Study protocol

1. Project title, version number, version date

Project title. Metabolic effects of a five-day remotely delivered hypocaloric and ketogenic program in healthy subjects – Fastreset-study –

Version number. 1.1

Version date. 08.03.2023

2. Abstract

Therapeutic effects of fasting are increasingly documented. There is an increasing demand for dietary interventions which can be delivered at home. In order to answer this demand, the Buchinger Wilhelmi Clinics have created a hypocaloric, ketogenic program with interval fasting that has positive effects similar to brief fasting. We hypothesise that this new five-day remotely delivered hypocaloric and ketogenic program can cause metabolic changes. In order to test this hypothesis, we will perform a randomized controlled trial in which a group of participants will perform the five-day programme and a group of participants will receive an eucaloric diet as a control group.

3. Responsibilities

- Lead Investigator:

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- Involved scientists: Franziska Grundler[1], Robin Mesnage[1,4]

- Involved institutions:

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- Financing

The expenditures for the study are financed by the institutions' own resources. Buchinger Wilhelmi Development und Holding GmbH is commissioned with the research of the Buchinger Wilhelmi clinics.

- Registration in a publicly available study registry:

The study will be registered after the receipt of the positive vote of the State Medical Chamber of Baden-Württemberg in ClinicalTrials.gov.

4. Scientific background

Therapeutic effects of fasting are increasingly documented. Recent studies on a cohort of subjects doing a long-term fasting lasting from 4 days to several weeks demonstrated beneficial effects on health and well-being (Wilhelmi de Toledo et al., 2020a). In recent studies on the therapeutic efficacy of the Buchinger Wilhelmi fasting program, long-term fasting significantly improved risk factors for a variety of chronic diseases including hypertension even in medicated subjects (Grundler et al., 2020), liver steatosis (Drinda et al., 2019), inflammation (Mesnage et al., 2019), oxidative stress (Wilhelmi de Toledo et al., 2020b) and lipoprotein distribution (Grundler et al., 2021).

There is an increasing demand for dietary interventions which can be delivered at home. This has been amplified by the Coronavirus-19 disease (COVID-19) pandemic during which many countries kept their population in lockdown at home in isolation. The COVID-19 pandemic has also influenced the lifestyle of individuals, with an increased consumption of unhealthy food and a sedentary behaviour resulting in mental (anxiety, depression, and others) and physical (weight gain, rise in blood pressure) health issues (Rentería et al., 2022 ; Daniel et al., 2022 ; Laffin et al., 2021). In order to adapt to this new context, the Buchinger Wilhelmi Clinics have created a hypocaloric, ketogenic program with interval fasting.

The five-day programme is designed to be easily integrated into everyday life and can be applied several times a year. The programme is based on ready-to-eat soups made with locally sourced organic ingredients. The ingredients represent a total daily intake of around 600 kcal per day – with a high share of fats and a limited quantity of carbohydrates and proteins. Minerals and micronutrients used at the Buchinger Wilhelmi Clinics are also supplied. Although the comprehensive medical care and assistance offered at the clinics cannot be replicated, medical professionals are available to support the customers remotely.

We hypothesise that the metabolic changes caused by this new five-day programme can be similar to a short-term fast. In order to test this hypothesis, we will perform a two-arm randomized controlled trial in which a group of participants will perform the five-day Fastingbox programme and a group of participants will receive an eucaloric diet as a control group.

References:

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5. Project objectives

- Primary hypothesis:
  To assess changes in body weight and blood pressure caused by this new five-day programme.

- Secondary hypotheses:
  1. To study if the 5-day hypocaloric and ketogenic programme causes an elevation in the excretion of ketone bodies in urine, reflecting a ketosis change.
  2. To study changes in lipid and glucose metabolism caused by this 5-day hypocaloric and ketogenic programme.
  3. To study if the 5-day hypocaloric and ketogenic programme increases antioxidant capacity in blood.
  4. To study if the 5-day hypocaloric and ketogenic programme increased well-being
  5. To study if the 5-day hypocaloric and ketogenic programme decreased inflammation
  6. To study if the 5-day hypocaloric and ketogenic programme changed gut microbiome composition

The following clinical endpoints are analysed:

