**Thesis**

# B-cell immune responses and body fat topography in immunocompromised and healthy individuals


Submitted by

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*Statutory Declaration*

*I declare on my honor that I have written this thesis independently and without assistance, I have not used other than the specified sources, and parts taken from other sources, verbatim or in substance, have been identified as such.*

*Graz, January 17th, 2023* *Thea Perl eh.*

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# Abbreviations

| | |
|---|---|
| ACE 2 | angiotensin-converting enzyme 2 |
| BMI | body mass index |
| BR | brachio radialis |
| CD | cluster of differentiation |
| Covid-19 | coronavirus disease 2019 |
| DAMPs | damage associated molecular pattern |
| Dexcl | distances in which embedded tissues are excluded |
| Dincl | distances in which embedded tissues are included |
| DNA | deoxyribonucleic acid |
| DT | distal triceps |
| ECMO | extracorporeal membrane oxygenation |
| ELISA | Enzyme-linked Immunosorbent Assay |
| ES | erector spinae |
| FT | front thigh |
| HC | healthy control group |
| Ig | immunoglobulin |
| IL | interleukin |
| INF | interferon |
| LA | lower abdomen |
| LT | lateral thigh |
| MC | medial calf |
| MCP | monocyte chemoattractant protein-1 |
| Md | median |
| MERS-CoV | middle east respiratory syndrome |
| MHC | major histocompatibility complex |
| mRNA | messenger ribonucleic acid |
| NK cells | natural killer cells |
| PAMPs | pathogen associated molecular patterns |
| PID | primary immunodeficiency |
| PRRs | pattern recognition receptors |
| RNA | ribonucleic acid |

| | |
|---|---|
| SARS-CoV | severe acute respiratory syndrome coronavirus |
| SAT | subcutaneous adipose tissue |
| SID | secondary immunodeficiency |
| SPSS | Statistical Analysis Software |
| TBE | tick-borne encephalitis |
| TH cell | T helper cell |
| TMPRSS2 | transmembrane protease serine subtype 2 |
| TNF | tumor necrosis factor |
| UA | upper abdomen |
| WHO | world health organization |

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Zusammenfassung

**Einleitung:**

Es ist bekannt, dass Übergewicht viele gesundheitliche Nachteile mit sich bringt. Unter anderem führt es zu einer chronischen geringgradigen Entzündung, welche sich auf das Immunsystem auswirkt. In dieser Studie wird untersucht, ob und wie sehr sich Menge und Verteilung des subkutanen Körperfetts auf die B-Zell Immunantwort auswirkt. Außerdem wird ein Zusammenhang mit dem Vorkommen des ACE2 Rezeptors gesucht, welcher im Körperfett verstärkt exprimiert wird und von SARS-CoV-2 verwendet wird um in die Zelle zu gelangen.

**Methoden:**

Die Daten wurden im Rahmen der CoVVac Studie der Medizinischen Universität Graz erhoben, welche sich mit der Antikörperproduktion nach einer Covid-19 Impfung beschäftigte. Dabei wurde eine Kohorte von immunsupprimierten Patient*innen (n= 64) und gesunden Kontrollpersonen (HC-Gruppe, n= 72) in die Untersuchung eingeschlossen. Die Gruppe der immunsupprimierten Patient*innen wurde wiederum in Personen mit primärer Immundefizienz (PID Gruppe, n= 24) und Personen mit sekundärer Immundefizienz (SID-Gruppe, n=40) unterteilt. Die PID-Gruppe schloss Personen mit genetisch bedingten Immundefekten ein, die SID Gruppe Menschen die aufgrund von hämatologischen Malignomen, immunsuppressiven Therapien oder hämatopoetischer Stammzelltransplantation eine B-Zell-depletierende Therapie erhielten. Als Teil der Studie wurden außerdem Ultraschallmessungen des subkutanen Fettgewebes durchgeführt, mithilfe eines Immunoassays die Antikörper bestimmt sowie die ACE2 Levels mit dem Human ACE2 ELISA Kit von ThermoFisher Scientific. Für alle Berechnungen zwischen gebildeten Antikörpern, Alter, Geschlecht, ACE2 Leveln und Körperfettverteilung wurde SPSS verwendet. Hier wurde die Verteilung mit dem Shapiro-Wilk-Test berechnet, Gruppenvergleiche mit dem Mann-Whitney U Test. Für Korrelationen wurde der Spearman-Korrelationskoeffizient bestimmt.

**Ergebnisse:**

Es konnten signifikante Unterschiede in der Menge der gebildeten Antikörper zwischen den Gruppen beobachtet werden. Während in der Gruppe der gesunden Kontrollpersonen alle einen hohen Titer erreichten (Md= 2500 U/ml), war der Median der Roche Anti-RBD sowohl in der PID-Gruppe (Md= 1572 U/ml, p =<0.001), als auch in der SID-Gruppe (Md= 0 U/ml, p =<0.001) im Vergleich zur gesunden Kontrollgruppe signifikant niedriger. Es konnten keine Alters- oder Geschlechterunterschiede bei den gebildeten Antikörpern gefunden werden. In der Gruppe der gesunden Kontrollpersonen gab es eine signifikante Korrelation zwischen Alter und BMI (p=0.032, r=0.254) sowie zwischen Alter und Taillenumfang (p=0.014, r=0.288). Ein signifikanter Unterschied im Körperfett zwischen Männern und Frauen wurde in allen Gruppen gefunden. In der PID-Gruppe gab es eine signifikante Korrelation zwischen den gebildeten Antikörpern und dem Gesamtkörperfett (p=0.004, r=0.433), sowie zwischen gebildeten Antikörpern und dem an der Wade gemessenen subkutanen Fett (p= 0.03, r= 0.462).
Bei den ACE 2 Levels konnte kein Zusammenhang mit Alter, Fettverteilung oder Geschlecht festgestellt werden, im Geschlechtervergleich zeigte sich jedoch, dass Männer signifikant mehr ACE2 Rezeptoren aufgewiesen haben als Frauen (p=0.039).

**Schlussfolgerung:**

Personen mit Immundefizienz unterscheiden sich sowohl in der Menge des Körperfetts, als auch in der Menge der gebildeten Antikörper nach einer Impfung. Eine Assoziation zwischen dem Körperfett und der Immunantwort konnte allerdings nur in der Gruppe der Personen mit primärer Immundefizienz gefunden werden. Hier bildeten Menschen mit höherer Fettmasse mehr Antikörper als Menschen mit weniger Körperfett. Für die anderen untersuchten Gruppen wurde eine homogene B-Zellantwort bei unterschiedlicher Alters- und Körperfettverteilung beobachtet, weshalb kein Einfluss der Körperfettmasse in dieser Kohorte angenommen werden kann.

