



Stress, anxiety, restlessness and sleep // Inflammation and pain // Biomarkers: cortisol and testosterone // Microbiome-gut-brain-interaction // Stress diagnostics // mHealth and wearables

Dear Reader,

Based on 30 years of neuroendocrine research and 15 years of contract research, we are increasingly experiencing the importance of our strategic and scientific consulting services for our customers and partners. We offer support from product development and study planning to results interpretation and presentation. This applies equally to our CRO and Saliva Lab.

A more detailed feedback shows what our customers particularly appreciate when working with us:

- Prompt communication channels between sponsor and daacro-PI, the daacro-team and experts of the daacro advisory board!
- Customized design with all services from a single source!
- Excellent price-performance through a flexible team: permanent employees, long-term freelancers and, if necessary, the highest-quality expertise from daacro's scientific advisory board!
- Rapid completion of clinical trials due to a large study participant pool!

We are pleased to introduce the latest edition of the daacro newsletter. Once again, we have compiled information that may be of interest to you in many ways.

Take advantage of our expertise and contact us. Our team will be happy to engage with your questions!
We look forward to an interesting exchange with you.

Sincerely,

your daacro team



Nadin Meyer (Senior Study Manager),
Juliane Hellhammer (CEO) and Anne Dubberke
(Head Saliva Lab)

SAVE THE DATE

VITAFOODS EUROPE

MAY 07-09, 2019
GENEVA, SWITZERLAND

ISPNE 2019

AUG 29-31, 2019
MILAN, ITALY

PHARMA HOUSE

SEP 02-03, 2019
FRANKFURT, GERMANY

FOOD MATTERS LIVE

NOV 19-20, 2019
LONDON, UK

FOOD INGREDIENTS

DEC 03-05, 2019
PARIS, FRANCE

OUR TOPICS:

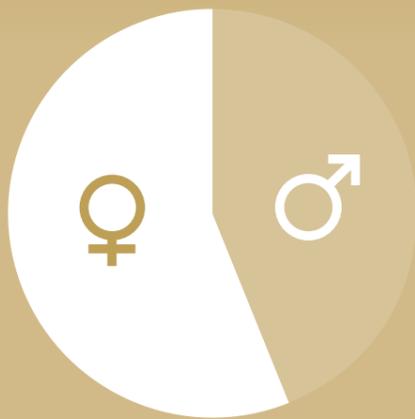
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STUDY SITE

A clinical trial begins with

- solid scientific planning, including literature review, experts, hypotheses formulation, endpoint selection, and statistical sample size calculation
- regulatory and methodological preparation such as the composition of study documents, selection of appropriate recognized methods, study registration and applications to authorities and ethics committee.

DAACRO'S VOLUNTEER-PANEL (AS OF MARCH 2019)



**4175 PERSONS, THEREOF
2331 WOMEN AND
1844 MEN**

1 DAACRO'S SUBJECT DATABASE

AN IMPORTANT QUALITY FEATURE IN CLINICAL TRIALS

A rapid and study tailored recruitment cycle, including all study-relevant criteria and quotas, is a necessity at the beginning of each study.

daacro recruits target group relevant individuals via ads and direct approaches, and also maintains a well-curated database to draw on a large pool of potential study participants. Thus, the existing stock of participants is continuously expanding with fresh and untested individuals, who can be invited to a study. As a result, we assure our clients of a quick and accurate recruitment for their study collective.

AGE GROUP	TOTAL NUMBER	WOMEN	MEN
10 to 18 years	48	14	34
18 to 30 years	1677	958	719
31 to 40 years	781	435	346
41 to 50 years	380	203	177
51 to 60 years	551	298	253
61 to 70 years	376	200	176
> 71 years	84	41	43
not specified	278	182	96

Since 2012, the participant database has maintained in the current format. An average of 521 new entries are added, annually.



PROGRAMMING THE ONLINE
DIARY IS PART OF DAACRO'S
SERVICES.

2 PROMPT DATA COLLECTION – ECOLOGICAL MOMENTARY ASSESSMENT FOR CLINICAL STUDIES – ONLINE DIARIES PROGRAMMING

ECOLOGICAL MOMENTARY ASSESSMENT, IN SHORT EMA, DESCRIBES A SURVEY STRATEGY THAT AIMS TO CAPTURE STUDY-RELEVANT OUTCOMES DIRECTLY AND IN A "NATURAL" ENVIRONMENT. IT IS THEREFORE A SPECIAL TYPE OF MEASUREMENT THAT SERVES TO INCREASE DATA VALIDITY.

