological stress effects by cortisol reactivity in saliva and hair. International Archives of Occupational and Environmental Health. 2012;85(8):849-52.
83. Vanaelst B, Huybrechts I, Bammann K, Michels N, de Vriendt T, Vyncke K, et al. Intercorrelations between serum, salivary, and hair cortisol and child-reported estimates of stress in elementary school girls. Psychophysiology. 2012;49(8):1072-81.
84. Kalra S, Einarson A, Karaskov T, Van Uum S, Koren G. The relationship between stress and hair cortisol in healthy pregnant women. Clinical and Investigative Medicine. 2007;30(2):103-7.
85. Van Uum SH, Sauvé B, Fraser LA, Morley-Forster P, Paul TL, Koren G. Elevated content of cortisol in hair of patients with severe chronic pain: a novel biomarker for stress. Stress. 2008;11(6):483-88.
86. Dettenborn L, Tietze A, Bruckner F, Kirschbaum C. Higher cortisol content in hair among long-term unemployed individuals compared to controls. Psychoneuroendocrinology. 2010;35(9):1404-9.

87. Steudte S, Kolassa IT, Stalder T, Pfeiffer A, Kirschbaum C, Elbert T. Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD. Psychoneuroendocrinology. 2011;36(8):1193-200.
88. Nassar GN, Leslie SW. Physiology, Testosterone. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK526128/2022.
89. Cumming DC, Quigley ME, Yen SS. Acute suppression of circulating testosterone levels by cortisol in men. Journal of Clinical Endocrinology and Metabolism. 1983;57(3):671-73.
90. Pletzer B, Poppelaars ES, Klackl J, Jonas E. The gonadal response to social stress and its relationship to cortisol. Stress. 2021;24(6):866-75.
91. Tang D, Comish P, Kang R. The hallmarks of COVID-19 disease. PLOS Pathogens. 2020;16(5):e1008536.
92. Böhmer MM, Buchholz U, Corman VM, Hoch M, Katz K, Marosevic DV, et al. Investigation of a COVID-19 outbreak in Germany resulting from a single travel-associated primary case: a case series. Lancet Infectious Diseases. 2020;20(8):920-8.
93. Kreidl P, Schmid D, Maritschnik S, Richter L, Borena W, Genger JW, et al. Emergence of coronavirus disease 2019 (COVID-19) in Austria. Wiener Klinische Wochenschrift. 2020;132(21-22):645-52.
94. WHO. Coronavirus disease 2019 (COVID-19) Situation Report – 40 [Internet]. 2020 [cited 2021 Aug 9]. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200229-sitrep-40-covid-19.pdf?sfvrsn=849d0665_2.
95. Sheervalilou R, Shirvaliloo M, Dadashzadeh N, Shirvalilou S, Shahraki O, Pilehvar-Soltanahmadi Y, et al. COVID-19 under spotlight: A close look at the origin, transmission, diagnosis, and treatment of the 2019-nCoV disease. Journal of Cellular Physiology. 2020;235(12):8873-924.
96. WHO. WHO Coronavirus (COVID-19) Dashboard [Internet]. 2021 [cited 2021 Aug 9]. Available from: https://covid19.who.int/.
97. Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. Zhonghua Liu Xing Bing Xue Za Zhi. 2020;41(2):145-51.
98. Cao Y, Hiyoshi A, Montgomery S. COVID-19 case-fatality rate and demographic and socioeconomic influencers: worldwide spatial regression analysis based on country-level data. BMJ Open. 2020;10(11):e043560.
99. Ritchie H, Ortiz-Ospina E, Beltekian D, Mathieu E, Hasell J, Macdonald B, et al. Coronavirus Pandemic (COVID-19) [Internet]. 2020 [cited 2021 Aug 9]. Available from: https://ourworldindata.org/coronavirus.
100. WHO. WHO Coronavirus (COVID-19) Dashboard Austria [Internet]. 2021 [cited 2021 Aug 10]. Available from: https://covid19.who.int/region/euro/country/at.
101. AGES, Österreichische Agentur für Ernährungssicherheit. Dashboard COVID19 [Internet]. 2021 [cited 2021 Aug 10]. Available from: https://covid19-dashboard.ages.at/.
102. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, et al. Coronavirus Disease 2019-COVID-19. Clinical Microbiology Reviews. 2020;33(4):e00028-20.
103. Zhao S, Lin Q, Ran J, Musa SS, Yang G, Wang W, et al. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in