# Abstract

**Introduction:**

It is well known that obesity has many health disadvantages. Among other things, it leads to chronic low-grade inflammation, which affects the immune system. In this study, we investigated whether and to what extent the amount and distribution of subcutaneous body fat also affects the B-cell immune response. In addition, a connection with the presence of the ACE2 receptor is sought, which is increasingly expressed in body fat and is used by SARS-CoV-2 to enter the cell.

**Methods:**

Data was collected as part of the CoVVac study at the Medical University of Graz, which focused on antibody production following Covid-19 vaccination. A cohort of immunocompromised patients (n= 64) and healthy controls (HC group, n= 72) was included in the study. The group of immunocompromised patients was divided into individuals with primary immunodeficiency (PID group, n= 24) and individuals with secondary immunodeficiency (SID group, n=40). The PID group included individuals with genetic immunodeficiencies, and the SID group included people who received B-cell-depleting therapy due to hematologic malignancies, immunosuppressive therapies, or hematopoietic stem cell transplantation. As part of the study, ultrasound measurements of subcutaneous adipose tissue were also performed. In addition, antibodies were determined using an immunoassay and ACE2 levels were measured using the Human ACE2 ELISA Kit from ThermoFisher Scientific. SPSS was used for all calculations between antibodies formed, age, gender, ACE2 levels and body fat distribution. The distribution was calculated with the
Shapiro-Wilk test, and group comparisons were calculated with the Mann-Whitney U test. For correlations, the Spearman correlation coefficient was determined.

**Results:**

Significant differences in the number of antibodies formed were observed between the groups. While in the healthy control group all reached a high titer (Md= 2500 U/ml), the median Roche anti-RBD was significantly lower in both the PID group (Md= 1572 U/ml, p =<0.001), and the SID group (Md= 0 U/ml, p =<0.001) compared to the healthy control group. No age or gender differences were found in the antibodies formed. In the healthy control group, there was a significant relationship between age and BMI (p=0.032, r=0.254) and between age and waist circumference (p=0.014, r=0.288). A significant difference in body fat between men and women was found in all groups. In the PGD group, there was a significant correlation between antibodies formed and total body fat (p=0.004, r=0.433), and between antibodies formed and subcutaneous fat measured at the calf (p=0.03, r= 0.462).

No correlation with age, fat distribution or gender was found for ACE 2 levels, but gender comparison showed that males had significantly more ACE2 receptors than females (p=0.039).

**Conclusion:**

Individuals with immunodeficiency differ both in the amount of body fat and in the number of antibodies formed after vaccination. However, an association between body fat and immune response could only be found in the group of individuals with primary immunodeficiency. Here, people with higher fat mass formed more antibodies than people with less body fat. For the other groups studied, a homogeneous B-cell response was observed with different age and body fat distribution, which is why no influence of body fat mass can be assumed in this cohort.

# 1 Introduction

The new virus Sars-CoV-2, which appeared in 2019, has caused a lot of new questions and inspired research around the world. The virus itself has been described in detail, it has been studied how it affects humans, how the immune system works against it, and what symptoms it causes. Consequently, countermeasures and vaccines with novel modes of action have been developed.

For a long time, people have been concerned with the effects of increasingly common obesity, as well as fat distribution, on people's health.
In this thesis I would like to combine the two topics and work on the question, what effect the fat distribution of a person has on the B-cell immune response in a Covid-19 vaccination. In addition, the group of people studied will be further divided into immunosuppressed and healthy participants.

First, I will discuss viruses in general and specifically the Covid-19 virus, as well as the immune system and the formation of an immune memory, among other things, through administration of a vaccination. Then a description of fat distribution and the effect on the immune system will follow. The second part will picture and discuss the results of the CoVVac study.

## *1.1 Functionality of a virus*

A virus is a small infectious agent that carries its genetic information as DNA or RNA. A single virus particle is called a virion, which consists of different parts. The first part is the nucleic acid, which carries the genetic information. Depending on which family the virus belongs to, it can be RNA, single stranded DNA or double stranded DNA. The second part is the capsid, which is built from proteins and covers the nucleic acid. Together, nucleic acid and capsid are referred as nucleocapsid. Some viruses are also covered in an envelope consisting of lipids. The danger of a virus depends on the damage done on the infected cell and the immune response. Some viruses use the infected cell´s synthesis machinery to do so much own production, that the cell is not able to maintain its own metabolism and perishes. Other viruses do not cause such extreme congestion. Here, the cell manages the increased load, but there are changes in the cell morphology. These visible changes are called cytopathic effect, which includes cell balling, giant cell formation, inclusion bodies and apoptosis. In addition to the direct damage of the virus to the cell, there is also damage caused by the immune response to the invading virus, whereby innate and adaptive immunity, interferons and inflammatory cytokines play a role. In addition, tissue damage is caused by cytotoxic T cells and NK cells, which are trying to destroy infected cells, but simultaneously also damage healthy parenchymal cells. Depending on the extent of the damage done, there are various clinical symptoms ranging from a mild cough to death. (Pellett et al., 2014)

A virus has no own metabolism or cell organelles, so it needs a living cell as host cell to replicate. It enters the cell by using a fitting receptor, which leads to receptor-mediated endocytosis or membrane fusion.  Then the genome is being unpacked and the synthesis of the proteins starts. The first step is the building of the non-structural proteins, followed by the genome and the structural proteins. Then the particles have to be assembled, which happens in the nucleus, cytoplasm or plasma membrane. When the virus particles are finished, they are released into the organism, where new cells can be infected, and the replication cycle starts again.  The infection can be limited to a certain area, most of the time the entry region, or become systemic. (Pellett et al., 2014)

### 1.1.1 Sars-CoV-2

Different types of the corona virus family have been reported to be exchanged between mammals and birds, where they usually lead to a minor infection of the airways. Over the years, there have been numerous cases related to zoonosis, where new types were being transmitted to humans. Those cases include SARS-CoV, MERS-CoV and SARS-CoV-2 and resulted in infections with severe health burden.