An electronic online diary for data collection in clinical trials offers an improved alternative to paper diaries and questionnaires. Data can be captured quickly and independently on location and thus distortion due to false

memory is reduced. In addition, it is already processed digitally, thus minimizing the time and effort concerning data entry. Questions about the subject's complaints or adverse effects can be recorded at specific times and over a specific period via a web platform. A simple design and the availability on portable electronic devices enhance the willingness to participate and guarantee temporal and spatial flexibility. To ensure data integrity and quality, a control function informs the study team about missing entries. This way, the study participant can be contacted and the data can be completed in a timely manner.

3 CHARACTERIZATION OF STUDY PARTICIPANTS: STRATIFICATION AS A USEFUL STRATEGY

The aim of a clinical study is to obtain reliable information on the efficacy of the investigational product. An important part is choosing a suitable study collective. While patient samples are often blurred due to diagnostic criteria, the main point in a clinical trial with healthy subjects is to define the criteria for a homogeneous study population that is appropriate for the study objective.

Students are a popular target group in clinical trials, because they are usually open to study participation, flexible with their time, and interested in study compensation. In older study collectives, on the other hand, there are more participants with age-related issues and health impairments. In any case, an accurate characterization of participants based on their lifestyle is recommended for each age cohort. The lifestyle of a person (diet, exercise, stress, social environment, etc.) has a significant impact on health and the aging process, and thus has important implications for evaluating the clinical efficacy of an OTC product.

Biomarkers and questionnaires for specific conditions can also be applied as inclusion criteria to provide further means of characterizing individuals and assigning them to homogeneous subgroups (stratify). In this way, it is possible to evenly assign persons with specific characteristics, such as high versus low anxiety or even high versus low stress levels, to the study arms. Accordingly, suitable characteristics for primary and secondary endpoints of the study need to be selected depending on the investigational medicinal product's mechanism of action.

Since stress factors are involved in many pathogenic processes and the development of diseases, stratification according to stress often provides insights when subgroup analyses are conducted. For this purpose daacro has developed two innovative diagnostic methods:

- the BASIC stress profile
- neuropattern™ stress diagnostics.

(For details, see article 4)

FROM THE DAACRO TEAM



Emilia Ellsiepen, PhD
Biostatistician

PhD in computational linguistics followed by a training in biostatistics. After stints in the UK and France, she has joined the daacro team in the summer of 2018. Her dynamism and eye for clear structures ensure optimal study planning and the professional evaluation of the study data.



Simone Kugel, MSc
Study Manager

A life scientist with additional qualifications in clinical trials has been reinforcing the team since the beginning of 2018. Her organizational skills, reliability and positive energy are appreciated by sponsors and study participants and, as well, by her colleagues in planning, implementation and evaluation of clinical trials.

SALIVA LAB TRIER

The analysis of hormones in saliva is closely linked to innovation and scientific expertise from Trier. daacro's Saliva Lab offers solid scientific advice and a comprehensive range of laboratory analysis to partners in science and industry. The Saliva Lab focuses on stress and sleep research, as well as hormone diagnostics and burnout.

ADVANTAGES OF BIOMARKERS IN SALIVA:

non-invasive / sampling several times a day in a natural environment / uncomplicated and easy for all age groups (newborns to the elderly) / also possible in many animal species

4 DIAGNOSTIC KITS FOR STRESS-RELATED HEALTH ISSUES – FOR PATIENTS, PHYSICIANS, CLINICS AND CLINICAL STUDIES

Whether job-related or at home - more and more people feel stressed out! But beware: the subjective perception does not necessarily match the physical signals. With the diagnostic procedures we have developed, we can assess stress levels in order to initiate individual interventions at behavioral, nutritional and medical levels.

BASIC STRESS PROFILE



EASY HANDLING – FAST RESULTS – INFORMATIVE FINDINGS

Scientifically approved procedure: the cortisol awakening response (CAR).

- 6 to 8 saliva samples for two morning measurements;
- descriptive instructions and detailed findings;
- as recommended by the Expert Consensus Guidelines (2016).