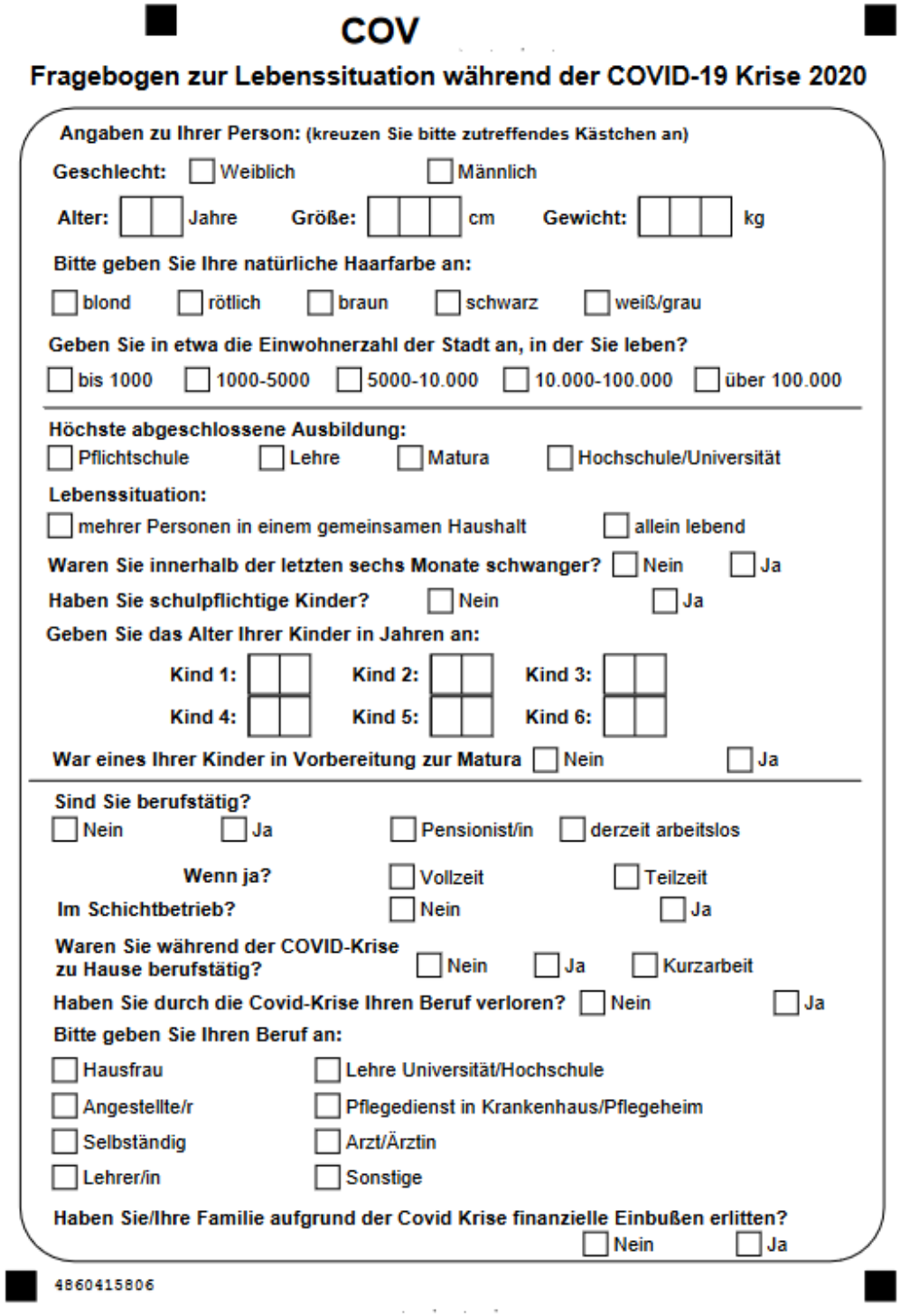
China, from 2019 to 2020: A data-driven analysis in the early phase of the outbreak. International Journal of Infectious Diseases. 2020;92:214-7.
104. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ. 2020;368:606.
105. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020;323(13):1239-42.
106. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
107. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. New England Journal of Medicine. 2020;382(18):1708-20.
108. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 Infection in Children. New England Journal of Medicine. 2020;382(17):1663-5.
109. Fricke-Galindo I, Falfán-Valencia R. Pharmacogenetics Approach for the Improvement of COVID-19 Treatment. Viruses. 2021;13(3):413.
110. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, Fotiou D, Migkou M, Tzanninis IG, et al. Emerging treatment strategies for COVID-19 infection. Clinical and Experimental Medicine. 2021;21(2):167-79.
111. Jirjees F, Saad AK, Al Hano Z, Hatahet T, Al Obaidi H, Dallal Bashi YH. COVID-19 Treatment Guidelines: Do They Really Reflect Best Medical Practices to Manage the Pandemic? Infect Dis Rep. 2021;13(2):259-84.
112. Yang C, Chen A, Chen Y. College students' stress and health in the COVID-19 pandemic: The role of academic workload, separation from school, and fears of contagion. PLoS One. 2021;16(2):e0246676.
