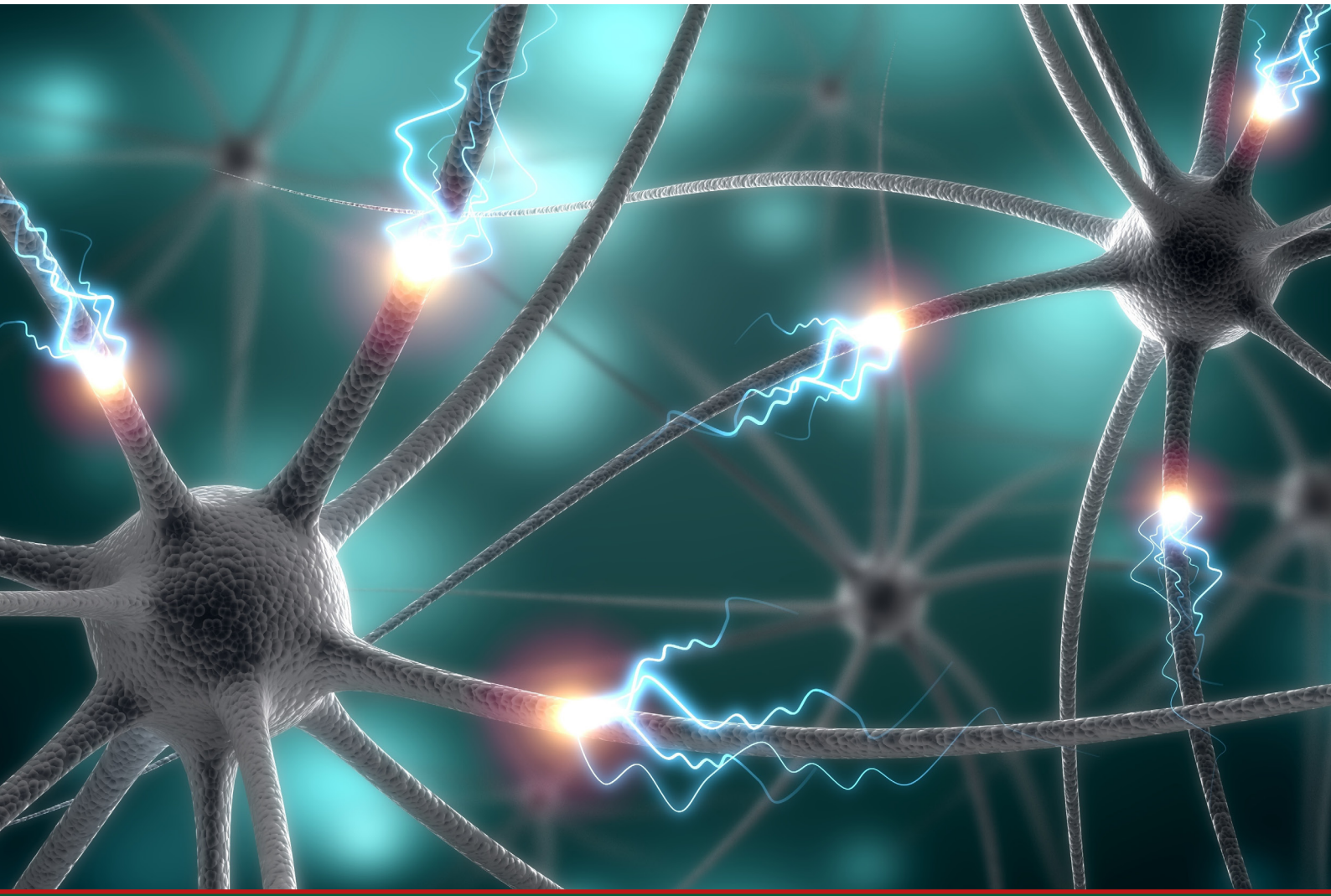
bio|vis' DIAGNOSTIK

Metabolome diagnostics

Anxiety and stress disorders after pandemics or worldwide crises



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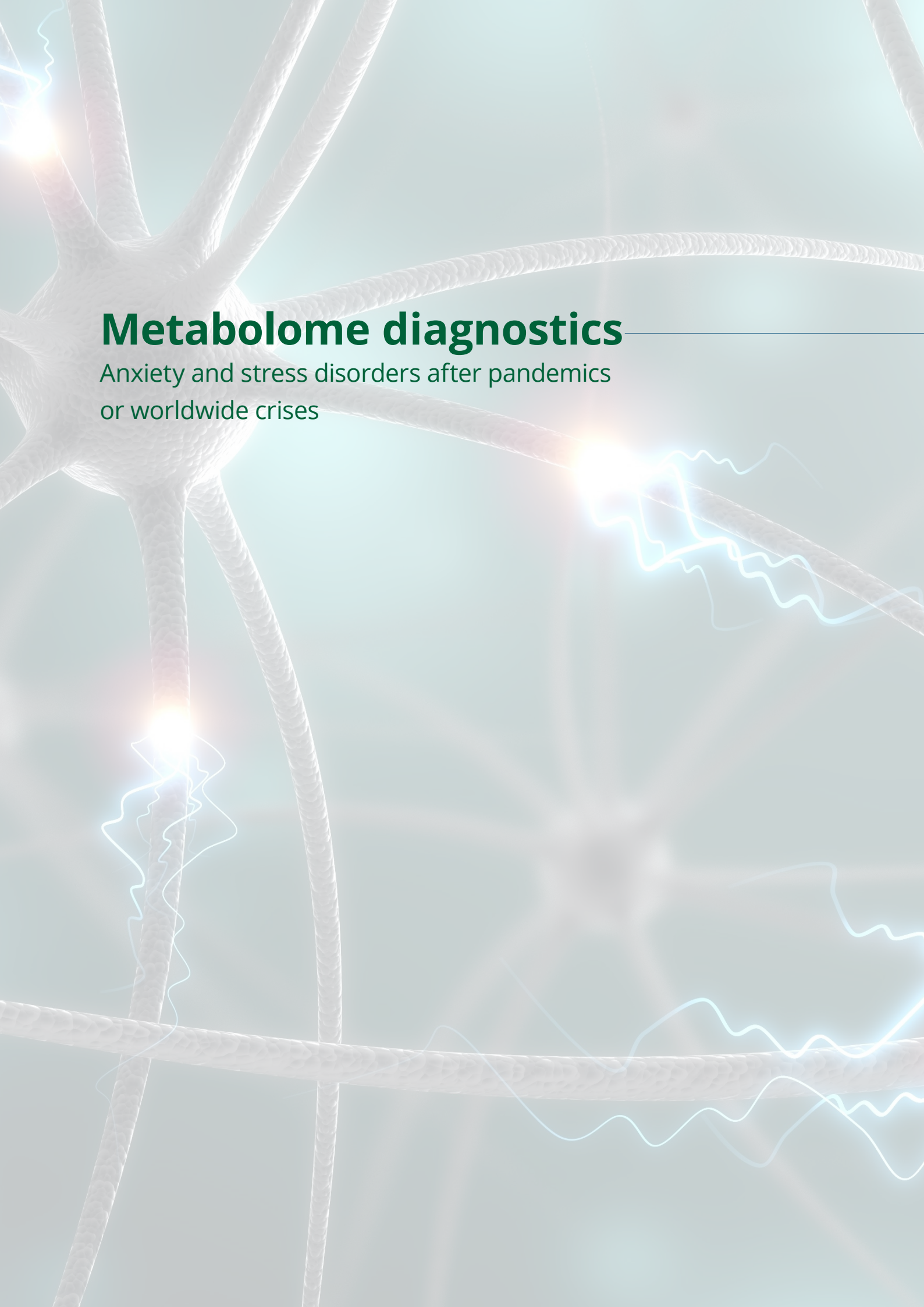