EASY ORDERING OF BOTH DIAGNOSTIC METHODS VIA INFO@DAACRO.DE

NEUROPATTERN™



STRESS HAS MANY PATTERNS – AN INDIVIDUAL CHARACTERIZATION FOR INDIVIDUAL STRATEGIES

Innovative stress diagnostics: neuropattern™ detects biological, psychological and physical stress reactions.

- 3 major stress systems: work, energy and recovery systems;
- performed with anamnesis, questionnaires, saliva samples and ECG measurements;
- illustrated results with the Stresstriangle™.

5 CORTISOL IN ANIMALS – APPLICATION IN PRECLINICAL STUDIES, ANIMAL BREEDING AND PRODUCTIVE LIVESTOCK

In addition to human saliva, the Saliva Lab Trier also analyzes biomarkers in animal saliva. The hormone cortisol is also a frequent focus here as a biomarker for stress and stress management.

In preclinical studies animals are often used to test the efficacy of a particular substance. If a stress- or anxiety-reducing effect is expected, researchers often use models such as the Forced Swim Test, the Restraint Test or the Novel Arena Test in rodents, cows, sheep and pigs. In such studies, cortisol is often analyzed in animal saliva as it can replace invasive blood sampling. Another important area of application is animal breeding, especially in dogs and horses. In dogs, stress can be caused by excessive demands (performance-related stressors due to hard training methods, road traffic, large crowds, etc.) or lack of demand (loneliness, species-inappropriate keeping) and promote aggression. At the same time, the need for specialized and well-trained dogs, such as guide dogs or police and military dogs, is growing. For effective training, it is important to select and train only dogs with appropriate personal characteristics, cortisol analyzes can be used for early assessment of a subjects stress management ability. In horses, the transport

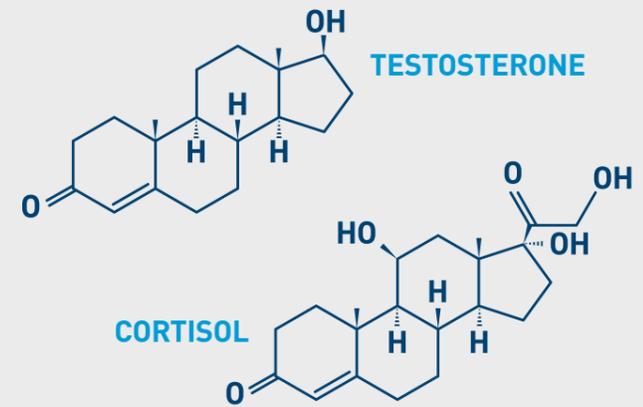
of animals is considered to be an additional stressor, as demonstrated by increasing cortisol levels that are further associated with the length of transport. Similarly, suboptimal length and intensity of exercise or extended resting periods without exercise demand can increase the level of stress.

Another domain is the analysis of the stress hormone in livestock husbandry. In pigs or cows, meat quality can suffer from stress in animal husbandry or animal transport. The burden of increased milking volume, which may also be harmful to the soft tissue, was also confirmed by cortisol measurements.

Important: Always implement relevant measurement times and the use of an appropriate collection system! Depending on the size of the animal, there are different versions of collection devices that can be used. Benefit from daacro's many years of experience in the lab and from our expertise in the field of hormone determination in animal saliva.

6 TESTOSTERONE/ CORTISOL- RATIO IN SALIVA

A BIOMARKER IN SPORTS PERFORMANCE AND SOCIAL BEHAVIOR



The interaction and ratio of testosterone and cortisol in saliva, the so-called T/C ratio, is currently gaining interest in the scientific community. A prominent hypothesis states that testosterone and cortisol work together as part of a biological system, directly influencing the response to threatening situations by adapting the Hypothalamic-Pituitary-Adrenal (HPA) axis and the Hypothalamic-Pituitary-Gonadal (HPG) axis. In a stressful situation, the human body increases the production of cortisol and decreases the production of testosterone. Balancing the T/C ratio supports a healthier lifestyle and can be used as a hormonal biomarker to detect overtraining and potential susceptibility to certain diseases.

A far-reaching influence of the T/C ratio is also assumed in the field of sociobiology. Initial evidence suggests a significant association between testosterone and aggressive social behavior in people with high cortisol levels, unlike those with average or low cortisol levels. The T/C ratio is thus described as a consistent hormonal marker of social aggression.