113. Schmidt AE, Rodrigues R, Simmons C, Steiber N. A crisis like no other? Unmet needs in healthcare during the first wave of the COVID-19 crisis in Austria. Eur J Public Health. 2022:136.
114. Barbosa J, Faria J, Carvalho F, Pedro M, Queirós O, Moreira R, et al. Hair as an alternative matrix in bioanalysis. Bioanalysis. 2013;5(8):895-914.
115. ORF. Coronavirus - Fahrplan bis Ende April steht [Internet]. 2020 [cited 2022 Jul 19]. Available from: https://orf.at/stories/3160816/.
116. Der Standard. Rückkehr an die Schulen, Geschäftsöffnung, Maskenpflicht: Die Maßnahmen der Regierung im Überblick [Internet]. 2020 [cited 2022 Jul 19]. Available from: https://www.derstandard.at/story/2000116573591/geschaeftsoeffnung-maskenpflicht-und-matura-die-massnahmen-der-regierung-im-ueberblick.
117. Schmid-Zalaudek K, Fredriksen PM, Fabiani E, Fredriksen MV, Brix B, Rössler A, et al. Children’s hair cortisol and hair testosterone concentrations in response to environmental changes: from summer holidays to school. Stress. 2021;24(6):998-1007.
118. Rajcani J, Vytykacova S, Solarikova P, Brezina I. Stress and hair cortisol concentrations in nurses during the first wave of the COVID-19 pandemic. Psychoneuroendocrinology. 2021;129:105245.
119. Rajcani J, Vytykacova S, Solarikova P, Brezina I. A follow-up to the study: Stress and hair cortisol concentrations in nurses during the first wave of the COVID-19 pandemic. Psychoneuroendocrinology. 2021;133:105434.