1. Height, body weight, body mass index
2. Waist circumference
3. Resting blood pressure, systolic/diastolic and heart rate
4. Acetoacetic acid in urine as biomarkers for ketosis
5. Total Antioxidant Capacity and lipid peroxidation in blood
6. Clinical routine laboratory examination (leucocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, thrombocytes, INR, Quick, PTT, GOT, GPT, GGT, AP, uric acid, urea, GFR (shortened), creatinine, TSH basal, total cholesterol, triglyceride, HDL, LDL, LDL/HDL-ratio, glucose, HbA1c, sodium, potassium, calcium, magnesium, high-sensitive C-reactive protein)
7. Inflammatory markers (IL6, IL8, TNFalpha)
8. self-reported lifestyle questions (physical activity level, nutrition, sleep, well-being)
9. possible adverse effects
10. Microbiome composition
11. Continuous glucose monitoring

6. Target figure.

- Changes in body weight and blood pressure before, during and after the intervention.
- Changes in energy metabolism before, during and after the intervention.
- Changes in antioxidant capacity before, during and after the intervention.
- Changes in general inflammation before, during and after the intervention
- Changes in gut microbiome composition

7. Study design

- Multicentric:
  1. Buchinger Wilhelmi Bodensee, Wilhelmi-Beck-Straße 27, 88662 Überlingen, Germany
  2. Buchinger Wilhelmi Marbella, Avda. Buchinger 15, 29602 Marbella, Spain

- Two-arm interventional study
  1. Fastingbox group (n=32)
  2. Control group that maintains an eucaloric diet (n=32)

- Randomization
  Participants will be randomized after signing the declaration of consent during the doctors interview on T0.

- No blinding

This study is a randomized controlled multicentric study.

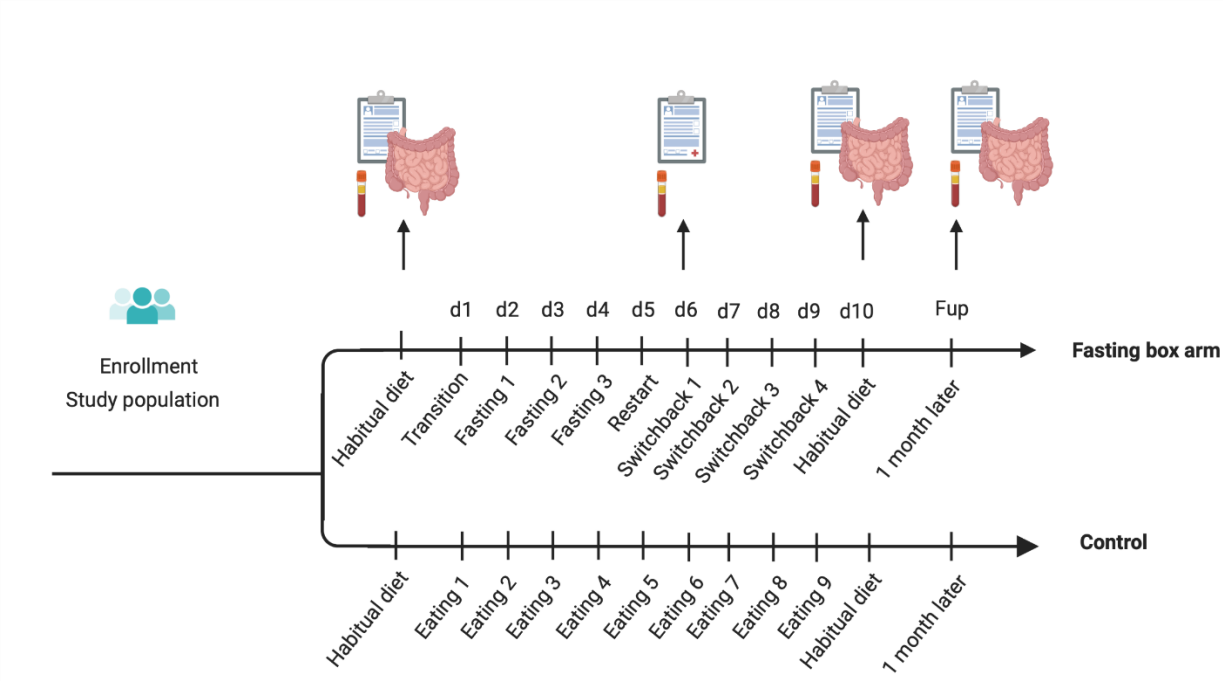

Figure 1. Presentation of the study design.

8. Study population

- inclusion criteria:
  - Men and women
  - Age: 18-80 years old
  - Signed informed consent

- exclusion criteria:
    - Not able to sign the informed consent
    - diagnosticated with cachexia, anorexia, nervosa, advanced kidney, liver or cerebrovascular insufficiency.
    - Intake of antibiotics up to 2 months prior the study
    - Medicated high blood pressure
    - Diagnosed hyperuricemia
    - Diagnosed diabetes mellitus type I and II
    - Diagnosed kidney stone
    - Active malignant diseases
    - Known substance addiction
    - Pregnancy or breastfeeding
    - Participation in another study

- number of participants:

Defined study population: 64 subjects. 32 subjects in each arm.