However, research on the health effects of the T/C ratio and their behavioral-biological influences is just beginning. Take advantage of our psychobiological expertise and our longstanding experience by contacting our laboratory team headed by Anne Dubberke, MSc.

7 QUALITY ASSURANCE IN THE LAB: COLLABORATIVE RING TRIALS

Quality assurance is indispensable nowadays, especially in the laboratory sector, and guarantees valid, usable and reproducible test results by adhering to defined standards. Annual participation in external collaborative ring trials examines the laboratory's actual analytical performance and demonstrates that the analyses provide accurate and correct results.

The Saliva Lab Trier participates annually in collaborative ring trials of the Salimetrics Center of Excellence Program, the American assay manufacturer, and other assay manufacturers - with exceptional results.

PROCESS OF A COLLABORATIVE RING TRIAL

Dispatch of identical samples of known concentration to several laboratories

Analysis of samples under specified conditions and under routine conditions

Investigation and statistical evaluation of the test results

= Evaluation of the measurement accuracy of the participating laboratories

8 mHEALTH AND WEARABLES FOR STUDY DESIGN AND -MANAGEMENT

In recent years, mobile health technologies, apps and portable devices have improved and contribute to accelerated mobile health (mHealth) innovation. The design and management of clinical trials also benefit from these new technologies:

- ✓ Improved characterization and recruitment of participants with a study-specific profile,
- ✓ Improved data quality when measuring health outcomes,
- ✓ Improved compliance with the study.



9 BRIEFLY NOTED: VIRTUAL TRIER SOCIAL STRESS TEST (TSST)

Virtual Reality (VR) versions of the TSST have been available for several years and can be used in both existing and new studies. In comparison with the in vivo Gold Standard TSST, similar reactions occur in the salivary cortisol and alpha-amylase endpoints as well as in the perception of stress. Depending on the question, the procedure can be used as a sensible alternative or supplement in a clinical trial.



FRESH OFF THE PRESS:

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Superiority of magnesium and vitamin B6 over magnesium alone on severe stress in healthy adults with low magnesemia: A randomized, single-blind clinical trial. PLoS ONE, 13(12): e0208454. <https://doi.org/10.1371/journal.pone.0208454>

Hellhammer, D., Meinschmidt, G., Pruessner, J. C. (2018).

Conceptual endophenotypes: A strategy to advance the impact of psychoneuroendocrinology in precision medicine. Psychoneuroendocrinology; 89: 147-160. <https://doi.org/10.1016/j.psyneuen.2017.12.009>

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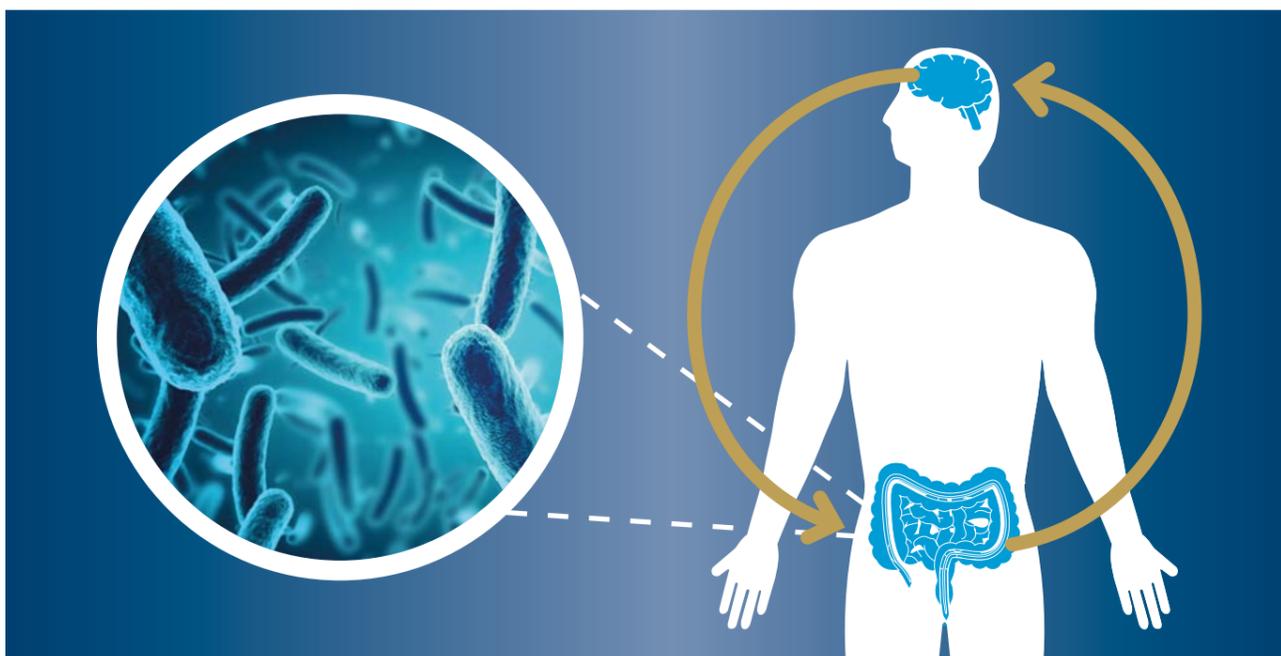
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10 PROBIOTICS - THE MICROBIOME-GUT-BRAIN-AXIS