The first pandemic caused by SARS CoV started in 2002. It spread from southern China and claimed 774 lives within half a year. Most of the new infected people were relatives of already infected and medical staff of hospitals. Almost exclusively adults were affected, children felt sick very rarely. The patients initially developed flu-like symptoms, such as fever, headache and chills. During the second week, an initially dry cough, and diarrhea appeared, furthermore a shortness of breath, which often had been so bad that oxygen was required. The mortality rate was estimated by the WHO at 11 %. (de Wit et al., 2016)

MERS-CoV, another corona virus species was identified in 2012. It also causes severe respiratory infections, pneumonia and kidney failure in humans, often combined with diarrhea. In healthy people, the infection is probably asymptomatic or with mild flu-like symptom, while patients with previous illnesses experience severe courses. MERS-CoV originated in Saudi Arabia, so far, it has been assumed, that it was sporadically transmitted to humans via dromedaries. The most patients are, like with SARS CoV, relatives of already infected and medical staff of hospitals, so the assumption is that it is not easily transmitted from human to human. (Mackay and Arden, 2015)

In 2019 a new disease resulting from corona virus infection emerged: Covid-19. It is caused by the beta corona virus type SARS-Cov-2 (severe acute respiratory syndrome coronavirus type 2), which was identified in 2020. SARS-CoV-2 is a membrane-enveloped single strand RNA virus with prominent surface projections, called spikes, and a 80-140 nm diameter. (Laue et al., 2021)

SARS-CoV enters the cell via receptor-dependent, pH sensitive endocytosis, which is also clathrin- and caveolae independent. (Wang et al., 2008)

In case of SARS CoV-2, the spike protein of the virus engages with the human receptor ACE 2 on the target cell. After docking, the spike proteins are split and activated as a fusion-transmembrane-protein, thus allowing the virus to be absorbed into the cell. The splitting and activating processes are induced by the TMPRSS2 enzyme, which means that SARS-CoV2 not only needs the ACE2 receptor, but also the TMPRSS2 for cell entry. (Oberfeld et al, 2020)

The primary site of infection is the respiratory system, where a high density of ACE-2 receptors and a higher amount of TMPRSS2 and TMPRSS11d can be detected. (Schuler et al, 2021) Of note, ACE-2 receptors are also enriched in adipose tissue, thus giving a potential explanation why obese patients more likely experience a severe course once they are infected with SARS-CoV 2. (Freuer et al, 2021)

The clinical picture of a Covid-19 infection is quite diverse. Pulmonic-, neurological-, gastrointestinal-, cardiovascular-, kidney-, and dermatologic manifestations can occur. Most of the time, patients complain about fever, coughing, fatigue and a loss of taste and smell. In addition, there can be also headache, body aches, diarrhea, eye irritations and more severe symptoms like shortness of breath, chest pain and confusion.

In severe cases, pneumonia can develop, which can progress to acute respiratory distress syndrome requiring ventilation. Under certain circumstances, it may even be necessary to enrich the venous blood with oxygen outside the body in a machine, and then return it to the arterial blood system. For this procedure a extracorporeal membrane oxygenation (ECMO) is used, which works like an external lung. Within patients requiring ventilation, acute renal failure is frequently observed. Furthermore, some of the patients with a severe course suffer from cardiovascular events such as myocardial damage, myocarditis, myocardial infarction, cardiac insufficiency, cardiac arrhythmias or thromboembolic events, others develop a hyperinflammatory syndrome, which can lead to multi-organ failure and ultimately cause death. (Sardu et al, 2020)

The treatment options depend on the severity of the symptoms and the phase of the disease. Asymptomatic patients and those with mild or moderate symptoms can be treated at home. Symptomatic treatment with antipyretics for fever and pain, adequate nutrition, rehydration and monitoring the vital signs is recommended by the WHO.  Patients developing severe symptoms should be hospitalized, where the essential therapy is the administration of oxygen. Additional evidence-based treatment options are the administration of anti-inflammatory agents like dexamethasone or tocilizumab, monoclonal neutralizing antibodies, anti-virial therapy and adjunctive therapy. For anticoagulation heparin can be used prophylactically. (Tsang et al., 2021)

### 1.1.2 Immune response to a virus

When the body is confronted with a new virus, the first contact is done by the nonspecific immune system, which is also called the “innate” immune system. It includes chemical, physical, cellular and humoral defense mechanisms and initiates the further immune response, which occurs via vasodilatation and increased vascular permeability, the activation, proliferation and docking of immune cells and the killing of the pathogen. Intact skin and mucous membranes, mucus production and coughing are parts of the chemical, physical and biological mechanisms. Part of the cellular parts are PRRs and DAMPs. Pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPS), which are special molecules for pathogens that do not occur in humans. PRRs generate an immune response on first contact. DAMPs are endogenous molecules that passively enter the extracellular space upon tissue injury with cell necrosis and trigger an inflammatory response or are actively released by immune cells. (Broggi and Granucci, 2015)

The humoral mechanisms are based on plasma proteins, which are mainly produced by the cells of the immune system. These proteins include acute phase proteins, which lead to an inflammatory reaction, and the complement system, which connects the innate and the acquired immune system by completing antibodies and proinflammatory cytokines. These cytokines include interleukins, interferons, colony-stimulating factors, prostaglandins and tumor necrosis factors. Cytokines activate the endothelium and leukocytes, have a chemotactic and proliferation-stimulating effect on leukocytes, stimulate the production of other cytokines, increase the production of acute phase proteins and initiate apoptosis.

Cytokines also play a special role when it comes to Sars-CoV-2: In case of systemic inflammatory response syndrome and severe sepsis, such as those that occur with a covid-19 infection, a strong overproduction of cytokines takes place, which leads to an overreaction of the immune system. (Primorac et al., 2022)

The first reaction is quick and leads to an inflammation. Due to the inflammation, the adaptive, specific immune system gets involved, which works with antigen-specific cells. These need to be activated, before they can act. Usually, the activation is done by antigen-presenting cells of the innate immune system.

The different response pathways of the acquired immunity are linked to three subtypes of T- cells. The first way is the direct cell lysis or induction of apoptosis by perforins or proteases of cytotoxic T-cells, which are previously activated by MHC I receptors. The second pathway is the cellular-mediated type 1 response by T-helper 1 (TH1) cells. TH1 cells interact with macrophages, cytokines are produced, and macrophages and cytotoxic T cells are stimulated. The third way is a humoral type 2 response of TH2 cells. Here, an interaction of helper cells with B lymphocytes is initiated via MHC II receptors, which leads to a T-cell dependent activation of B cells. As a result, immunoglobulins are produced.

As the immune response of the acquired immune system needs to be activated, it occurs later than the innate one. However, it is then highly specialized and therefore more effective. After the first infection, the adaptive immune system develops an immunological memory, which reacts quickly and with reduced inflammatory processes if the same virus gets into the body again. (Primorac et al., 2022)

### 1.1.3 Development of the immunological memory

As described above, the development of an immunological memory is necessary for a quick and effective reaction when the body is confronted with a specific virus for a second time.