- recruitment measures:

The study will be conducted at the Buchinger Wilhelmi clinics, Überlingen and Marbella. Participants will be recruited during a period of 12 months. Information about the study will be offered in social media and per mail. Interested persons can get in touch via an online questionnaire that captures contact details (name, address, mail address, phone number), queries in- and exclusion criteria and the possible date for the intervention as well as the motivation of the interested person. The online questionnaire is built with an online tool that is certificated to adhere data security (GDPR). Only representatives of the principal investigator who are employees of the Buchinger Wilhelmi Development und Holding GmbH, Wilhelm-Beck-Strasse 27, 88662 Überlingen, and who are bound to confidentially have access to the data. All registered persons get feedback about their registration. Potential study participants are contacted to provide them with further information about the study protocol via mail and telephone. Unsuitable candidates get information about their rejection. All data captured with this online questionnaire will be deleted when the recruitment is completed, at the latest on the 30/06/2024.

9. Order of study

- Procedure of information and obtaining the informed consent:

The investigators will inform the subjects(information document for the patient) on the purpose of the study. During the doctors interview an informed consent discussion and the written declaration of consent will take place. The declaration of consent will therefore be obtained before the start of any study relevant measurements are performed. Subsequently, the participants will be randomized for the participation in the fastingbox or control group. The randomization will take into account a balanced gender and BMI distribution.

- measures (intervention/control)

Fastingbox group. The fastingbox is a five-day program. The first day is a transition day to prepare to reduce food intake and to initiate the change in metabolism. Participants receive a yogurt, cashew nuts, two soups supplemented by a spoon of linseed oil each (about 600-700 kcal in total). During the day 2,3 and 4, namely the fasting days, the participants consume each day two meals composed of a vegetarian soup supplemented by 30 g Hummus and 2.5 tbsp linseed oil. On day 5, namely the restart day, the participants receive an apple puree together with cashew nuts as first solid food, and then a soup supplemented with 1.5 tbsp oil. Meals during the switchback phase of four days are also standardised and include vegetarian meals, which are designed to progressively increase caloric intake.

An important part of the fasting box is the accompanying Buchinger Wilhelmi app. It provides a daily support with questionnaires and video messages.

Control group. The control group receives an eucaloric diet. Daily breakfast, lunch and dinner is offered at the clinic or as take away. Alternatively, recipes are offered. The used food is organic and local. Buchinger Wilhelmi is certified as a teaching clinic for nutritional medicine "Lehrklinik für Ernährungsmedizin" by the German Academy of nutritional medicine (Deutsche Akademie für Ernährungsmedizin).

- Recording of the target figures (examinations, measurements, data collections)

The following measurements are conducted:

measuring method:	**parameter:**
Clinical health	height, body weight, body mass index, waist circumference, resting blood pressure (systolic/diastolic) and heart rate
Blood analysis: ca. 30 mL venous blood is collected at each of the 4 time points.	Blood cell formulation (leucocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, thrombocytes, INR, Quick, PTT, GOT, GPT, GGT, AP, uric acid, urea, GFR (shortened), creatinine, TSH basal, total cholesterol, triglyceride, HDL, LDL, LDL/HDL-ratio, glucose, HbA1c, sodium, potassium, calcium, magnesium, high-sensitive C-reactive protein), IL6 and IL10, TNFalpha Total Antioxidant Capacity, lipid peroxidation.
Continuous glucose measurement	Glucose measurement with sensors (e.g. FreeStyle Libre, Dexcom) that have a valid CE certification
Acetoacetic acid in urine	Ketosis
DNA sequencing in faecal samples	Gut microbiome composition using shotgun metagenomics, qPCR, SCFA
In addition, samples are deep-frozen and provided for analysis at a later time point. Rest material of samples are stored in a biobank up to 10 years if subjects gave their written informed consent.	

- Time frame and study length for each participant:

Four main visits are conducted at the Buchinger Wilhelmi clinic as follows:

T1: Baseline: one to three days before start of the intervention

T2: in the morning of the sixth day

T3: in the morning of the tenth day

T4: follow-up after 1 month

Each of the 4 visits will last up to 30 minutes. Additionally, daily check-ups at the Buchinger Wilhelmi clinic (each approx. 10 minutes) are performed to measure body weight, and blood pressure. The blood drawings and measurements are conducted by trained staff of the Buchinger Wilhelmi clinic. Furthermore, daily questionnaires are included in the data collection. In total, the study duration is 12 days for each participant.