"Gut-Brain-Axis" refers to the biochemical connection between the gastrointestinal tract and the central nervous system. The addition of the word "microbiome" explicitly emphasizes the role that the intestinal flora plays in signal transmission between the two components. Bidirectional communication takes place on the neural, hormonal and immunological levels. Changes in these systems can modify health and stress responses. While the brain-gut direction has already been studied extensively, current research focuses on the interaction

of both organs, in particular the potential influence of intestinal bacteria on brain and behavior. Much attention is focused on probiotics, living microorganisms, which, if adequately ingested, can provide the user with health benefits. Clinical studies indicate that some bacterial strains of probiotics not only have health effects on digestion, but have additional impact on the immune system, anxiety and stress responses.

11 NOOTROPICS - COGNITIVE ENHANCER - SMART DRUGS - ANTI-DEMENTIA DRUGS

The market for nootropics is very large, since these medicines, dietary supplements or other substances are attributed a beneficial effect on the CNS. Therefore, terms such as brain doping or smart pills are well known. Nootropics are investigated in clinical studies in many areas (pharmacology, complementary medicine, wellness and anti-aging) often focusing on

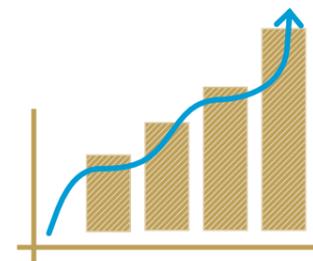
- optimizing the balance of neurotransmitters and hormones in the brain and body,
- improving cognitive functions, including comprehension, focus, memory and learning,
- mood enhancement and reduction of stress and anxiety,
- increasing the blood and oxygen flow to as well as in the brain,
- attenuation of oxidative stress and neuronal inflammation,
- and reducing the risk of suffering from neurodegenerative diseases and cognitive dysfunctions.

After all, these body functions are particularly affected by stress, sleep disorders, lack of exercise, etc. in today's time.

Nootropics act in different ways, mainly via an activity change of neurotransmitters in the CNS. Neurotransmitters can have a stimulating or inhibitory action potential and thus "excite" or "calm down" the brain. Examples of nootropics are phosphatidylserine or the medicinal herb ginkgo, which is presumed to improve memory performance and promote blood circulation.

12 DAACRO'S IN-HOUSE STATISTIC

Many contract research institutes offer services in the area of study management and study execution. Other services such as data management and statistics are often taken over by external service providers. This makes sense in many areas, but is limited when it comes to more complex scientific questions and data analysis or in explorative studies. For this reason, biostatistics with all available methods is an integral part of the team at daacro, thus ensuring optimal study planning in the entire course of a study.



Our statisticians are not detached from the rest of the team, but are closely interlinked with each other and in constant exchange, right from the start. Statistical expertise is already required when planning the study: In determining primary endpoints and control variables, the statistics team is consulted in order to ensure a meaningful and efficient analysis later on. This early involvement of the statisticians also pays off in the analysis because they already have a sound understanding of the data collected and the evaluation strategy is largely established.



VISIT OUR WEBSITES AT:

WWW.DAACRO.COM // WWW.STRESSZENTRUM-TRIER.DE // WWW.WERDEPROBAND.DE