120. Ibar C, Fortuna F, Gonzalez D, Jamardo J, Jacobsen D, Pugliese L, et al. Evaluation of stress, burnout and hair cortisol levels in health workers at a University Hospital during COVID-19 pandemic. Psychoneuroendocrinology. 2021;128:105213.
121. Stalder T, Steudte-Schmiedgen S, Alexander N, Klucken T, Vater A, Wichmann S, et al. Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. Psychoneuroendocrinology. 2017;77:261-74.
122. Kirschbaum C, Tietze A, Skoluda N, Dettenborn L. Hair as a retrospective calendar of cortisol production—Increased cortisol incorporation into hair in the third trimester of pregnancy. Psychoneuroendocrinology. 2009;34(1):32-7.
123. Fischer S, Duncko R, Hatch SL, Papadopoulos A, Goodwin L, Frissa S, et al. Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol in a South London community sample. Psychoneuroendocrinology. 2017;76:144-53.

7. Appendix

7.1 Questionnaire

In figures 26 and 27, the questionnaire used in the study is shown.



COV

Fragebogen zur Lebenssituation während der COVID-19 Krise 2020

Angaben zu Ihrer Person: (kreuzen Sie bitte zutreffendes Kästchen an)

Geschlecht: ☐ Weiblich ☐ Männlich

Alter: ☐☐ Jahre **Größe:** ☐☐☐ cm **Gewicht:** ☐☐☐ kg

Bitte geben Sie Ihre natürliche Haarfarbe an:

☐ blond ☐ rötlich ☐ braun ☐ schwarz ☐ weiß/grau

Geben Sie in etwa die Einwohnerzahl der Stadt an, in der Sie leben?

☐ bis 1000 ☐ 1000-5000 ☐ 5000-10.000 ☐ 10.000-100.000 ☐ über 100.000

Höchste abgeschlossene Ausbildung:

☐ Pflichtschule ☐ Lehre ☐ Matura ☐ Hochschule/Universität

Lebenssituation:

☐ mehrer Personen in einem gemeinsamen Haushalt ☐ allein lebend

Waren Sie innerhalb der letzten sechs Monate schwanger? ☐ Nein ☐ Ja

Haben Sie schulpflichtige Kinder? ☐ Nein ☐ Ja

Geben Sie das Alter Ihrer Kinder in Jahren an:

Kind 1: ☐☐ **Kind 2:** ☐☐ **Kind 3:** ☐☐

Kind 4: ☐☐ **Kind 5:** ☐☐ **Kind 6:** ☐☐

War eines Ihrer Kinder in Vorbereitung zur Matura ☐ Nein ☐ Ja

Sind Sie berufstätig?

☐ Nein ☐ Ja ☐ Pensionist/in ☐ derzeit arbeitslos

Wenn ja? ☐ Vollzeit ☐ Teilzeit

Im Schichtbetrieb? ☐ Nein ☐ Ja

Waren Sie während der COVID-Krise zu Hause berufstätig? ☐ Nein ☐ Ja ☐ Kurzarbeit

Haben Sie durch die Covid-Krise Ihren Beruf verloren? ☐ Nein ☐ Ja

Bitte geben Sie Ihren Beruf an:

☐ Hausfrau ☐ Lehre Universität/Hochschule

☐ Angestellte/r ☐ Pflegedienst in Krankenhaus/Pflegeheim

☐ Selbständig ☐ Arzt/Ärztin

☐ Lehrer/in ☐ Sonstige

Haben Sie/Ihre Familie aufgrund der Covid Krise finanzielle Einbußen erlitten?