The protocol will be conducted according to the following schedule:

Control	Habitual diet	Eating 1	Eating 2	Eating 3	Eating 4	Eating 5	Eating 6	Eating 7	Eating 8	Eating 9	Habitual diet	1 month later	
Fastingbox	Habitual diet	Transition	Fasting 1	Fasting 2	Fasting 3	Restart	Switchback 1	Switchback 2	Switchback 3	Switchback 4	Habitual diet	1 month later	
	T1						T2				T3	T4	
	D0 to D - 3	D1	D2	D3	D4	D5	D6	D7	D8	D9	D 10	D 11	**Measurements**
	x						x				x	x	Blood analysis
	x										x	x	Stool analysis
	x	x	x	x	x	x	x	x	x	x	x	x	Weight, blood pressure, pulse
	x	x	x	x	x	x	x	x	x	x	x	x	Waist circumference
		x	x	x	x	x	x	x	x	x	x	x	Ketone bodies in urine
	x	x	x	x	x	x	x	x	x	x	x		Continuous glucose measurement
	x	x	x	x	x	x	x	x	x	x	x	x	Daily questions: health condition, well-being
	x						x				x	x	Questionnaires: WHO-5 index, IPAQ, PSQI, short healthy eating index, questionnaire of highly processed food consumption (sQ-HPF), life style and well-being questions

- Total duration of the study:

The study will take about 12 months for the data collection.

10. Risk-benefit ratio

- individual benefit associated with the study participation:

Participants in the Fastingbox arm get a regular potentially effective therapy. In the course of the study a systematic and careful clinical documentation occurs. These results in a predictable benefit.

- related burden and risks with the study participation:

The sampling procedures present no relevant health risk for the patient. By taking venous blood, small bruises may occur. The introduction of blood collection cannula causes complications such as infection, nerve damage or bruising in less than 1% of cases. Even greater venous bleeding is harmless, but results in a palpable hematoma in the adipose tissue that completely regresses over 6-8 weeks. When attaching the sensor for continuous glucose measurement, slight bleeding or the formation of bruises may occur. Some people may be sensitive to the adhesive film that fixes the sensor to the skin. The development of a contact allergy to the sensor is possible. The new fasting programme in study here is only uses natural ingredients and provide a total calorie intake around 600-700 kcal which is not associated with health risks. The safety of long-term fasting according to the Buchinger Wilhelmi program, with a lower caloric intake of 250 kcal per day, has been documented in 1422 subjects who underwent fasting periods of 4 to 21 days (Wilhelmi de Toledo et al., 2019). This intervention had numerous beneficial outcomes with few mild adverse effects, like cardiac arrhythmia, hyponatremia, or hypoglycemia, that have been reported in less than 1% of the participants.

- termination criteria

In case of adverse event, it will be documented by the study physician assessing the seriousness and the possible relation to the investigational treatment. Severe adverse events have to be communicated by the study physician within 24 hours per telephone, telefax or telegram to the study leader.

If the patient retracts his declaration of consent the study physician has to terminate its participation in the study. This will be indicated in the documentation forms.

The study could be aborted if it is recognizable that the study will not meet the specified requirements. Such as:

- occurrence of severe protocol violation
- the documentation forms are filled inadequate or intentionally false
- legal or ethical provisions are not complied

An abort for the above-mentioned reasons is only possible in accordance with the study leader and study physician.

If severe adverse events or serious side effects occur or not severe adverse events accumulate, the study leader could terminate the study in its sole decision. The study has to be aborted if the safety of the therapy is uncertain due to the occurrence of adverse events.

- statement about the medically justifiability:

The in-patient treatment is inspired by the treatments that are conducted on approx. 6000 patients each year in the both Buchinger Wilhelmi clinics in Überlingen and Marbella since 1953. The intervention has been documented as presenting extremely low-risk and being totally justifiable.

This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements

11. Biometry

11.1 Statistical power

Based on previously collected data for changes in systolic blood pressure (Grundler et al., 2020), we calculated that a sample size of 32 participants per arm is required to detect a decrease in 1 mm/hg per day with three groups and an effect size of 0.33 with 90% power and an alpha error probability of 0.05 with an ANOVA test including repeated measurements (G*Power version 3.1.9.7).