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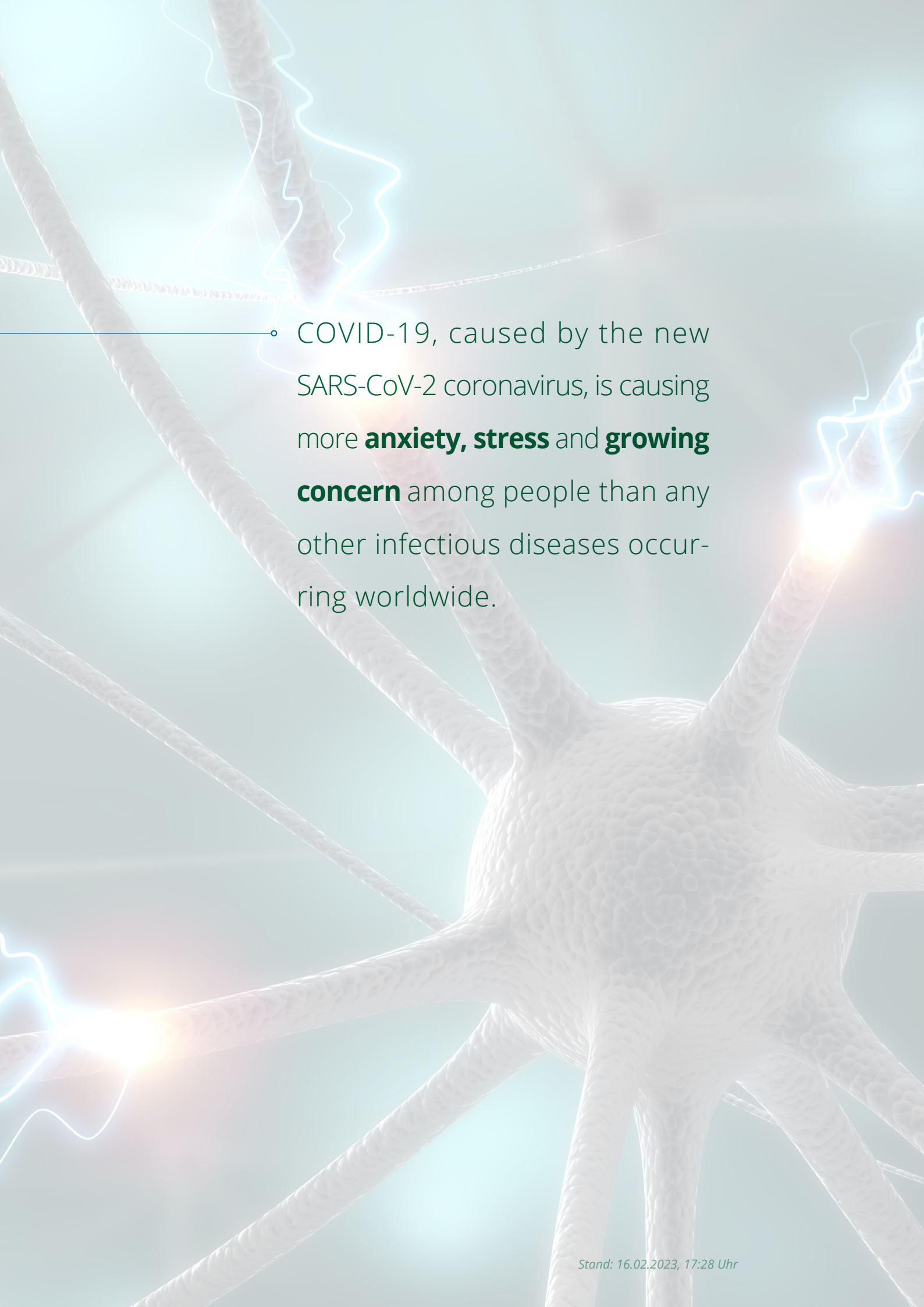

COVID-19, caused by the new SARS-CoV-2 coronavirus, is causing more **anxiety, stress** and **growing concern** among people than any other infectious diseases occurring worldwide.

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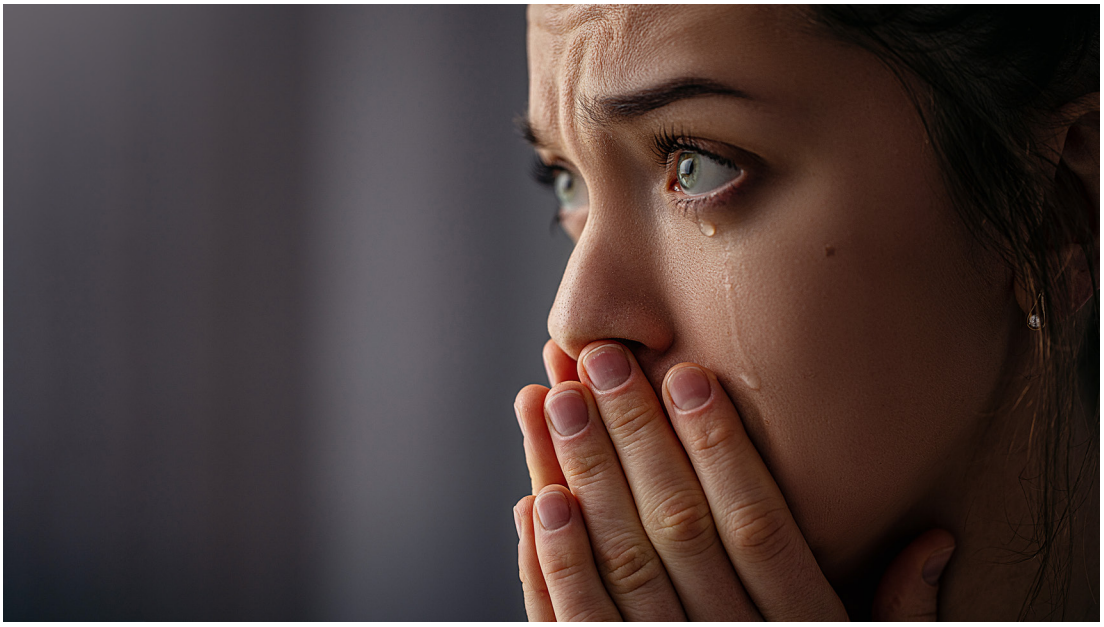


Stress-induced diseases

Pandemics are more than medical phenomena; they affect individuals and communities on multiple levels. They cause psychological disorders. Anxiety and stress have been linked to disease outbreaks. As concerns grow about the perceived threat, people begin to stockpile flour, pasta, toilet paper, or even face masks and other medical supplies. This is often followed by anxiety-related behaviors, sleep disturbances and overall worsened self-perceived health. People with mental illness may be particularly vulnerable to the effects of pervasive fear and threat.

Chronic diseases are often associated with mental disorders [1, 2]. Rates of depression also rise rapidly after infections [3, 4]. Although the impact of the coronavirus pandemic on mental health has not yet been systematically studied, it is reasonable to assume that COVID-19 will have consequences for months to come, also due to its intense media exposure. COVID-19 fuels fear at a communal level. On the individual level it can increase **anxiety**, lead to **stress**, **depression**, or nonspecific mental disorders. Common observations are mood problems, sleep disturbances and phobia- or anxiety-like symptoms, leading to an increase in **hand eczema, skin eczema** and **allergies** [5, 6].

In addition to health problems as one of the pandemic's results, the severe collapse of the global economy will also put a strain on people's health as unemployment rates and numerous company insolvencies grow. A study by Ioannis Laliotis et al [7] in "The Lancet Public Health" examined the health consequences of the sovereign debt crisis in Greece. In addition to an increase in overall mortality, a significantly rising **suicide rate** is particularly striking. People report higher **stress** due to fear of layoffs, loss of salary, or changes in the work environment [8]. Anxiety and changed working conditions often lead to **insomnia** [9], **burnout symptoms** [10] and a higher occurrence of **depression** [11].

Psychological stress is an important factor in the development of **irritable bowel syndrome** (IBS). Indications from clinical and experimental studies show that stress has significant effects on intestinal motility, secretion, and permeability. Stress-induced influences affect the gut-brain axis and often increase the symptoms [12]. The influence of stress in crises and the resulting burdens not only effect patients with IBS, they also influence the course of chronic complex diseases, such as **Crohn's disease** or **ulcerative colitis** [13].

When we confront a crisis, whether as a product of the COVID 19 pandemic or a severe financial or economic crisis resulting from it, this has consequences for our health. When we are anxious and stressed, the delicate balance of the psyche, nervous and immune systems is disturbed. People become more vulnerable to infections, inflammations and allergies. Emotional strain and stress not only put pressure on the mind but also on the immune system [14]. There is a persistent elevated release of cortisol and catecholamines with negative effects for the organism. Stress, burnout or depressions are frequently combined with chronic subclinical inflammations, which lead to an influence on important metabolic pathways [15]. The resulting consequences can significantly influence the development and course of diseases.

Influence of anxiety and stress

Stress can be caused by a wide variety of factors: Fear of infection, partnership or family problems as a result of social distancing, as well as existential fears in an economical crisis due to the threat of losing one's job or negative changes in everyday working life.