☐ Nein ☐ Ja

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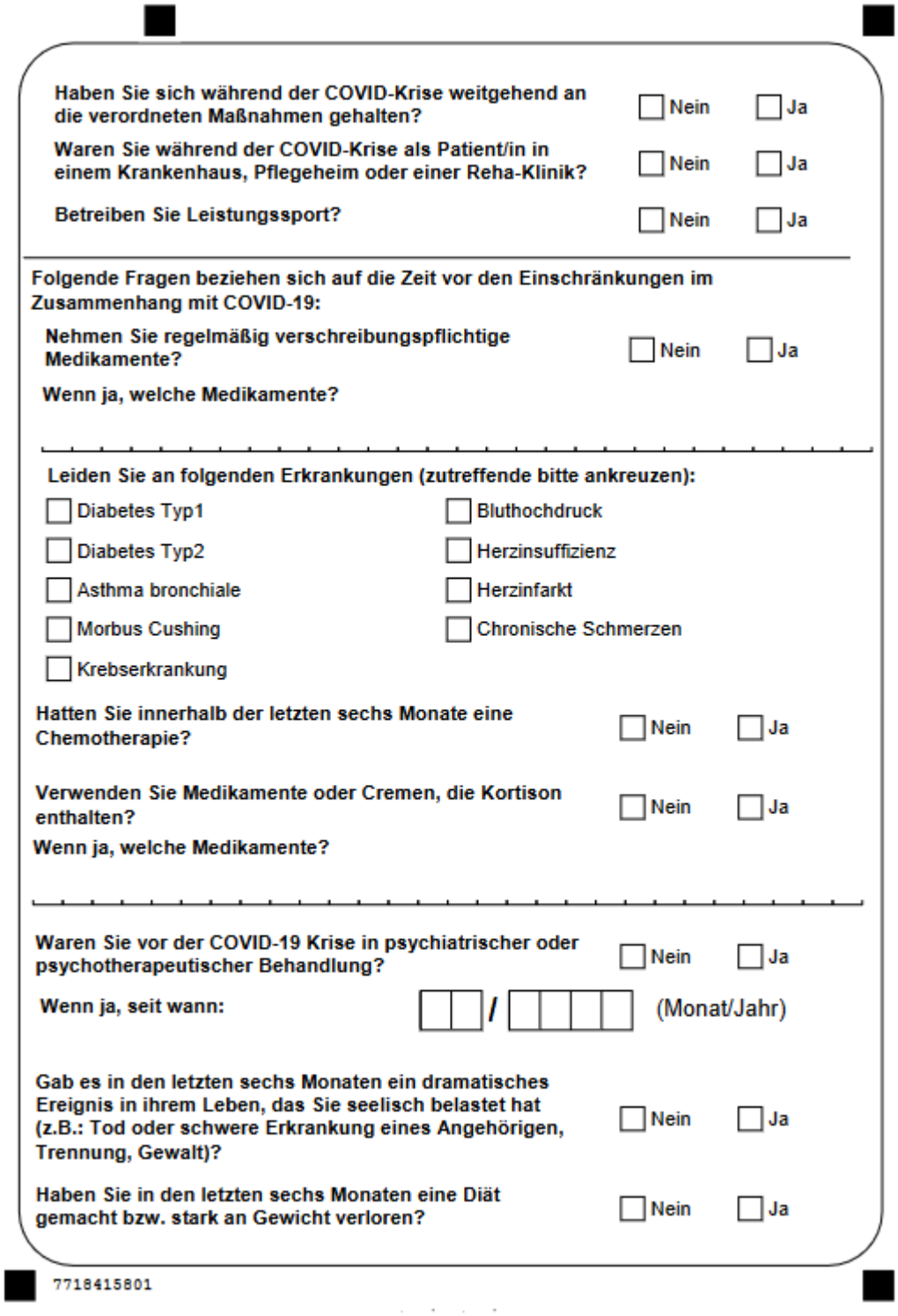
Figure 26: Questionnaire page one



Haben Sie sich während der COVID-Krise weitgehend an die verordneten Maßnahmen gehalten? ☐ Nein ☐ Ja

Waren Sie während der COVID-Krise als Patient/in in einem Krankenhaus, Pflegeheim oder einer Reha-Klinik? ☐ Nein ☐ Ja

Betreiben Sie Leistungssport? ☐ Nein ☐ Ja

Folgende Fragen beziehen sich auf die Zeit vor den Einschränkungen im Zusammenhang mit COVID-19:

Nehmen Sie regelmäßig verschreibungspflichtige Medikamente? ☐ Nein ☐ Ja

Wenn ja, welche Medikamente?

Leiden Sie an folgenden Erkrankungen (zutreffende bitte ankreuzen):

☐ Diabetes Typ1
☐ Diabetes Typ2
☐ Asthma bronchiale
☐ Morbus Cushing
☐ Krebserkrankung
☐ Bluthochdruck
☐ Herzinsuffizienz
☐ Herzinfarkt
☐ Chronische Schmerzen

Hatten Sie innerhalb der letzten sechs Monate eine Chemotherapie? ☐ Nein ☐ Ja

Verwenden Sie Medikamente oder Cremen, die Kortison enthalten? ☐ Nein ☐ Ja

Wenn ja, welche Medikamente?

Waren Sie vor der COVID-19 Krise in psychiatrischer oder psychotherapeutischer Behandlung? ☐ Nein ☐ Ja

Wenn ja, seit wann: ☐☐ / ☐☐☐☐ (Monat/Jahr)

Gab es in den letzten sechs Monaten ein dramatisches Ereignis in ihrem Leben, das Sie seelisch belastet hat (z.B.: Tod oder schwere Erkrankung eines Angehörigen, Trennung, Gewalt)? ☐ Nein ☐ Ja

Haben Sie in den letzten sechs Monaten eine Diät gemacht bzw. stark an Gewicht verloren? ☐ Nein ☐ Ja