11.2 Statistical analysis plan

The statistical analysis of the data will be performed using R software for statistical computing. Given that our study has a longitudinal design with multiple repeated measurements, a mixed model for repeated measures will be used for quantitative variables. Missing data will be handled with complete case analyses for the dependent variables, or will be imputed using the median for auxiliary variables with less than 20% missing values. The statistical models will be adjusted for potential confounders such as age, gender, or baseline body weights. A parsimonious approach will be used. If a covariate does not influence the variance of the model, it will be excluded from the model.

11.2.1 Primary endpoints

The changes in blood pressure and body weights will be modelled with linear mixed models using the subject unique ID as a random effect and the timepoint as a fixed effect. The Bonferroni correction will be used to counteract the multiple comparisons problem. Effects will be accepted as statistically significant if the p-values of the statistical test is below 0.05.

11.2.2 Secondary endpoints

Changes in lipid and glucose metabolism, inflammatory parameters, total antioxidant capacity, and well-being will also be analysed using linear mixed models

11.2.3 Exploratory endpoints

All other parameters are considered as exploratory endpoints.

12. Data management and data protection

The legal basis for the data processing is the voluntary informed consent (Art. 6 para. 1 letter a, and Art. 9 para. 2 letter a, EU general data protection regulation (GDPR)).

The responsible person is the principal investigator, Dr Françoise Wilhelmi de Toledo, Buchinger Wilhelmi Development und Holding GmbH, Wilhelmi-Beck-Strasse 27, 88662 Überlingen (francoise.wilhelmi@buchinger-wilhelmi.com). The data protection officer of the Buchinger Wilhelmi clinic is Ulf Neumann, Lederstraße 134, 72764 Reutlingen (info@neumann.law).

12.1 Data recording

The study relevant primary data (patient data and medical findings) will be documented on case report forms in the patient file. The data will be pseudonymized and captured in electronic data files, which are protected by a password and stored safely. A representative of the principal investigator who is obliged to maintain confidentially will conduct the transcription. The list to match the pseudonyms with the patient name is stored separately in strict confidence in the institution of the principal investigator. The Buchinger Wilhelmi Amplius App will be used to self-report well-being and symptoms. Additionally, data will be collected by means of an online questionnaire that fulfils the GDPR standards like Typeform or EasyFeedback. Participants will enter a code that they received separately to pseudonymize their answers.

Data will be treated confidentially at all times and data protection regulations will be complied with.

12.1.1 technical characteristics of the app

The Buchinger Wilhelmi Amplius App is a smartphone app and can therefore also be used flexibly by the user at home. In addition to study participants, the app is primarily aimed at commercial users of the fasting box and patients of the clinic, who will find accompanying material (texts and videos) to their fasting programs in the app.

a. Video films can be viewed by means of the app. It records and stores which videos have already been viewed and the temporal position of the currently viewed video. The video films are embedded Vimeo and YouTube videos. The usage data is collected using Matomo. Matomo in turn is self-hosted.
b. For the study participants there are questionnaires in the app in which the study participants can document their well-being. (see Fig 2)
c. The app is developed as a cross-platform app for iOS (Apple) and Android (Google) operating systems. The app supports the following operating system versions:
    i. iOS: support for iOS 12 and iOS 13 versions, as well as the current iOS 14 version.
    ii. Android: support for versions starting from 5.0 to 9.0, as well as the current version 10.0.
d. Data collected with the app will be written from the servers to the database via an interface yet to be implemented. (see Fig 1)
e. The app is four-language in German, English, French and Spanish.
f. The existing user account of Buchinger Wilhelmi will be used to place the app in the App Store of Apple or in the Play Store of Google.

12.1.2 data acquisition - cloud-based data processing system

a. App content is managed via CMS Strapi, which is hosted on our servers.
b. Database systems on cloud servers for pure data storage. Data is stored in PostgreSQL on encrypted Hetzner volumes in Nuremberg and Falkenstein (provider Hetzner).

12.1.3 technical information about the initialization of the app and the reporting process

a. The native Buchinger Wilhelmi Amplius app can be downloaded either via a search function in the App Store, in the Google Play Store or via a link (QR code). The app download can happen before the fasting experience begins.
b. However, a 6-digit activation code is required to create a user account and successfully log in to the app.
c. Each study participant receives an individual Buchinger Wilhelmi identification (BW ID), which is assigned to a defined user group. The BW ID guarantees that only study participants can view and complete the study questionnaires.

12.1.4 technical information on the transmission of data entered in the app.