All of this causes stress and triggers a neuroendocrine functional axis that leads to increased release of cortisol and catecholamines.

Cortisol is without a doubt our most important stress hormone. It is formed from cholesterol and leads to reactions in the body that will allow us to cope with acute stress situations. To do this, cortisol causes the blood sugar and triglyceride level in the blood to rise in order to quickly supply the body with large amounts of energy. For evolutionary purposes, a rapid supply of energy was important in order to be able to fight or flee from danger. However, current stress triggers can hardly be managed by fight or flight. Persistent fears lead to stress and thus to a release of cortisol, which burdens the body [16]. The body cannot use the sudden supply of energy, but must process it differently, for example by releasing more insulin. Under ongoing stress, other cortisol effects also cause difficulties. Rising blood pressure and increased blood sugar levels are examples. Reduced blood flow to the skin and intestines often leads to digestive disorders. On top of that, cortisol inhibits the cellular immune response: both natural killer cells and T-helper cells are suppressed [17]. This makes people under stress more vulnerable to infections and promotes tumor progression.

If chronic stress persists over a long period of time, the stress hormone production in the adrenal cortex becomes exhausted [18]: the affected person becomes tired, lacks drive, has a variety of physical complaints and everyday tasks represent an excessive effort for them.

The catecholamines adrenaline, noradrenaline and dopamine are released within seconds upon a stressful event. The adrenaline produced in the adrenal medulla leads to a higher pulse rate, cardiac output, blood pressure and mental activity. It also inhibits cellular immune defense [19]. Norepinephrine also causes blood pressure to rise, promotes motivation, focus and motor activity. Just like adrenaline, noradrenaline also has an inhibitory effect on the cellular immune response. After all, dopamine is an important stimulating neurotransmitter.

Similar to noradrenaline, it has a positive effect on motility, focus, drive, motivation and cognitive performance [20]. All three catecholamines are formed from tyrosine, a non-essential amino acid, which in turn can be synthesized from the essential amino acid phenylalanine. Catecholamines, unlike cortisol, are broken down very rapidly by the body. Their half-life lasts only a few minutes. Catecholamine levels are elevated under stress [21]. If the patient suffers from long-term stress exposure, burnout syndrome or CFS (chronic fatigue syndrome), the measurement results are often below the normal range. Both the adrenal glands and the neurons have have minimized themselves in the production of neurotransmitters over the long period of stress.

Serotonin is an important inhibitory neurotransmitter and precursor of the sleep hormone melatonin. For the production of serotonin, the body depends on an adequate supply of tryptophan, which is found in protein-containing food [22]. Important cofactors for serotonin synthesis are vitamins of the B complex, especially vitamin B6 and folic acid. In addition, niacin has an influence, too. Finally, serotonin counteracts stress, regulates blood pressure, intestinal motility, has a relaxing, mood-lifting, sleep-regulating effect, anti-anxiety, anti-depressant and exerts a positive influence on many brain functions [23, 24].

Long-term stress, severe pain or chronic inflammations lead to an increased conversion of tryptophan into L-kynurenine, which is no longer available for serotonin production. This may result in disturbances of the emotional life and depression [25].

GABA, ɣ-aminobutyric acid, is another important inhibitory neurotransmitter in the central nervous system in addition to serotonin. It counteracts the stimulating catecholamines and also dampens the stress response mediated by cortisol [26]. GABA stabilizes blood pressure, regulates appetite, and has anxiety-relieving and sleep-inducing effects. GABA is formed from glutamic acid, a non-essential amino acid that acts as an excitatory neurotransmitter in the central nervous system and can be considered as GABA's antagonist. **Glutamic acid** promotes motor activity, learning and memory [27].

Classical and new analysis methods

In addition to classical diagnostic approaches for the detection of acute or chronic stress on the basis of cortisol determinations in saliva or neurotransmitter detections in urine, we would also like to present new analytical methods, so-called **metabolome analyses**. They are based on a highly modern technology and allow completely new insights into the complex but very interesting world of **stress-** or **anxiety-related diseases**.

Classical methods for the detection of acute and chronic stress loads

Cortisol Daily Profile

Simple saliva tests give you indications of acute or chronic stress loads in your patients. In a **cortisol daily profile**, the cortisol levels are measured in the morning, at noon and in the evening in one saliva sample each. The normal course of a cortisol daily profile shows a maximum in the morning, about one to two hours after waking up. Thereafter, the values decrease continuously during the course of the day. In the early afternoon, there may increase until the lowest level is reached in the evening (see Fig. 1).

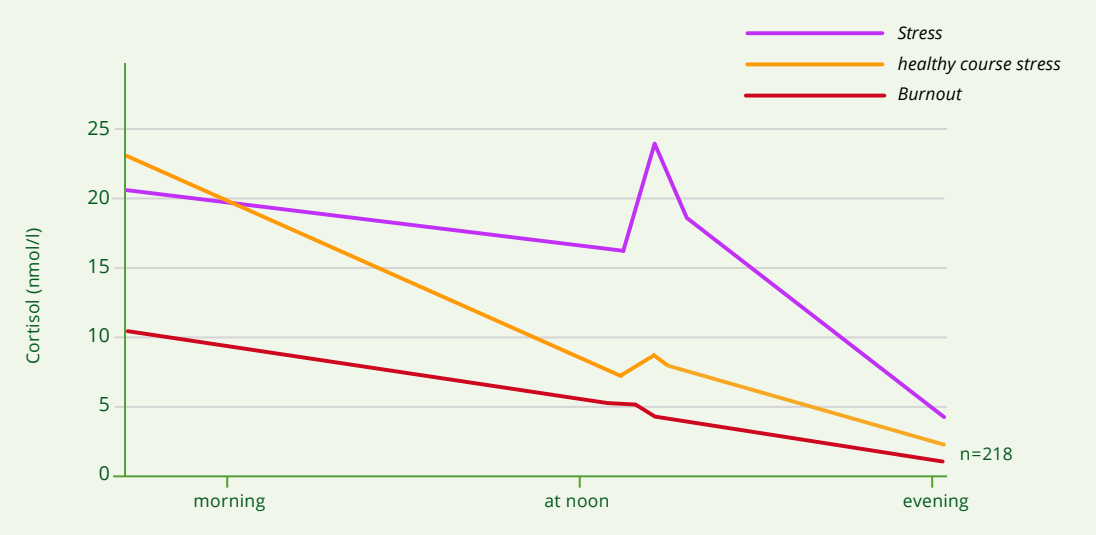


Fig. 1
Daily course of cortisol levels in saliva
The graph shows how the daily cortisol profiles differ between healthy individuals, patients under acute stress and burnout patients.
(Source: Own illustration)

Under acute stress, cortisol levels rise temporarily. From one, two or all three measured values can be affected in the daily profile.

In the case of long-term chronic stress impacts, a period of increased cortisol levels is followed by a drop in salivary hormone concentrations [18]. The measured values fall below the normal area. At the beginning, only decreased values are shown frequently in the morning, later the other daily values also fall below the norm. Decreased cortisol levels are commonly found in patients with CFS or burnout syndrome

➔ **Profil: O200 Cortisol daily profile i. saliva Test Kit Bio1**

Cortisol DHEA Daily Profile

An important antagonist of cortisol is **DHEA**, which is able to neutralize negative cortisol-mediated stress effects [28]. It has a positive effect on blood lipid levels (LDL reduction and HDL increase), stimulates the cellular immune response, has an anti-inflammatory effect and increases insulin sensitivity [29]. If one wishes to include the balancing effects of DHEA, there is an extended profile available, called the cortisol-DHEA daily profile. Saliva samples are also required for this. The corresponding test material is provided within the test set (Bio1).

➔ **Profil: O220 Cortisol DHEA Daily Test Kit Bio1**

Neurostressprofil

The catecholamines **epinephrine**, **norepinephrine**, and **dopamine** are released within seconds after a stressful event. Their lifespan only lasts a few minutes.released during a stress event. Their half-life is only a few minutes.

For the diagnosis of catecholamines and **serotonin**, the **second morning urine** is required. Catecholamine levels are elevated under severe stress.However, if the patient suffers from long-term massive stress or if CFS or burnout syndrome is already present, the measured values are often below the standard range.syndrome, the measured values are often below the normal range.

Long-term stress also leads to decreasing serotonin levels. Tryptophan is increasingly converted into L-kynurenine and is no longer available for serotonin synthesis. This can result in negative effects on mental health and lead to depressions. A sample report for the profile F500 is provided in Fig. 2.