The immunological memory consists of B- and T- memory cells, which are differentiated B- and T- lymphocytes. The cells carry characteristic molecules in the cell membrane, which are called differentiation antigens. These act as antigens, against which specific antibodies can be produced. T-lymphocytes mature in the thymus, where they form an antigen-recognizing, but self-tolerant t-cell receptor, as well as the typical surface molecules CD4 (helper cell) or CD8 (cytotoxic T-cell). B- cells arise in the bone marrow, where they already have their immature B-cell receptor, which is later differentiated in secondary lymphatic organs. After the differentiation, the cells circulate between these and the blood, until they encounter the appropriate antigen. Then, they transmute into plasma cells and start producing antibodies.

To get B- and T- cells with the right antigens, it is important to know, what the virus antigen looks like. This information is shared through MHC I and MHC II complexes.
To get MHC-I complexes, an infected cell produces interferons, which are signal proteins that lead other cells to produce antiviral proteins and induce the expression of MHC-I Molecules. MHC II is presented by antigen-presenting cells like dendritic cells, monocytes, macrophages and B lymphocytes, after they have been in contact with the foreign particles. If now a CD4 T-cell connects with a MHC II molecule, it comes to a process called priming. When the same CD4 T-cell then meets a B-cell, which is presenting the same antigen, it comes to the production of B and T memory cells and plasma cells. (Primorac et al., 2022)

The course of the antibody response is divided into a primary and a secondary response. After the first contact with a specific antigen, the body produces IgM antibodies, of which the concentration increases exponentially in the first eight days, but then starts sinking. This is called the primary response. If there is a second contact with the same antigen within a certain time, it comes to the secondary response, where IgG antibodies are produced. Consequently, the number of built antibodies is higher and lasts longer. With each renewed infection, new memory cells are formed, that can act faster, more effective and more specific against the next infection. (Sundling et al., 2021)

### 1.1.4 Vaccinations

The capability of the body to build an immunological memory is the basis for the function of vaccinations. Broadley, two forms are used: active and passive immunization. Active immunization is the administration of a vaccine containing an attenuated pathogen or parts thereof, leading to the development of endogenous, longer lasting immunity. Passive immunization is the administration of ready-made immunoglobulins for immediate protection against an infectious disease. It does not result in the development of longer-term endogenous immunity. It is administered, for example, when a particularly vulnerable person has been exposed to a dangerous pathogen, such as to prevent rubella infection during pregnancy. In this Diploma thesis I will focus on the active immunization given the scope of the presented project.

Active immunization is created to prevent a disease without being exposed to a certain virus. The aim is to build a long-lasting immunity with few side effects, without ever going through the full clinical picture. Vaccination primarily usually only protects the person who is vaccinated. If, however, a sufficient number of people are vaccinated, virus spread is limited and thus even not-vaccinated people are protected. This phenomenon is called herd immunity. (Pandhi et al., 2017)

Due to the Covid-19 pandemic new vaccination strategies, including mRNA and viral vector vaccines have been implemented. Today, there are different types of active vaccination available:

1. Live vaccines: These contains replicable, but attenuated pathogens. Even a single vaccination provides vaccine protection, a second serves as a booster. Examples include the vaccination against mumps, measles, rubella and varicella.

2. Inactivated vaccinations: These contain inactivated pathogens or components of them. Here, a single dose is not sufficient, several vaccinations are necessary. Examples are Hepatitis A and B, TBE and meningococcus.
3. Gene-based vaccines: These contain the genetic information for target antigens of the pathogens. The synthesis of the antigens occurs in the cells of the vaccinated person. An example is the Covid-19 mRNA vaccine, which will be discussed in the following chapter.

#### 1.1.4.1 mRNA Vaccines

There are now several vaccines against Covid-19, which are based on different principles. The vaccines Comirnaty (BioNTech Pfizer) and Spikevax (Moderna biotech) are gene-based vaccines, they contain the genetic information to build just the spike protein of SARS-CoV-2. This information is written on mRNA, which gets absorbed into the cell. The ribosomes start translating the mRNA and building the spike protein, which then gets recognized as foreign to the cell and split up again. The parts are presented on the cell surface, where the immune system reacts with building antibodies and memory cells. (Cagigi and Loré, 2021)

Vaccine protection exists after two vaccinations but must always be boostered thereafter. In people with immunodeficiency, there is probably an attenuated effect. For pregnant woman and in the breastfeeding period the vaccination is not approved but still recommended. The application is muscular, after the injection the vaccinated person must be observed for 15 minutes to detect rare anaphylactic reactions early. After vaccination, pain at the injection site may occur, as well as fatigue, headache, myalgia, chills, fever or similar. Rarely, anaphylaxis or myocarditis have been observed. (Cagigi and Loré, 2021)

#### 1.1.4.2 Vector Vaccines

This method is used by the vaccines by AstraZeneca, Johnson&Johnson and Sputnik V. Again, the information brought into the cell by the vaccine is the blueprint to produce just the Spike protein. However, this time it is built into a vector, which in this case is a non-replicating recombinant chimpanzee adenovirus, used as shuttle into the cell. Since the vector virus is a DNA virus, also the information for the spike protein is written on DNA in prefusion conformation, in which it is present in SARS-CoV-2 infection prior to fusion of the virus with a host cell. The DNA has to be translated to mRNA first, then the same procedure is followed as with the mRNA vaccine.

For immunization, the combination of vector vaccination and mRNA vaccination after four weeks is currently recommended, followed by regular booster vaccinations. The application is also muscular, after which an observation period of 15 minutes should be appended. If immune thrombocytopenia is known, platelets should be monitored after vaccination because thrombosis with thrombocytopenia has been observed as a rare complication of vaccination. Other adverse effects may include injection site pain, myalgias, arthralgias, gastrointestinal symptoms, fever and other mild symptom of disease. (Kaur, 2020)

### 1.1.5 Covid-19 Vaccination

Vaccination against SARS-CoV-2 is the most important measure to contain and control the Covid-19 pandemic. The benefit of a vaccination against SARS-CoV-2 is a significantly reduced probability of a symptomatic disease. If patients still get sick, the vaccine protects against a severe course, hospitalization and death. Also, vaccinated patients with covid-19 are less infectious to others than those without a vaccination.

In principle, everyone who is capable of being vaccinated should be vaccinated. However, caution should be exercised in the case of very old people or people in very poor general health, in whom undesirable side effects such as fever, diarrhea or vomiting could already be life-threatening.

The choice of vaccine is based on age. Only for pregnant women or breastfeeding mothers, just the mRNA vaccine is currently recommended from the 2nd trimester.