Within the overall system, several data transfers are performed between the subsystems. During data transmission between any subsystem of the overall application, end-to-end secured data transmission is always used, which realizes the protection goals of confidentiality, integrity and authenticity of both the data and the communication partner according to the current state of the art. Wherever possible, forward security (PFS) is also used. For example, TLS can be used with a suitable cipher suite. Different application containers, different virtual systems as well as different physical systems are necessarily considered as different subsystems. In case of doubt, a different subsystem is always assumed.

a. Inter-app container transfer and general inter-subsystem transfer

Different sub-functions of the system may be provided on different subsystems. These subsystems may be, for example, application containers, virtualized systems, or physical systems. Examples of such data transfers can be the retrieval of data from a database system or the transfer of this just retrieved data to a system for further processing of this data. The same minimum requirements for data transfers described at the beginning apply here.

b. Smartphone app <-> cloud services

Communication connections exist between the smartphone app and components of the cloud system, via which data is exchanged between the app and the individual components. These connections are always implemented as https connections, with at least TLS 1.3 to be used as the security framework.

On the server side, the identity must be proven by a certificate that has been digitally signed by a public certification authority, and both the validity of the digital signature as such and the check for its current validity must be carried out by the smartphone app using suitable measures (e.g. OCSP, CRL). In the event of a negative result, all communication connections are terminated immediately by the app.

The identity of the user is proven to the server in a suitable manner. In addition to the user ID and password of the user account, a unique identifier of the smartphone is also used, so that the use of an unknown end device is not possible without further ado and the

account must first be unlocked again via a separate communication channel, for example e-mail, or via a function within the application on a second end device.

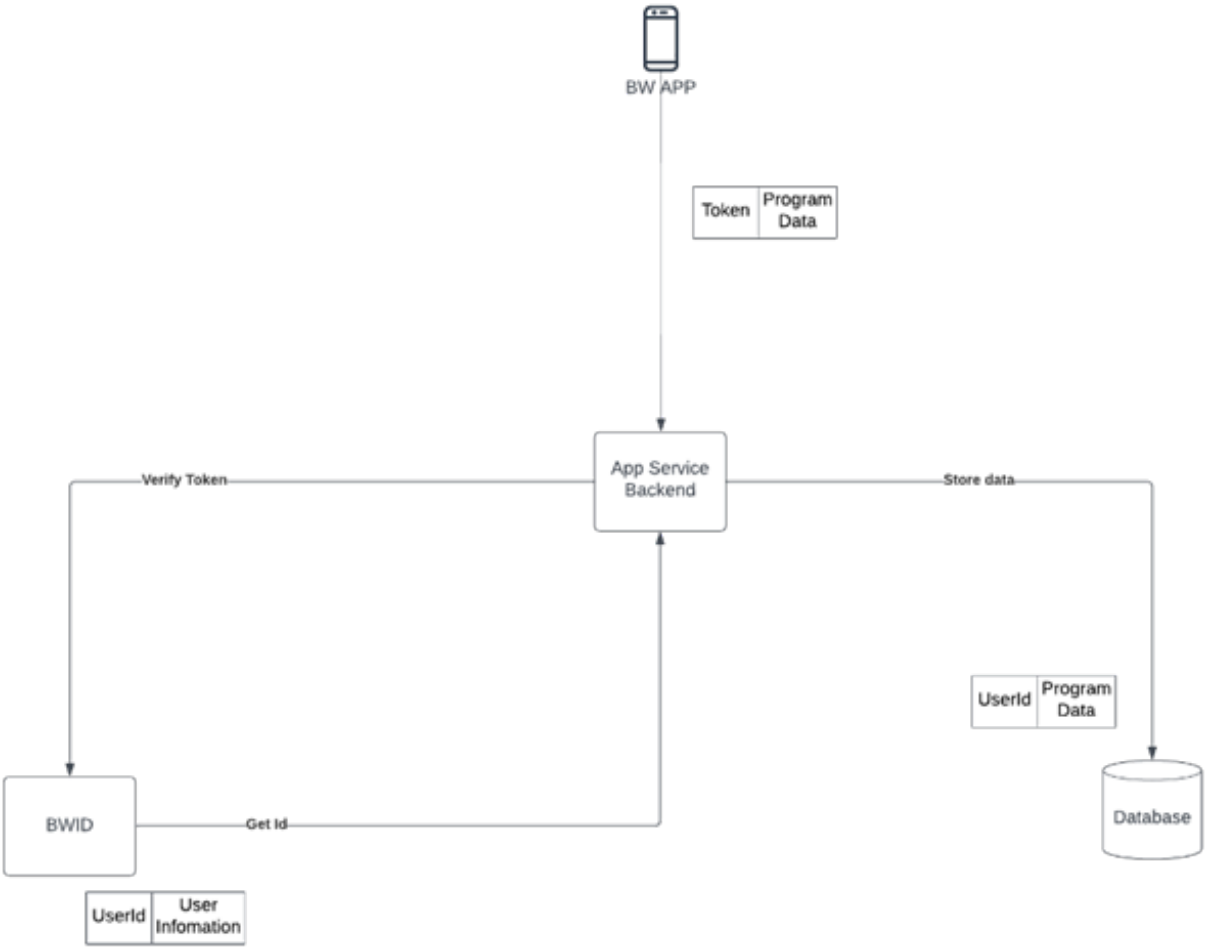

Figure 2. Data transfer of the app data to the database. The data is stored via a token in combination with the BW ID (User ID).