➔ **Profil:** **F500 Neurotransmitter base** **Test Kit 908**
catecholamines (adrenaline, noradrenaline, dopamine), serotoni

Test	Ergebnis	Einheit	Normbereich	Vorwert
Endokrinologie				
Neurotransmitter Basis				
Serotonin	108,40	µg/g Crea	80 - 190	
Katecholamine				
Adrenalin	1,86	µg/g Crea	2,0 - 5,5	
Noradrenalin	41,15	µg/g Crea	15 - 36	
Dopamin	256,64	µg/g Crea	130 - 240	
Noradrenalin/Adrenalin Quotient	22,16	Quotient	3 - 6	

Fig. 2
Sample findings Profile F500
Norepinephrine and dopamine are increased, epinephrine is decreased. Serotonin is unremarkable. Indication of strong Stress load and disruption in catecholamine buildup.

Metabolome analyses - New paths to future-oriented laboratory diagnostics

In addition to the profiles described above, which biovis has been offering for several years now, more comprehensive profiles have been developed as part of a metabolome project. They do not only include cortisol-intake during the course of the day and a few neurotransmitters but also cover the complete metabolic pathways, including important metabolites, enzymes and cofactors.As a result, disorders can be localized much more precisely and therapeutic approaches can be provided in a more targeted and efficient manner.

Metabolome analyses (see Fig. 3) represent new future-oriented approaches in laboratory diagnostics. They require sophisticated analytical methods and are mostly based on LC-MS/MS technology (LC tandem mass spectrometry). Unlike in the past, individual parameters are not analyzed and then combined into smaller profiles. Metabolome analyses allow the simultaneous analysis of **up to 70 metabolites** or low molecular weight analytes, regardless of whether they are amino acids, peptides, lipids, sugars or organic acids. **Complete metabolic pathways** can be illustrated by specific combinations of the analytes they contain. Starting with amino acids, for example, all **biologically active metabolites** can be taken into account that are involved in the regulation of immune reactions, neuronal functions or inflammatory processes. By quantifying dependent metabolites, **enzyme activities** or influences of **important cofactors** can be identified. On the basis of three or four measured metabolites, there is no need to speculate about the consequences anymore.

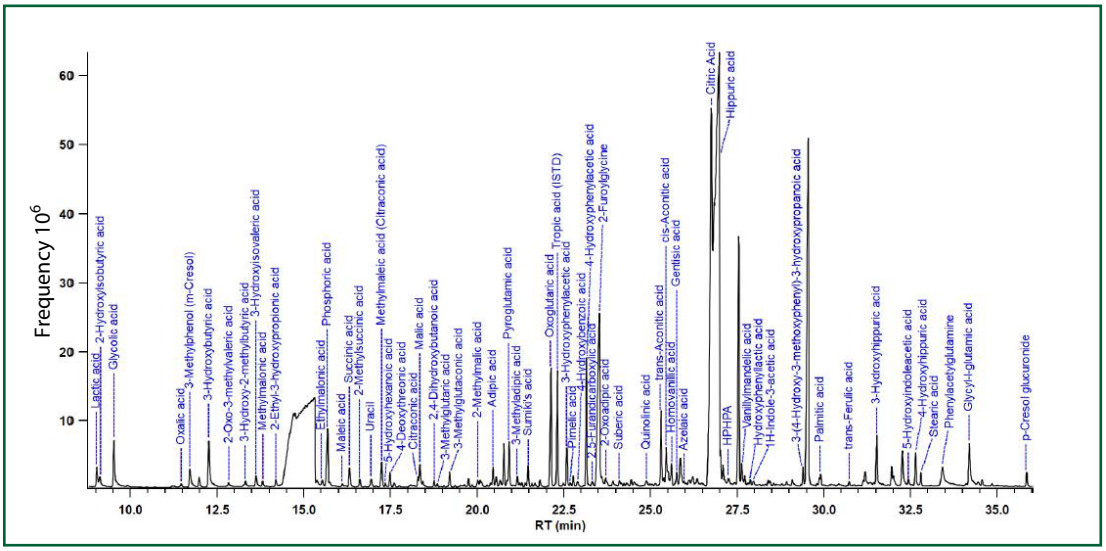
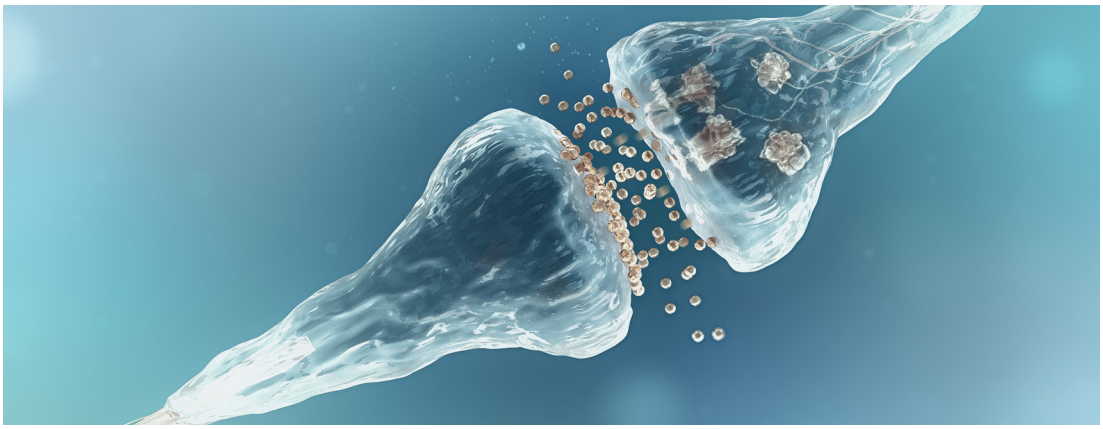


Fig. 3

Metabolome analysis: complex metabolic analysis instead of single parameter tests
Example of a metabolome analysis in urine via mass spectrometry: More than 100 chromatographic peaks are detectable and more than 70 organic compounds can be identified.
(Quelle: Wishart DS. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol Rev. 2019;99(4):1819-1875. doi:10.1152/physrev.00035.2018)

With using metabolome analyses, end products of the relevant metabolic pathways are recorded as analytes which makes previous assumptions turn into **facts.**

New diagnostic approaches do not always have to lead to rising prices, despite using modern measurement methods. This also applies to the new metabolome diagnostics. Profiles consisting of 25, 30 or more analytes can be obtained **efficiently** with the new methods rather than through conventional individual tests.



NT-TRP metabolism

The NT-TRP metabolism profile doesn't just include the analysis of catecholamines and serotonin but also provides a complete insight into tryptophan metabolism (see Fig. 4) with all important metabolites and enzyme activities.

Tryptophan metabolism is of monumental importance for human health. It regulates important neurochemical functions and parts of the immune system [30].

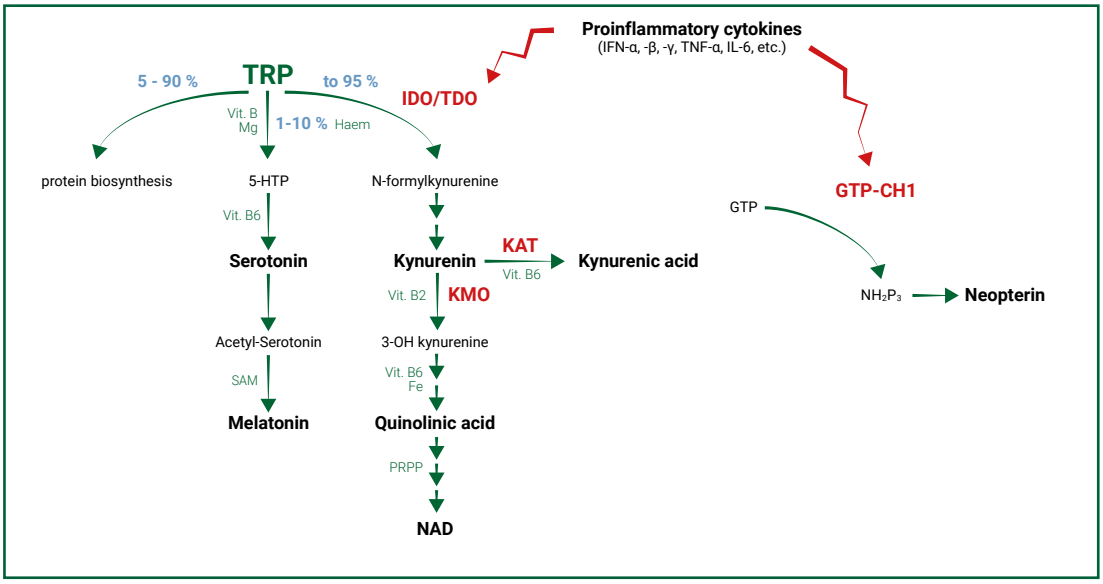


Fig. 4
Tryptophan metabolism
*Explanations: **TRP** = tryptophan; **TDO** = tryptophan 2,3-dioxygenase (esp. liver, heart, lung, brain); **IDO** = indoleamine 2,3-dioxygenase (remaining tissue); **5-HTP** = 5-hydroxy-tryptophan; **KMO** = kynurenine monooxygenase; **KAT** = kynurenine oxoglutarate transaminase; **NAD** = nicotinamide adenine dinucleotide (reduction equivalent, cofactor); **SAM** = S-adenosylmethionine; **PRPP** = α-5'-phosphoribosyl-1'-pyrophosphate.*
(Source: Own illustration)

Disruptions in tryptophan metabolism caused by anxiety, stress or subclinical inflammatory reactions can have negative effects on the course of disease and the potential for recovery.