Despite, everybody without an absolute contraindication should be vaccinated, there are people suffering chronic diseases which increase the risk of a severe course. These diseases include obesity and various clinical pictures that require immunosuppressive therapy. Since these patients depend on the effectiveness of the vaccine, it is particularly important to know how well their immune system responds to the vaccination. (Haas et al., 2021)

## *1.2 Impact of body fat on the immune response*

Overweight, as well as underweight influences the health in many ways. Obese patients have a higher risk of developing type 2 diabetes, heart disease or cancer. The adipose tissue has also an impact on the immune system regarding the defense against infection and the efficiency of vaccines. (de Leeuw et al., 2021)

Speaking of influenza, obese patients have a significantly increased risk of undergoing a severe course, while at the same time they experience a poor initial and adaptive immune response to vaccination. (Honce, 2019)

Worldwide observations of Covid-19 patients showed that a significant high part of the patients suffering from respiratory failure are overweight. Studies revealed a correlation between the probability of hospitalization and BMI, independent of other risk factors. In addition, the proportion of patients who required invasive ventilation was significantly higher among patients with a high BMI. (de Leeuw et al., 2021)

### 1.2.1 Anatomy

Adipose tissue is found throughout the body around the organs and subcutaneously. In healthy quantities, it has many important functions in the body. On the one hand, it serves as building and "insulating material" in the skin and organs, and on the other hand, it is our energy store for longer periods of fasting or severe illness. It is also important for hormone production. (Frigolet and Gutiérrez-Aguilar, 2020)

Adipose tissue can be divided into two types, white and brown. Brown adipose tissue can be deployed quickly and is mainly used by newborns to regulate their temperature by uncoupling the respiratory chain in the mitochondria. It mainly found in infants, in adults usually it has already regressed. White adipose tissue is used as energy storage, which increases in anabolic metabolic state by hypertrophy of lipid vacuoles and hyperplasia of adipocytes and decreases in catabolic metabolic state by lipolysis. If there is a persistent high calorie intake, which exceeds the daily requirement, a pathological increase of white adipose tissue can occur. A body mass index (BMI) of 30 $kg/m^2$ indicates obesity, which is associated with an increased risk to develop different diseases. (Frigolet and Gutiérrez-Aguilar, 2020)

About 85 % of the adipose tissue is stored subcutaneously. 15 % is visceral fat, which is located for example around the internal organs, soles, retrobulbar and in the joints. (Frigolet and Gutiérrez-Aguilar, 2020)

### 1.2.2 Inflammatory response of the adipose tissue

Excess dietary energy is stored as fat in the white adipose tissue, which contains not only fat-storing adipocytes, but also immune cells that influence the overall homeostasis of the body through metabolic, endocrine and immune functions. Obesity can severely imbalance these functions, as there are different hormones and inflammatory mediators released by the adipocytes. Is the released amount to high, it leads to high leptin secretion, chronic low-grade inflammation and the release of pro-inflammatory mediators. (de Leeuw et al., 2072)

Low-grade inflammation is a major risk factor for the development of cardiovascular disease, diabetes mellitus and fatty liver disease. Because of the inflammation, also cells necessary for the immune response are limited in their function. This limitation can lead to a poorer defense against pathogenic agents, a higher probability of reinfection and excessive inflammatory reactions. (Weisberg e al, 2003)

#### 1.2.2.1 Effect on the immunological memory

As discussed in chapter 1.1.3, the building of an immunological memory involves the function of B and T cells. In adipose tissue, an impairment of these cells can occur.

In healthy adipose tissue, sufficient Th1 cell levels can be found. Th1 cells induce the differentiation of anti-inflammatory M2 macrophages, which have a high phagocytic capacity, as well as regenerative effects by releasing growth factors and anti-inflammatory cytokines. At the same time fewer Th17 cells can be found. This shortage leads to the reduced formation of M1 macrophages. This type of macrophages has a toxic effect to other cells which produce proinflammatory cytokines and thus establish an inflammatory environment. In obese patients the differentiation is reversed, due to high levels of CD11c and Th 17 cells, there are high rates of M1 macrophages and low rates of M2 macrophages. Also, the adipocytes change and become dysfunctional, hypertrophic and necrotic. (Lumeng, 2007)

The B – cells of obese individuals are characterized by a high level of activation, increased differentiation in antibody-producing plasma blasts, higher levels of IL-6 and TNF α. This defect leads to decreased B-cell responses and to elevated production of proinflammatory cytokines. (Wiggins, 2021)

In regard to Covid-19, an infection with SARS-CoV-2 in obese people may lead to a cytokine storm, which is characterized mainly by the presence of IL-6, TNF-α IL-2, IL-7, INF-Y, MCP-1. In severe cases, high levels of IL-6 are found regularly, since IL-6 can be produced and secreted in adipose tissue. In addition, the cytokines of hypertrophic adipocytes can enter the bloodstream and cause high cytokine levels systemically, which counteract the termination of the antiviral immune response in the lungs in patients with severe disease.(de Leeuw et al., 2021)

## *1.3 Immunosuppression*

Immunosuppressive pharmaceuticals are used in medicine to temper or deactivate the function of the immune system. This can be necessary if either the immune system is not supposed to do its job, for example after a transplantation, or if it comes to a harmful overreaction, which happens in allergies or autoimmune diseases. Regardless of the reason for prescription, immunosuppressive drugs have the main side effect to decrease the immune response for infection and against malignancies, beside unintended side-effects as loss of appetite, nausea, vomiting, anemia, hair loss and gain of weight. (Meneghini et al., 2021)

### 1.3.1 Consequences on the immune response

The effects on the immune system depend on the active ingredient and the mechanism of action. Consequently, immunosuppressive pharmaceuticals are divided into subgroups:

- The first group is formed by cytotoxic immunosuppressants like Methotrexate, Azathioprine and Cyclophosphamide. They act cytostatically, which means that they inhibit the clonal expansion and the function of B- and T-lymphocytes, or the synthesis of DNA and RNA. Indications for the use are rheumatoid arthritis, arthritis psoriatica, vasculitis, scleroderma or tumor deseases.

- The second group are immunosuppressants with an inhibitory effect on antigen-induced T-cell activation. This group includes Ciclosporin and Tacrolimus, which antagonize a step in the T-cell receptor signal transduction pathway, so the clonal expansion of the T-cells fails to appear, as well as glucocorticoides, which are inhibiting the antigen-induced activation of the cellular immune response and Abatacept, which alleviates T-cell activation. The indications are organ transplant patients and patients suffering from severe autoimmune diseases.