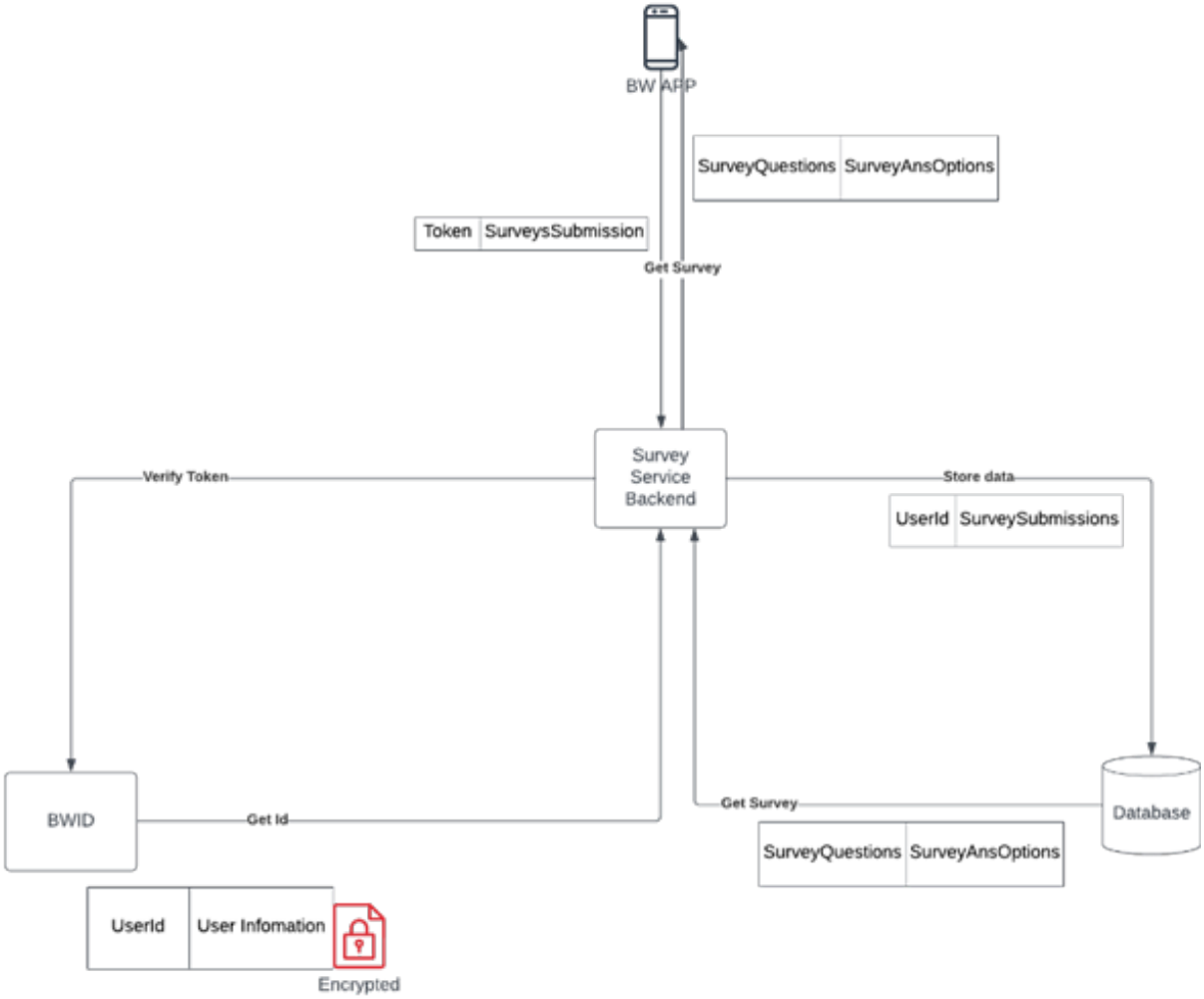

Figure 3. Data transfer of the questionnaire data into the database. The data is stored via a token in combination with the BW ID (User ID).

c. End devices of the project participants <-> systems

The security of this data transmission is also of central importance. The developers always make sure about the identity of the remote station and always use secured connections.