NT-TRP Metabolism contains a complete analysis of tryptophan metabolism with all major bioactive metabolites and relevant enzymes in addition to the analysis of catecholamines and serotonin. We decided to include following **13 parameters**:

- **Initial substance:** *Tryptophan*
- **Serotonin pathway:** *Serotonin*
- **Kynurenin Path:** *L-kynurenine*
  Kynurenic acid
  3-OH-kynurenine
  Quinolinic acid
  NAD (nicotinamide adenine dinucleotide)
- **Relevant enzymes:** *IDO (indoleamine 2,3-dioxigenase)*
  KMO (Kynurenin-Monooxigenase)
- **Important cofactors:** *Vitamin B3 (as NAD)*
- **Immune activation:** *Neopterin (activation by proinflammatory cytokines)*

Tryptophan (TRP) is known for a long time as a precursor of **serotonin**. Under normal circumstances, barely more than 2 - 4 % of TRP is converted into serotonin. The largest part is added to the kynurenine metabolic pathway. **L- kynurenine** is formed, a metabolite that is further converted to 3-OH- kynurenine and **quinolinic acid**, which is mainly located in the liver [31]. Quinolinic acid is converted into **NAD**, which is needed for effective energy production in the mitochondria. More than 90% of the tryptophan is converted into NAD and about 4% is also converted into **kynurenic acid**, another important metabolite that has anti-inflammatory effects, protects against oxidative stress and, above all, has neuroprotective properties [32].

Anxiety leads to **stress**, which is triggering an increased release of **inflammatory cytokines** (e.g. IFN-α, -ß, -γ, TNF-α, IL-6), causing a considerable impact on tryptophan metabolism via enzyme activation in immune cells [33]. The enzymes **IDO** and **KMO** in particular are activated, which has consequences. They convert considerably more tryptophan via L-kynurenine into quinolinic acid, which cannot be further converted into NAD in immune cells as they do around the liver.

It accumulates in the circulation and is metabolized by the kidneys [34]. Increasing **quinolinic acid** levels have a negative effect because it has neurotoxic, proinflammatory and prooxidative effects [35]. Since it cannot be converted to NAD in immune cells, **NAD levels** decrease dramatically by more than 90%. This is not without consequences for themitochondria. An important cofactor is missing due to a loss of energy.

Due to the inflammation-induced displacement in tryptophan metabolism, there is not only a lack of NAD but also significantly less tryptophan that gets converted into **serotonin** and **kynurenic acid**. The amount of these substances is reduced by 50 - 70 % with negative consequences for the affected person [36].

Consequences of anxiety and prolonged stress on human tryptophan metabolism

What happens in case of anxiety, chronic stress, a stress-induced burnout syndrome or depression? What effects and consequences can be derived from an altered TRP metabolism for patients? What is proven by clinical research?

TRP metabolism can influence the physiology of the body at various levels and intervene in functional processes. Disturbances and imbalances are associated with numerous diseases. Regulation is the basis for effective therapeutic approaches.

Stress induces an activation of IDO via increased expressions of proinflammatory cytokines (mainly IFN-γ, IL-1β and IL-6) [37]. Chronic stress in crises thus leads to depletion of available TRP due to the kynurenine pathway and less TRP is reaching the brain, impairing serotonin synthesis there. Increased levels of quinolinic acid promote neuronal dysfunction and damage the blood-brain barrier. Stress triggered by stressful situations can therefore cause mental illness through TRP metabolism [38]. Major **depression** may also be associated with shifts in TRP metabolism [39]. An imbalance of **neurotoxic** and **neuroprotective kynurenine** metabolites is characteristic for such cases [40]. Thus, elevated levels of kynurenine, 3-OH- kynurenine, and quinolinic acid are often found in patients with major depression, as well as decreased levels of kynurenic acid and especially low levels of NAD [25]. It is no surprise that, due to NAD deficiency, there is often a significant impairment of cellular respiration as a result of NAD deficiency [41].

Within a BHI measurement (**BHI: Bioenergetic Health Index**) it is visible in the form of a decreasing maximum respiration (=> Mitochondrial Diagnostics => BHI). It is no longer possible to compensate for an increasing energy demand by raising cellular respiration. Affected patients become **powerless and low in energy**. Stress-induced changes in tryptophan metabolism have profound consequences. A drop in serotonin formation in the CNS has a negative effect on people's **temper**. The ability to learn and concentrate also decreases [23]. Since less melatonin is produced as serotonin levels decrease, **sleep disturbances** are a common result [42]. The sleep-wake rhythm is increasingly disturbed with all the resulting consequences. Serotonin and kynurenic acid are also of crucial importance for the control of intestinal functions [24]. Anxiety- and stress-induced changes in TRP metabolism are therefore regularly accompanied by a significant increase in **irritable bowel syndromes**, characterized by **diarrhea**, **constipation** or associated **pain symptoms** [43, 44]. In order to successfully treat these diverse symptoms, it is necessary to understand their causes and to understand tryptophan metabolism.

NT-TRP Metabolism Plus

The most advanced profile that biovis' now offers is the NT-TRP Metabolism Plus Profile (NT-TRP-Plus for short). It is especially suitable for patients with chronic stress, burnout symptoms, CFS or depressive patients. It contains **34 parameters**. In addition to the **catecholamines** and a complete analysis of the **tryptophan metabolism**, many **important factors** are taken into account that directly or indirectly influence the metabolic processes taking place (see also Fig. 5):

● **Other amino acids:**	*Tyrosine*
	Phenylalanine
● **Other neurotransmitters:**	*GABA*
	Glutamate
● **Other neurotransmitters:**	*Vitamin B6 (cystathionine)*
	Vitamin B12 (methylmalonic acid)
	Vitamin B3 (nicotinic acid, NAD, nicotinamide)
	Tetra hydrobiopterin (BH4) - indirectly
● **Methyl group donors::**	*SAM*
	Betaine
	Choline
● **NO stress/mitochondria:**	*Citrulline*
	Methylmalonic acid
	Suberic acid
	Citrate
	Lactate/pyruvate
● **Fatty acid combustion:**	*L-Carnitine*
● **Arteriosclerosis/inflammation**	*TMAO, TMA*