- The third group includes immunosuppressant's with inhibitory effects on the IL-2 receptor and it´s signaling. An example is Basiliximab, which is used in organ transplanted patients to prevent rejection. It leads to a blockage of the IL-2 receptor, resulting in the IL-2induced autocrine and paracrine signals for T-cell maturation and proliferation being attenuated. (Meneghini et al., 2021)

### 1.3.2 Vaccination under immunosuppression

Since immunosuppression potentially impair some facets of the immune system of patients, vaccinations are particularly important to prevent diseases and severe courses. People with autoimmune diseases, who are not having a immunosuppressive therapy, usually have proper vaccine responses. The extent of the immune deficiency or immune suppression is decisive for the development of the protective immune response. To determine the success of vaccination, it is possible to measure the B-cell immune response at certain intervals after the vaccination.

Generally, it is recommended to administer vaccinations before starting immunosuppressive therapy. If this is not possible, as it is in the case of the newly developed Covid vaccination, it must be considered whether the risk of the disease and the expected success of the vaccination outweigh the risk of vaccination.
For patients receiving strong immunosuppressive medication, live vaccines should be avoided because of the risk of uncontrolled multiplication of the vaccine virus. Inactivated vaccines are considered safe for people with autoimmune diseases or those receiving immunomodulatory therapies. (Schulz et al., 2021)

## *1.4 Aims and Hypotheses*

This thesis aimed to investigate potential effects of elevated body fat mass on the B-cell response after Covid-19 vaccination in immunocompromised patients and healthy controls.

The hypotheses were as follows:

- The amount of subcutaneous adipose tissue differs significantly between the immunocompromised and healthy cohort.
- Levels of circulating ACE 2 are correlated to the amount of body fat.
- The amount of subcutaneous adipose tissue is associated with the B-cell response to the Covid-19 vaccination.

# 2 Material and Methods

## 2.1 Study design

The CoVVac Study was an open-label, phase IV, prospective, monocentric study at the Medical University of Graz which was scheduled between April 2021 and May 2024. The study compared different groups of people who got a vaccination against SARS- CoV-2 according to the Austrian vaccination plan. For this thesis two subgroups of the overall setting (which was comprised of haematological, autoimmune deficient patient groups and Covid-19 recovered and healthy participants as controls) were selected. In the first study arm, a total of 195 patients with primary or secondary immunodeficiency was planned to be included, in the second arm 195 healthy control people were considered for recruitment. Both groups underwent the same diagnostic procedures, which consisted of serology and analysis of the immune status. Figure 1 shows the final study cohorts of this thesis.

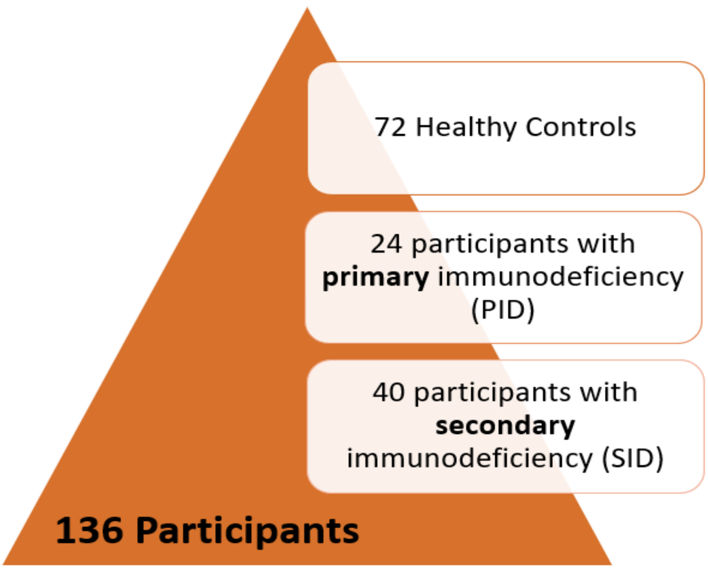


*Figure 1 Summary of the study cohort of this thesis*

The primary outcome measures were the levels of anti-SARS-CoV-2 spike antibodies, which were measured three to four weeks after the second vaccination by SARS-CoV-2 antigen-binding lg assay comparing immunocompromised patients to healthy controls. Also, the secondary outcome measures were of importance to this thesis, which included serum ACE2 Levels and body fat topography.

As figure 2 shows, the study participants had to take part in a total of 6 visits. The first visit took place before vaccination, the second up to two weeks after vaccination, the third three to four weeks after vaccination, and the last three at 6-month intervals starting after the second vaccination. The body fat assessment took place at the third visit.

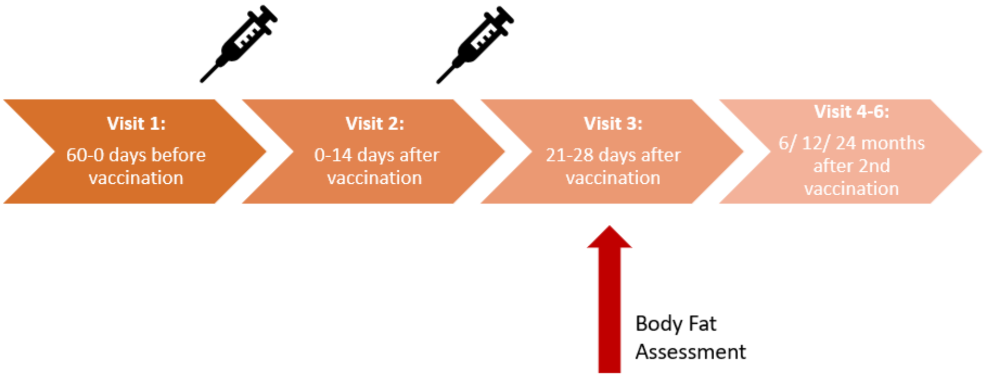


*Figure 2 The study timeline*

## *2.2 Ultrasound measurement of subcutaneous fat patterning*

The most accurate method to receive the exact amount of body fat, as well as the topography, is the standardized ultrasound technique of the subcutaneous adipose tissue by Müller et al. (Müller et al., 2016) Within this method, ultrasound pictures are taken at eight standardized sites, which are upper abdomen (UA), lower abdomen (LA), erector spinae (ES), distal triceps (DT), brachioradialis (BR), lateral thigh (LT), front thigh (FT) and medial calf (MC). Each site is exactly defined and marked before the ultrasounds are taken.

The points are measured in relation to the body size, the percentage, the body position during the marking and a reference muscle is standardized and stated in the description.

In addition to the body height and the exact marking points, other parameters are required for the precise calculation of the body fat mass and fat distribution. For this purpose, the body weight is captured and seat height, leg length and the circumference of the hips, waist, biceps and thighs are measured. The ethnicity of the participants and whether they are active in sports, and if so, how much, were recorded. The persons had to indicate how many hours they train per week and what their highest training level was (hobby sport, competitive sport, high performance sport). Although this information is not required for the calculation, it does allow statistical evaluations.