12.1.5 technical measures to protect against misuse and unauthorized access

a. Activation code

A 6-digit activation code must be entered to create a BW ID. By means of the app, a user account can be created within the cloud-based software application. For this purpose, the study participants, a 6-digit code is provided. Based on this code, the user can then enter an email address used by him within the app to create an individual user account together with a password chosen by him. After registration, the app is then operated exclusively in the context of this user account.

b. Data security of BW ID two-factor authentication

The 2FA is initiated via the app. The code is then used via an SMS to a mobile device. After entering the code, the login process is executed in the app.

c. Encryption of data and access to databases.
    a. Cloud applications and cloud-based database systems.

All productive and backup systems as well as all productive and backup data within these overall system parts shall be stored on data carriers encrypted according to the current state of the art. This is due to the lack of enforceability of preventing physical access to the server and computer systems along with the number and type of data.

d. Storage of password hashes

The storage of passwords is not in plain text and likewise not by exclusive use of a hash function - of any type. For example, it is permissible to add a random string (SALT) individual to each account with to the plaintext password (<plaintext password> | <SALT>), so that the stored hash value then results in hash(<plaintext password> | <SALT>). The hash function itself is one that is considered unbroken by the state of the art. This approach serves to prevent the use of rainbow tables in the potential theft of username and password hashes.

The data is stored on the hard disks in encrypted form. This means that the data cannot be read even if the hard disk is physically removed.

12.2 Storage (type, location, length)

In accordance with the rules of good scientific practice, research data will be safely stored on stable and secured carriers at the Buchinger Wilhelmi Clinic in Überlingen in an electronic data base. The long-term archiving will take at least 10 years.

Microbiome data will be stored anonymously on a data repository.

12.3 Transfer of data

For the scientific analysis pseudonymized data will be transferred to involved scientists and their teams (mentioned under point 3). The evaluating scientists are only provided with the data they need to answer their questions.

12.4 Ensurance of data safety

The compliance with the data protection law according to § 4 (1) Federal Data Protection Act (BDSG) is ensured. There will be no other use of the data than for the research purpose describes. It is guaranteed that the provisions of data protection are complied with and that only the data required for the research purpose are evaluated.

The pseudonymized data may also be transferred to involved scientists that are located outside the EU (e.g. Great Britain, Switzerland). These countries may have a lower data protection. To ensure the data protection the principal investigator will conclude contracts with these scientists according Art. 46 and 47 GDPR. The code (pseudonym) can only decrypted within the institution of the principal investigator.

12.5 Anonymised/pseudonymised

Within the context of the present study all patient data in the case report forms and database are only identified by a patient number and are herewith pseudonymised. The pseudonymised study relevant data will be used for purposes of research and statistical analysis. Decryption will only take place in the event of withdrawal from the study for the purpose of data destruction. As soon as it is possible according to the research or statistical purpose, the personal data will be anonymized.

12.6 Revocation and data deletion

The participants have the right of revocation of the declaration of consent. In this case, all patient-related data will be deleted. If data were already anonymized a deletion is not possible. Additionally to the case of revocation, data will be deleted after the expiry of the prescribed 10-year storage period after termination of the study. They will be deleted after 15 years at the latest.

12.7 Right of access and information

The participants have the right to get access to their captured data as well as a complimentary copy of their data. They have the right to rectification and erasure, this means they can demand the correction or deletion of their data and samples/images. Moreover, they have to the right to restriction of processing and the right to object to collection, processing and/or use as well as the right to data portability. They have the right to withdraw the consent at any time for the purpose in question. The lawfulness of processing the personal data on the basis of the consent provided by the participant remains valid until the withdrawal of consent has been received. The participants have the right to complain to a supervisory authority.

13. Handling of biomaterials

- Blood samples

  Blood samples are collected in the morning in fasting subjects by an educated and authorized staff. If necessary, the blood samples are centrifuged immediately after collection (3500 rpm for 15 min). Part of the blood samples are sent directly to the laboratory in Ravensburg/ Málaga. The other part of them are stored in plastic tubes at a temperature of -70 °C.

- Stool samples
  Stool samples are collected at T0 and T2 as well as T3 and stored by –20 °C.

14. Participant insurance

There is no study specific insurance.

15. Publication policy

The publication is performed in an accepted scientific journal independent of the results by the involved scientists that are mentioned in the section ‘responsibilities’.

16. Signature: lead investigators

Überlingen, 08.03.2023	
place, date	signature