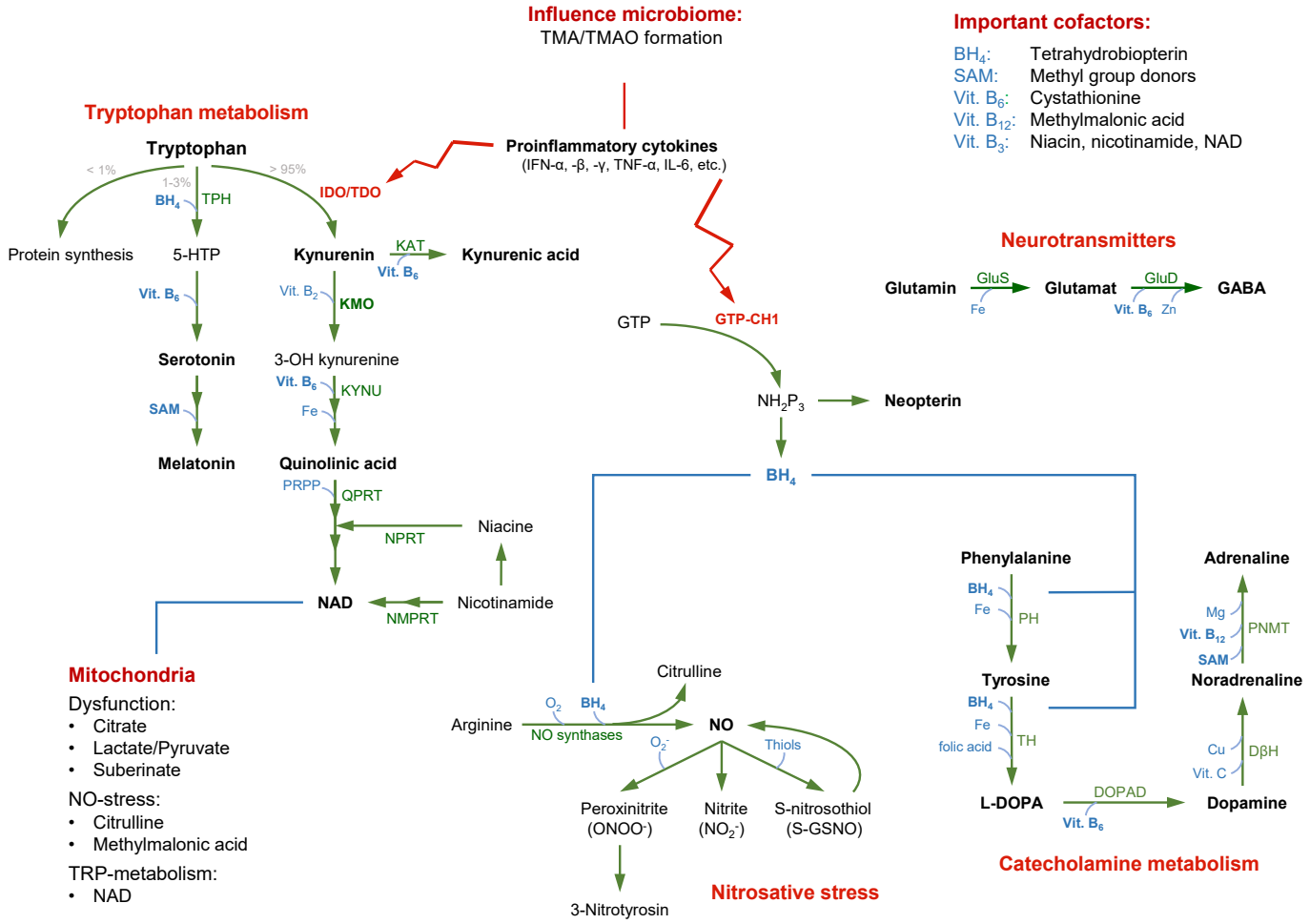


Fig. 5

Relationship between tryptophan and catecholamine metabolism and other metabolic processes The figure illustrates the profile content added in the NT-TRP metabolism plus (compare with Fig.3). (Source: own illustration)

NT-TRP-Plus - why?

Why are the supplementary profile contents important and why does it make sense to record them simultaneously as part of a metabolome analysis? **Metabolome examinations** provide a **simultaneous insight** into all relevant levels of metabolism. In contrast, step-by-step diagnostics are performed in several steps, which may take days or weeks to complete. Stepwise diagnostics always bear the risk that by the time of follow-up examinations, important influencing factors with regard to epigenetics, organ physiology or external effects (e.g. food) have changed, so that previously suspicious constellations become blurred or are no longer traceable. Metbolome examinations represent a meaningful snapshot, taking all **currently relevant influencing factors** into account. They allow a higher clarification of causal factors than single parameter analyses or stepwise concepts.

Above all, **budgetary concerns** lead to the use of single parameter analyses or multi-stage concepts. However, the selection of suitable single parameters for the detection of complex processes is often difficult, sometimes even nearly impossible. Step concepts often do not work for the reasons mentioned above, too. What about the budgetary concerns? The new metabolome assays are based on sophisticated analytical methods. They capture **34 parameters** from urine in the case of the NT-TRP Plus profile, and for a price that is barely **50% of the usual GOÄ-based costs**. This might be one of the many reasons why metabolome tests are a real alternative for various people.

Influencing factors included in the NT-TRP Plus

➔ Other amino acids

Tyrosine and **phenylalanine:** The essential amino acid phenylalanine is the starting point of catecholamine synthesis. Under the influence of the cofactors tetrahydrobiopterin (BH4) and iron, phenylalanine is converted by hydroxylation into tyrosine, which is further metabolized into dopamine through L-dopa (see Fig. 6). The amino acid tyrosine can also be used for the formation of catecholamines. Phenylalanine can thus be partially, but not completely, substituted by tyrosine. Phenylalanine can also be decarboxylated to produce phenylethylamine, which has mood-lifting, analgesic and attention-improving effects.

➔ Other neurotransmitters

γ-aminobutyric acid (GABA)) is the largest and most important inhibitory neurotransmitter in the central nervous system in terms of quantity [26]. Endogenous synthesis of GABA occurs with the help of the enzyme glutamate decarboxylase from the excitatory neurotransmitter glutamate. The concentrations of the transmitters glutamate and GABA are about a 1000 times higher than those of norepinephrine or dopamine. Glutamate is, among other things, of great importance for learning, memory and motor function [27].

➔ Other cofactors

Vitamin B6 is an important cofactor in neurotransmitter synthesis and tryptophan metabolism. If the vitamin is missing, L-dopa cannot be converted into dopamine and therefore not into adrenaline. In tryptophan metabolism, the metabolization steps from 5-HTP to serotonin, from kynurenine to kynurenic acid and from 3-OH-kynurenine to quinolinic acid require vitamin B6 as a cofactor [45]. The profile does not include vitamin B6 but **cystathionine**, a functional marker for the supply of **bioactive vitamin B6** [46].

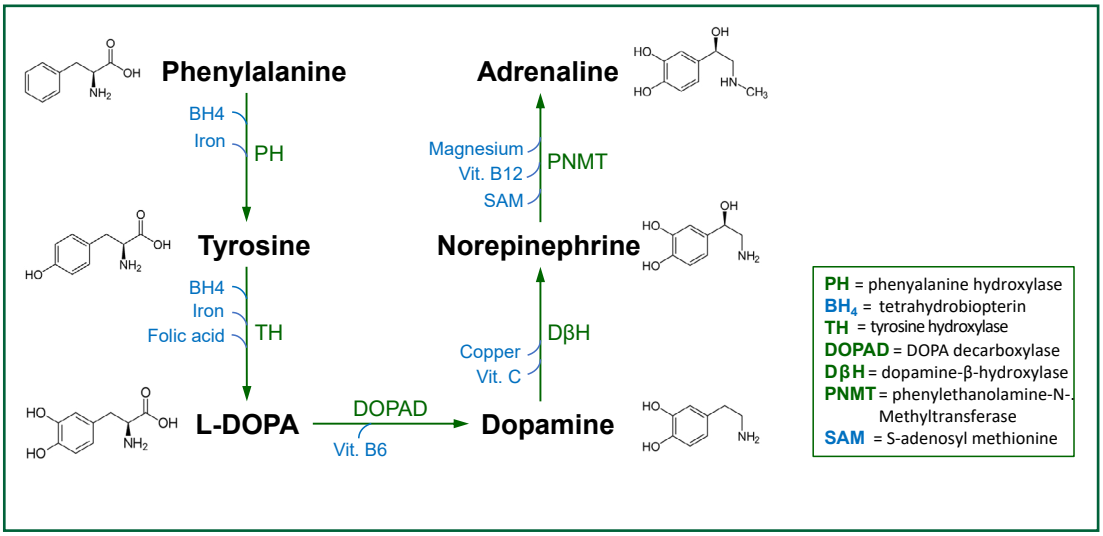


Fig. 6
Catecholamine metabolism with relevant cofactors (Source: own illustration).

Vitamin B12 is a cofactor of methyltransferase, which is responsible for the conversion of noradrenaline into adrenaline. Vitamin B12 is also not measured directly, but rather **methylmalonic acid**, one of the most sensitive markers for preclinical vitamin B12 deficiency[47].

Vitamin B3 is influencing the kynurenine metabolic pathway. A proper supply of niacin leads to a down-regulation of L-kynurenine synthesis [48]. However, the profile includes not only the determination of **niacin**, but also of **nicotinamide** and **NAD**. Nicotinamide is mainly supplied by food. NAD, on the other hand, which is formed from quninolinic acid, originates from tryptophan metabolism and is indispensable for sufficient ATP production in the mitochondria [41].