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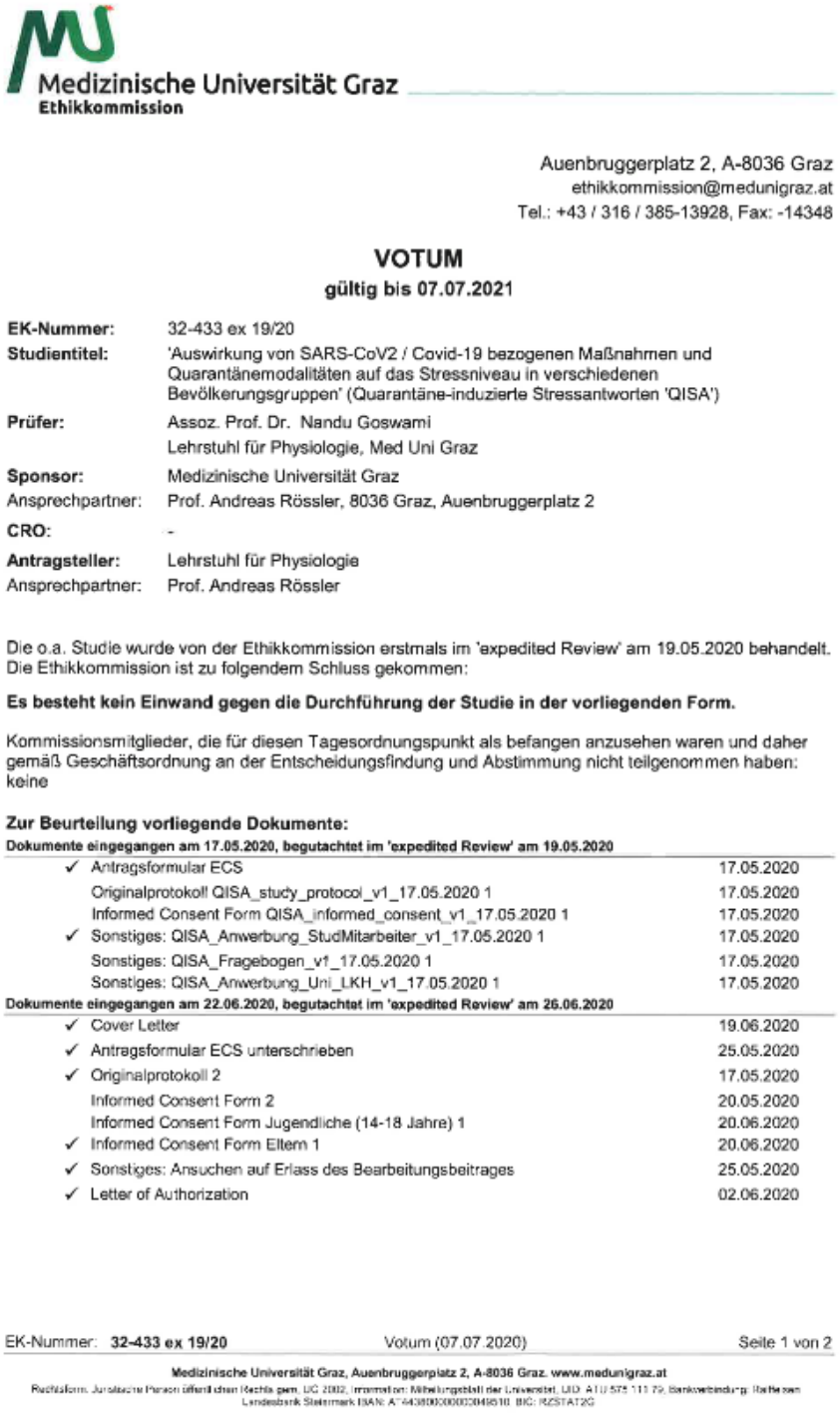
Figure 27: Questionnaire page two

7.2 Ethics approval

In the figures below, the ethics approval of the study is shown.