### 2.2.1 Marking

All points are marked in relation to body height, so the key anthropometric parameters of the study participants must be measured first. The marking is done on the right side if it is possible, if the left side is used it must be documented separately. For accurate marking a caliper, tape measure, measuring stick, a box to step on, table and an accessible wall are required.

1. UA: Upper abdomen is marked in standing position. 2% of body size is measured first from the center of the umbilicus to the right, then 2% upwards.
2. LA: Lower abdomen is determined the same as UA, except that the second 2% is measured downwards.
3. ES: To mark the erector spinae, the person must sit on a straight, solid surface (table) with the upper body extended, thighs horizontal and the legs unsupported. First 14% of the body height is measured upwards from the table, then 2% of the body height lateral to the spinous process of the vertebra.
4. DT: To mark the distal triceps, the participants must put the forearm on a hard straight surface with the hand in the mid prone position. Then measure 5% of the body height upwards on the most posterior aspect of the arm.

5. BR: To mark the brachioradialis, first the forearm must be in "Shake Hands Position" on a support table, then the brachioradialis can be contracted and the most anterior surface of the brachioradialis muscle can be marked. Then 2% of body height is measured distally from the anterior surface of the biceps brachii tendon.
6. FT: For Front thigh, the leg must be placed on an elevated surface, which is in front of a wall, with a right angle at the hip. The knees and toes must touch the wall. The point has to be marked at a horizontal distance of 14% of the body height from the wall.
7. MC: After marking FT, the knee and foot can be brought to a right angle. The marking should be 18 % of body height above the surface at the most medial aspect.
8. LT: The measuring point for Lateral thigh is located in the middle of the horizontal line between the lateral thigh and gluteal fold and is marked in standing position. (Müller et al., 2016)

### 2.2.2 Ultrasound Measurement

For the ultrasound images, it is important for the participants to be in the right position. All ultrasound measurements are done in a lying position.

1. For upper and lower abdomen, the participant has to lie in a supine position and stop breathing at mid-tidal expiration while capturing the image.
2. The picture of erector spinae has to be taken in a prone position.
3. For distal triceps, the participant has to lie in a prone position and the image has to be captured with the dorsal surface of the hand on the table, the probe orientation has to be perpendicular to the skin.
4. The brachioradialis image has to be taken in a supine lying position, the arm has to be in a mid-prone position and in contact with the thigh. The muscles of the arm should be relaxed, and it should be avoided to catch the vein in the vicinity.
5. Front thigh has to be done lying in a supine position.
6. For medial calf the participant has to be lying in a rotated position to the right side with the right knee bent at 90° angle so that the lateral aspect of the right led is supported.

7. For lateral thigh, the participant has to be lying in a rotated position to the left side, with both knees bent at a 90° angle, the right leg over the left leg.(Müller et al., 2016)

As well as the marking, also the ultrasound must be done in specific positions, to get comparable results. Since the procedure was primarily developed for athletes, in this study sometimes it was not possible for our patients to stay in certain positions. In these cases, the ultra-sounding was done in a position as close to the standard as possible and documented in the protocol.

To get ultrasound pictures, that can be edited with the specially developed software, each evaluable picture must include necessarily the skin, subcutaneous adipose tissue, muscle fascia and muscle, an example is shown in figure 3. Since the adipose tissue has a high compressibility, the ultrasound transducer has to be placed on a thick layer of gel over a certain site on the skin.

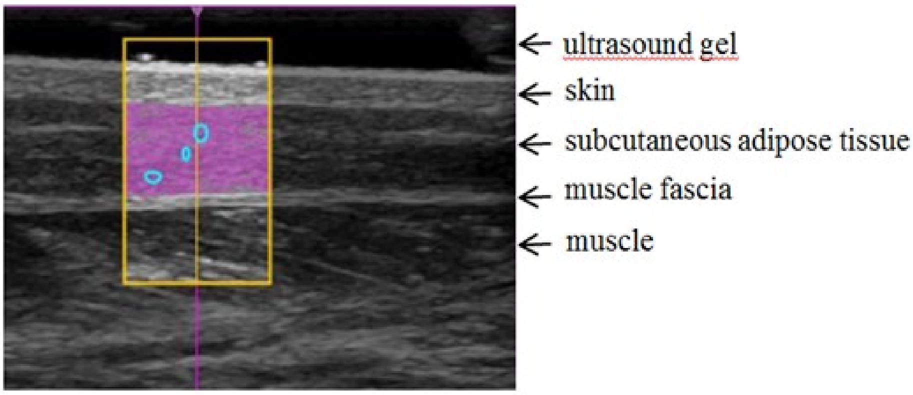


*Figure 3 shows an example of ultrasound image of the subcutaneous adipose tissue.*

A main advantage of the ultrasound technique is the possibility to remove the embedded fibrous structures of the measurement, which is shown in figure 4. Especially athletes and people doing sports regularly own a higher mount of these structures in their subcutaneous adipose tissue, but it would be wrong to calculate them to the body fat mass.

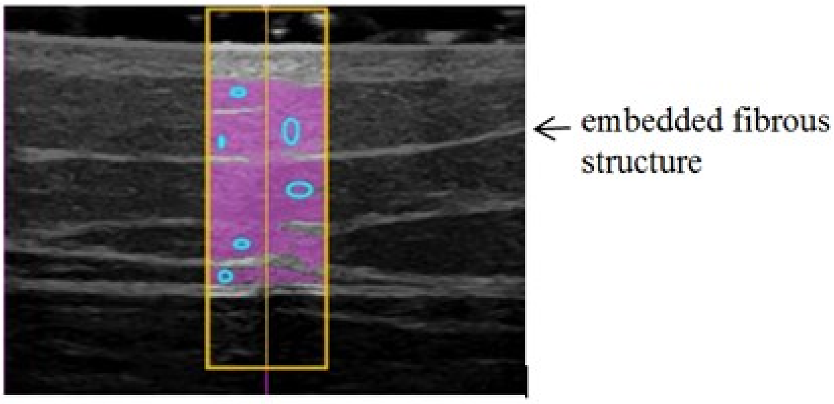


*Figure 4 shows an example of embedded fibrous structure*

The ultrasound investigations were performed with a conventional ultrasound system (GE Logiq-e, General Electric) and linear probes (L8-18i RS and 12L RS) operated at 8-16 MHz were used for the image generation.