Tetrahydrobiopterin (BH4) is an important cofactor in human metabolism. Unlike riboflavin or folic acid, which are involved in electron-transferring reactions similar to BH4, biopterin can be synthesized by the body itself. Biopterin (BH2) itself is not biologically active, but the tetrahydroform **(BH4)** can act as a cofactor in redox reactions. The main metabolic reactions in which BH4 appears as a cofactor of an electron transfer are shown in Fig. 7. The metabolic reactions include **catecholamine** and **serotonin synthesis** and the conversion of arginine to **nitric oxide** (NO) and citrulline. If BH4 is missing, this inhibits the enzymatic processes taking place.

- Conversion of **phenylalanine → Tyrosin** *(Enzyme: phenylalanine hydroxylase)*
- Conversion of **tyrosine → L-dopa** *(Enzyme: tyrosine hydroxylase, TH)*
- Conversion of **arginine → NO + citrulline** *(Enzyme: NO synthase, NOS)*
- Oxidation of **tryptophan → 5-HTP** *(Enzyme: tryptophan hydroxylase, TPH)*

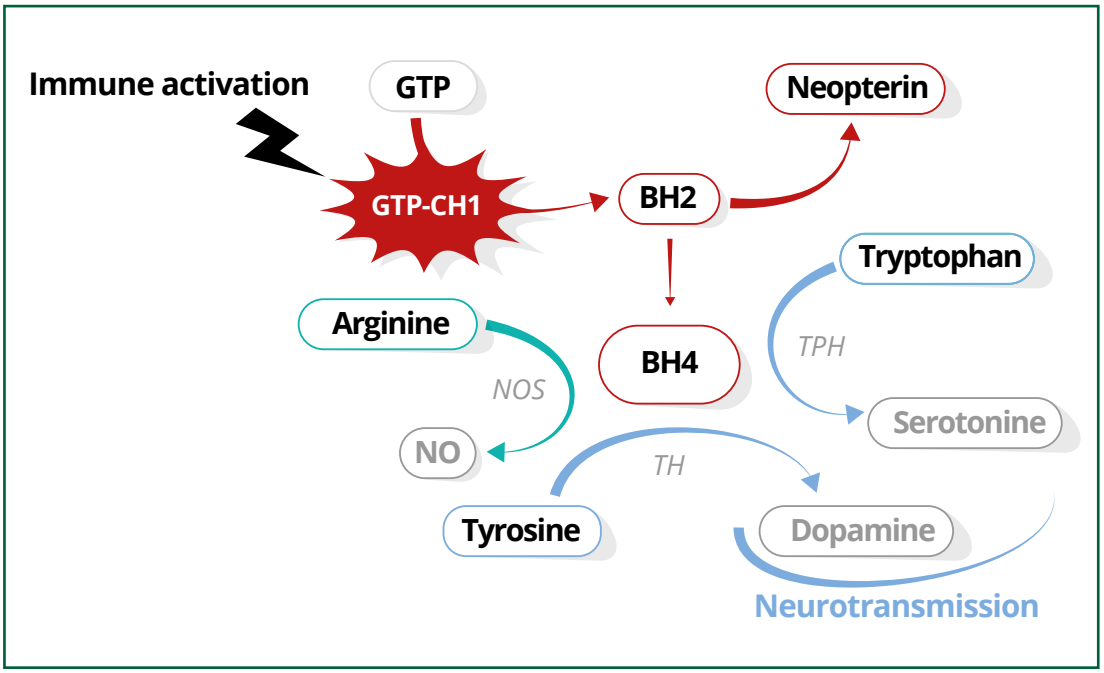


Fig. 7
The influence of immune activation on the availability of BH4
In the case of immune activation, neopterin is increasingly produced instead of BH4, which reduces important metabolic reactions such as serotonin, dopamine and NO synthesis.
***GTP-CH1**: guanosine triphosphate cyclohydrolase 1; **BH4**: tetrahydrobiopterin; **BH2**: dibiopterin.*
(Quelle mod. nach: Castanon N et al. Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity. Front Neurosci. 2015;9:229. doi:10.3389/fnins.2015.00229)

→ Methyl group donors

Methylation is the transfer of methyl groups from one molecule to another within a chemical reaction. **S-adenosylmethionine (SAM)** is one of the most important methyl group donors in detoxification and synthesis reactions. For example, the methyl groups of **adrenaline** are mainly derived from SAM. Other important methyl group donors are **betaine** and **choline**. If methyl group donors are not available in sufficient quantities, this has consequences for adrenaline synthesis. The levels drop.

Valuable information:

Methylations also affect DNA, RNA, proteins and lipids. DNA methylations have important biological functions. They are part of the epigenetic code and represent the most important factor influencing **epigenetic changes**. The importance of methylations in detoxification reactions has already been indicated before. The conversion of toxic **homocysteine** into methionine or **histamine methylation** by the enzyme HNMT (histamine N-methyltransferase) are common examples (see Fig. 8).

➔ NO stress/mitochondria

Inflammation due to anxiety or chronic stress affects serotonin and catecholamine synthesis through misdirection of tryptophan metabolism and BH4 cycle. Increased consumption of neurotransmitters under stress and impaired new synthesis result in increasing depletion of neurons. People feel drained, tired and powerless.

But not only the mentioned **neurostress** can be the cause of these symptoms. Lack of energy can also be due to **mitochondrial causes**, such as a deficiency of NAD due to activation or breakdown of the kynurenine pathway. This results in impaired ATP synthesis. Mitochondrial dysfunction can also be caused by **oxidative** or **nitrosative stress**, which, by damaging the inner mitochondrial membranes (proton leakage), leads to a decrease in coupling efficiency. In some cases this also reduces mitochondrial ATP production considerably.

For an actual understanding of the impact on chronic stress and a resulting silent inflammation on human health and targeting measures to help patients, it is not enough to study catecholamine and tryptophan metabolism. Initial **mitochondrial markers** must also be included to provide a complementary conclusion on mitochondrial function.

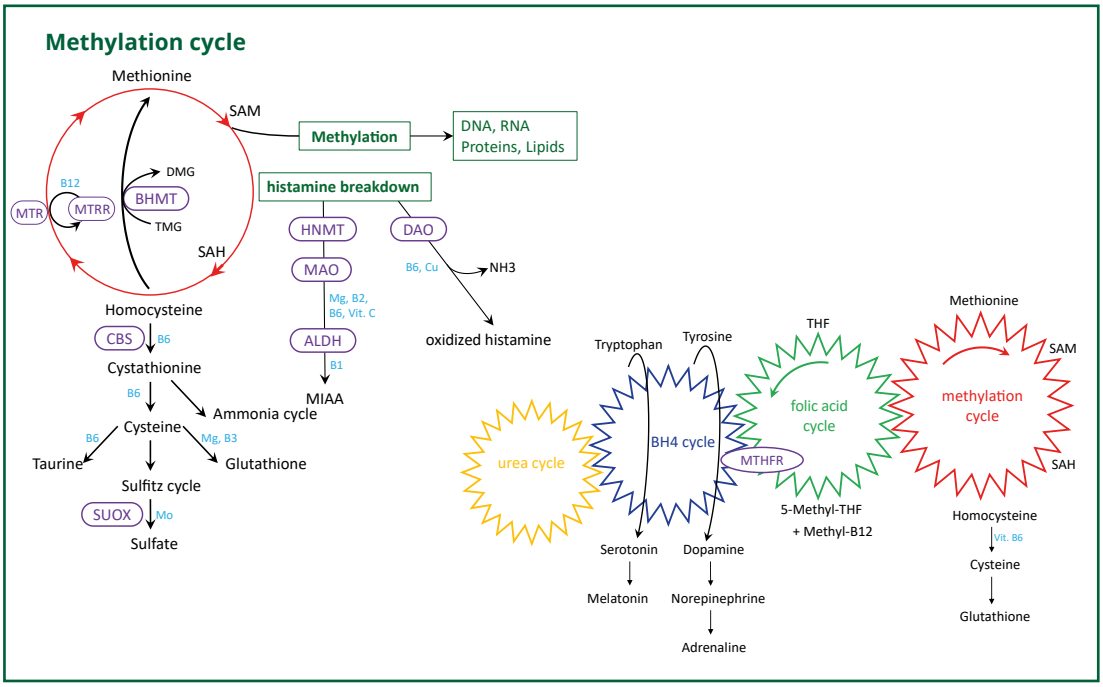


Fig. 8

Methylation cycle and its linkage to folic acid, BH4 and urea cycles.
(Quellen: All Natural Advantage.Methylation. URL: http://www.allnaturaladvantage.com.au/home/wp-content/uploads/2014/10/Methylation.pdf. [Abruf am: 10.09.2020.]
VidaAiD Professional Therapeutics. 2016. Methyl-Cycle & Its Collateral Pathways - Addressing the Whole Picture. URL: https://www.vitaaid.com/main/FTF_info.asp?ID=70. [Abruf am: 10.09.2020.])