Medizinische Universität Graz
Ethikkommission

Auenbruggerplatz 2, A-8036 Graz
ethikkommission@medunigraz.at
Tel.: +43 / 316 / 385-13928, Fax: -14348

VOTUM
gültig bis 07.07.2021

EK-Nummer:	32-433 ex 19/20
Studientitel:	'Auswirkung von SARS-CoV2 / Covid-19 bezogenen Maßnahmen und Quarantänemodalitäten auf das Stressniveau in verschiedenen Bevölkerungsgruppen' (Quarantäne-induzierte Stressantworten 'QISA')
Prüfer:	Assoz. Prof. Dr. Nandu Goswami Lehrstuhl für Physiologie, Med Uni Graz
Sponsor:	Medizinische Universität Graz
Ansprechpartner:	Prof. Andreas Rössler, 8036 Graz, Auenbruggerplatz 2
CRO:	-
Antragsteller:	Lehrstuhl für Physiologie
Ansprechpartner:	Prof. Andreas Rössler

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 19.05.2020 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 17.05.2020, begutachtet im 'expedited Review' am 19.05.2020		
✓	Antragsformular ECS	17.05.2020
	Originalprotokoll QISA_study_protocol_v1_17.05.2020 1	17.05.2020
	Informed Consent Form QISA_informed_consent_v1_17.05.2020 1	17.05.2020
✓	Sonstiges: QISA_Anwerbung_StudMitarbeiter_v1_17.05.2020 1	17.05.2020
	Sonstiges: QISA_Fragebogen_v1_17.05.2020 1	17.05.2020
	Sonstiges: QISA_Anwerbung_Uni_LKH_v1_17.05.2020 1	17.05.2020
Dokumente eingegangen am 22.06.2020, begutachtet im 'expedited Review' am 26.06.2020		
✓	Cover Letter	19.06.2020
✓	Antragsformular ECS unterschrieben	25.05.2020
✓	Originalprotokoll 2	17.05.2020
	Informed Consent Form 2	20.05.2020
	Informed Consent Form Jugendliche (14-18 Jahre) 1	20.06.2020
✓	Informed Consent Form Eltern 1	20.06.2020
✓	Sonstiges: Ansuchen auf Erlass des Bearbeitungsbeitrages	25.05.2020
✓	Letter of Authorization	02.06.2020

Figure 28: Ethics approval part 1

Dokumente eingegangen am 29.06.2020 (in der nächsten Begutachtung mitbegutachtet)

✓ Informed Consent Form 3	26.06.2020
Informed Consent Form Kinder/Jugendliche 6-18 Jahre 2	26.06.2020
✓ Fragebögen undatiert	
✓ Fragebögen Kinder/Jugendliche undatiert	

Dokumente eingegangen am 03.07.2020, begutachtet im 'expedited Review' am 07.07.2020

✓ Informed Consent Form Jugendliche (14-18 Jahre) 3.0	03.07.2020
✓ Informed Consent Form Kinder (8-14 Jahre) 1.0	02.07.2020
✓ Sonstiges: mail Stellungnahme	03.07.2020

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung nach AMG bzw. MPG handelt.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

zusätzliche Auflagen: Die behördlich vorgeschriebenen Maßnahmen hinsichtlich der COVID-19 Pandemie müssen beachtet werden. Der Prüfer und der Sponsor müssen in ihrem jeweiligen Wirkungskreis unter allfälliger Beachtung von Leitlinien gewährleisten, dass keine zur Bekämpfung der Pandemie benötigten Ressourcen gebunden werden bzw. ausreichend Personal vorhanden ist und die TeilnehmerInnen durch ihre Studienteilnahme keiner zusätzlichen Infektionsgefahr ausgesetzt werden.

Dieses Votum gilt für ein Jahr ab dem Datum der Ausstellung. Bei längerer Studiendauer ist rechtzeitig vor Ablauf der Gültigkeit des Votums ein Zwischenbericht vorzulegen (Berichtsformular), um eine etwaige Verlängerung zu erlangen.

Graz, 07. Juli 2020

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

Univ.Prof.Dr.Hans Dimai
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. UG 2002, Information: Mitteilungsblatt der Universität, UID: ATU 070 111 75, Bankverbindung: Raiffeisen Landesbank Steiermark IBAN: AT443800000000049510, BIC: RZSTAT2G

Figure 29: Ethics approval part 2