Nitrosative stress and orienting mitochondrial function markers.

Citrulline and **citrate** allow conclusions about **nitrosative stress**. An increase in citrulline indicates an increased NO synthesis from arginine (see Fig. 9), while increasing citrate values suggest a mitochondrial dysfunction. The affinity of NO for iron-containing enzymes inhibit aconitase in the citrate cycle. Further conversion of citrate to isocitrate is thereby disturbed. **Methylmalonic acid** is a functional marker for a deficiency of vitamin B12, which, as an NO antagonist, is able to unblock iron-containing enzymes by NO.

The organic acids **lactate** and **pyruvate** contained in the profile also indicate mitochondrial disorders. If there is an additional deficiency of L-carnitine due to nutritional factors, long-chain fatty acids from ß-oxidation can no longer be supplied to mitochondrial energy production. **L-carnitine** provides fuel to mitochondria by transporting fatty acids from the cytosol to the mitochondria. A deficiency of L-carnitine can therefore exacerbate energy deficits and associated symptoms.

If ß-oxidation is dysfunctional or L-carnitine is absent, fatty acids are alternatively broken down by ω-oxidation to form medium-chain dicarboxylic acids, which are then excreted in the urine. This also includes **suberic acid**. An increase in this profile's marker thus indicates the above-mentioned disorders.

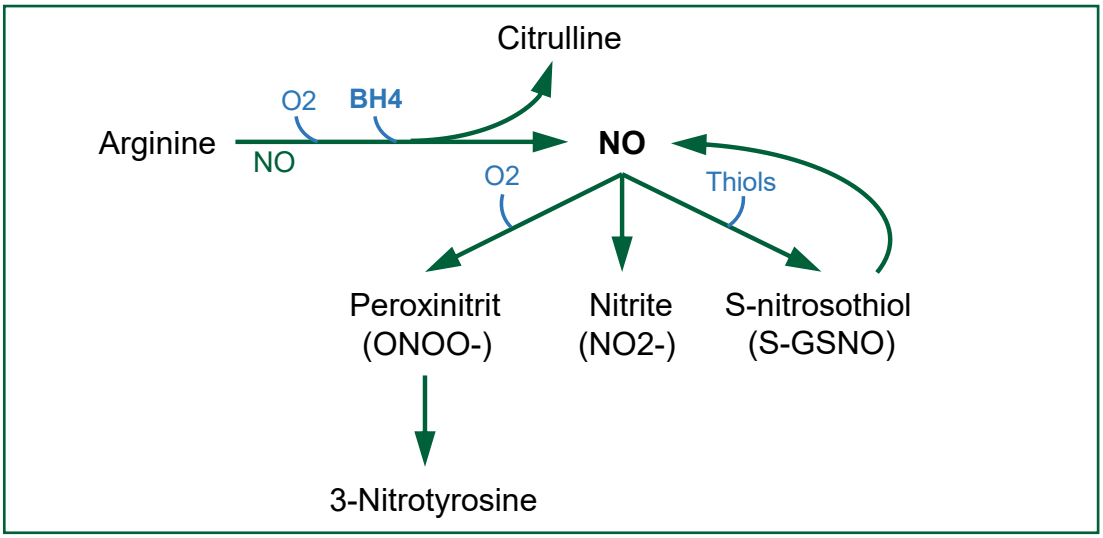


Fig. 9
Conversion of arginine and O2 via NO synthases to nitric oxide (NO) and citrulline. Further metabolization into including peroxinitrite and 3-nitrotyrosine, or 3-nitrophenylacetic acid (NPE)
(Source: Own illustration)

→ TMAO (Trimethylamin-N-Oxid)

Certain intestinal bacteria are able to metabolize choline, betaine, or L-carnitine to produce TMA (trimethylamine), which is further degraded to TMAO in the liver [4, 5]. TMAO has been associated with **inflammation**, atherosclerosis, and more broadly, cardiovascular disease [1, 2]. In the profile TMAO was primarily included because of its **proinflammatory properties**, which affect tryptophan metabolism through increased release of proinflammatory cytokines. **Serotonin** and **NAD synthesis** decrease. At the same time, catecholamine formation is also disturbed by impairment of the BH4 cycle.

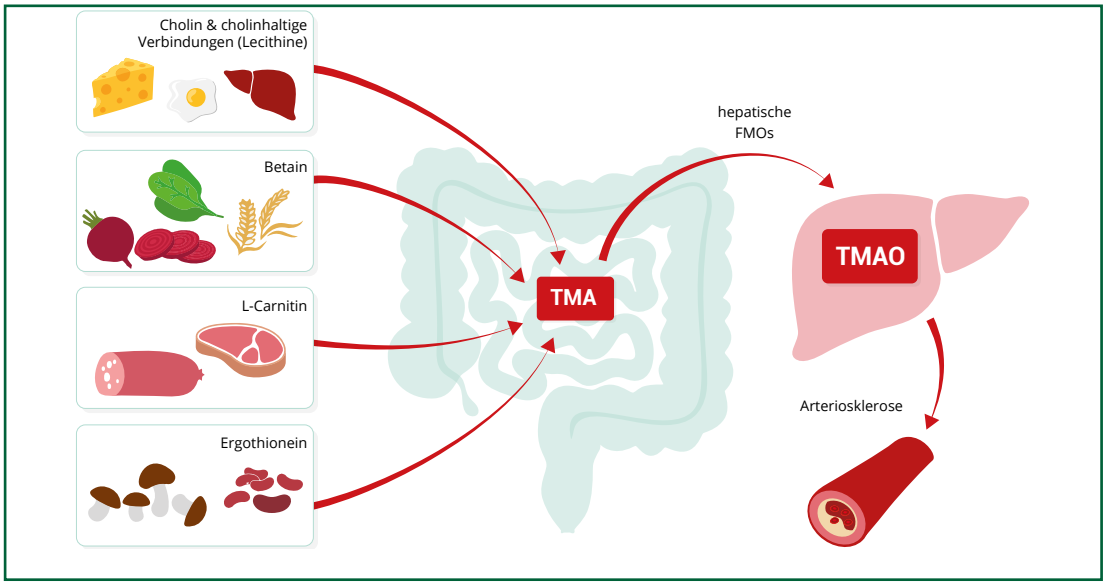


Fig. 10

Formation of TMAO. Choline, betaine and L-carnitine from various foods are enzymatically converted to TMA in the intestine by some bacteria and further converted to TMAO in the liver. TMAO is associated with inflammation, atherosclerosis / cardiovascular disease. (mod. according to M. H. Janeiro et al., 2018) (Source: Own illustration).

Anxiety- and stress-induced subclinical inflammation may be exacerbated by collateral factors. **Changes in the intestinal microbiome** represent an important and, above all, frequent factor here.

The quantity makes the difference

Choline, **betaine** and **L-carnitine** are consumed as **semi-essential micronutrients** in the diet. They have protective properties, represent methyl group donors (betaine and choline) or are required for the transport of fatty acids into the mitochondria (L-carnitine). Choline or choline-containing compounds contribute to the stabilization of cell membranes or maintain acetylcholine (neurotransmitter) levels [5]. Too much choline, betaine or L-carnitine can lead to inflammation or arteriosclerosis in people with TMAO-forming bacteria; too little can result in the positive and protective properties described above, which are not effective (see Fig. 10).

A **concentration measurement** for the distinction between protective and potentially harmful effects is therefore indispensable. This is also accomplished by the new metabolic.

Stress - a frequent cause of irritable bowel

Stress negatively affects the gut-brain axis, which is a bidirectional neurological pathway connecting the brain and the digestive system. Stress-induced factors have a significant impact on the development of IBS [49]. Chronic stress can disrupt **central pain circuits** as well as affect **motility** and **permeability** in the entire gastrointestinal tract [50]. If stress-related disorders like anxiety or depression are present, the risk of developing IBS is doubled [51, 52].

Patients with anxiety and depression should therefore be evaluated for irritable bowel syndrome. Studies have shown that treatment of gastrointestinal disorders can have a very positive effect on psychiatric complaints [53, 54].

If you would like to learn more about the connection between stress and irritable bowel development, please read our upcoming information brochure on this topic or reach out to our scientific field service.

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