### 2.2.3 Evaluation of ultrasound images

For the evaluation of the obtained ultrasound images the software NISOS body composition analysis v 4.1 (Rotosport, Stattegg Austria) was used. The software calculates the value $D_{INCL}$ (the sum of the eight measurement sites) and $D_{EXCL}$ (the sum of the eight measurement sites without the embedded fibrous structures).

The NISOS body composition analysis software is a special developed image editing software which is able to detect very minor fat mass changes. At first, the anthropometric information of the study protocol has to be transferred to the system, then each of the eight pictures per person has to be analyzed. At first, the useful area has to be selected, then the subcutaneous fat areas are marked. Here, the embedded fibrous structures are also identified by the software. The edge of the muscle fascia should be marked by the designation of the associated measuring point. After choosing the right areas, the program calculates the exact amount of subcutaneous fat from the marked areas together with the entered data.

The results of the image evaluation are illustrated into a document, which includes the BMI, the total amount of subcutaneous fat ($D_{INCL}$) including the fibres, excluding the fibres and the total amount of the fibres alone. This division is also calculated for each measured point and stated in the document, so the examined person gets information about the personal bodyfat topography. Additionally, information on the amount of SAT in Kilogram and percentage of total body weight is obtained from a certain algorithm.

## *2.3 Detection of anti-SARS-CoV-2 antibodies*

Before the patients and the healthy control persons got their first vaccination, and 21-28 days after they got their second vaccination, blood was obtained. Then two commercially available CE-certified serological tests (Immunoassays) were performed in batches to get the number of antibodies against SARS-CoV-2. In this thesis, I will focus on the Roche Immunoassay. Immunoassays are used to determine laboratory values through an antigen-antibody reaction. The starting point is always a monoclonal antibody that is as specific as possible for the target substance sought. The target molecule must be a potential antigen which is capable of generating an immune response. The detection limit can be lowered by connected detection reactions. For specific IgG determination, the Roche Elecsys anti-SARS-CoV-2 S electrochemiluminescence immunoassay was used, which is targeting the receptor-binding domain of the viral spike protein. As analytical unit a Cobas e 801 analytical unit (Roche Diagnostics GmbH, Mannheim, Germany) was used, which is a high throughput immunochemistry module, that performs a broad range of heterogeneous immunoassays using the highly innovative and patented ElectroChemiLuminescence technology. The quantification range lies between 0.4 and 2500 U/ml, the cut-off for positivity is 0.8 U/ml. (Schulz et al., 2021)

## *2.4 Measurement of ACE-2 concentrations*

Concentrations of circulating ACE-2 were measured using the Human ACE2 ELISA Kit made by ThermoFisher Scientific, which is able to analyze ACE2 in human serum, plasma or cell culture medium. It detects both, natural and recombinant human ACE2, using the sandwich method: it measures the amount of target molecule bound between a matched antibody pair. For this purpose, the microtiter plates are equipped with wells that are pre-coated with target-specific antibodies. The coated samples then bind to the immobilizing antibody, the sandwich is formed by adding the second detector antibody. A substrate solution is then added, which reacts with the enzyme-antibody-target complex. This reaction produces a signal that is directly proportional to the concentration of the target present in the sample and can be measured. (ThermoFisher Scientific)

## *2.5 Statistical analysis*

Statistical analyses were performed using SPSS Statistics v27 (IBM, Armonk, NY, USA). First, the distribution of the data was assessed by the Shapiro-Wilk-Test. Since most of the data was not normally distributed, non-parametric tests have been used for the statistical analyses. For group comparison the Mann-Whitney U test was applied. To calculate correlations between two variables scaled at least ordinally, Spearman's rank correlation coefficient was used. It indicates whether two variables are correlated, if so how strong this correlation is and in which direction it exists.

# 3 Results

## *3.1 Study Population*

To answer the question of whether body fat has an influence on the B-cell response of healthy and immunocompromised individuals, subcutaneous fat mass and immune response are correlated. The data was collected as part of the CoVVac study.
Data of 136 participants were included in the efficacy analysis. Of these, 72 were healthy participants (HC), 24 had a primary immunodeficiency (PID) and 40 had a secondary immunodeficiency (SID). Two participants with a primary immunodeficiency and all participants with a secondary immunodeficiency took B-cell depleting medication (rituximab or ocrelizumab).
We observed a significant difference in age (p= 0.011) between the group of participants with SID and the HC group, as well as a significant difference in BMI (p= 0.026) and waist circumference (p= 0.001) between the group of participants with PID and the HC group. Detailed participant characteristics are listed in Table 1.

| Variable | healthy (n= 72) | PID (n=24) | P HC-PID | SID (n=40) | P HC-SID |
|---|---|---|---|---|---|
| **Sex** | w=40<br>m=32 | w=13<br>m=10<br>d=1 | | w=28<br>m=12 | |
| **Age (years)** | 50 (21-64) | 55.5 (20-76) | 0.055 | 55 (27-76) | **0.011** |
| **BMI (kg/m²)** | 23.6 (18.1-38.7) | 25.6 (17.2-38.1) | **0.026** | 24.7 (17.5-41.6) | 0.202 |
| **Waist (m)** | 0.81 (0.63-1.08) | 0.91 (0.62-1.3) | **0.001** | 0.85 (0.63-1.3) | 0.242 |
| **Hip (m)** | 1 (0.85-1.27) | 1.01 (0.83-1.24) | 0.419 | 1.02 (0.86-1.23) | 0.098 |
| | | | | | |
| **Medikation** | | | | | |
| **none n (%)** | 100 (72) | 91,7 (22) | | 0 (0) | |
| **B-cell depleting therapy n (%)** | 0 (0) | 8,3 (2) | | 100 (40) | |

*Table 1: Study population characteristics*

## 3.2 Antibody levels in HCs/PID/SID

Among the three groups the antibody formation after vaccination differed significantly, as can be seen in figure 5. While in the group of healthy control subjects all reached a high titer (Md= 2500 U/ml, Min-Max= 1.131 – 2.500), the median of the Roche Anti-RBD total Abs was significantly lower in the primary immunodefiencies (Md= 1572 U/ml, p =<0.001) and secondary immunodefiencies (Md= 0, U/ml, p =<0.001). Since the clinical significance of antibody levels near the detection limit was unclear, this thesis adopted the term "stringent response" used in the publication associated with this study. The ”stringent response” was defined as Antibody level >1000 U/ml, which is also the lowest measured antibody level in a healthy control subject. Overall, all participants in the healthy control group showed this stringent response (100%), whereas only 15 of 24 participants in the group of individuals with PID (63%) and 4 of 40 participants in the group of individuals with SID (10%) were able to achieve this antibody level.

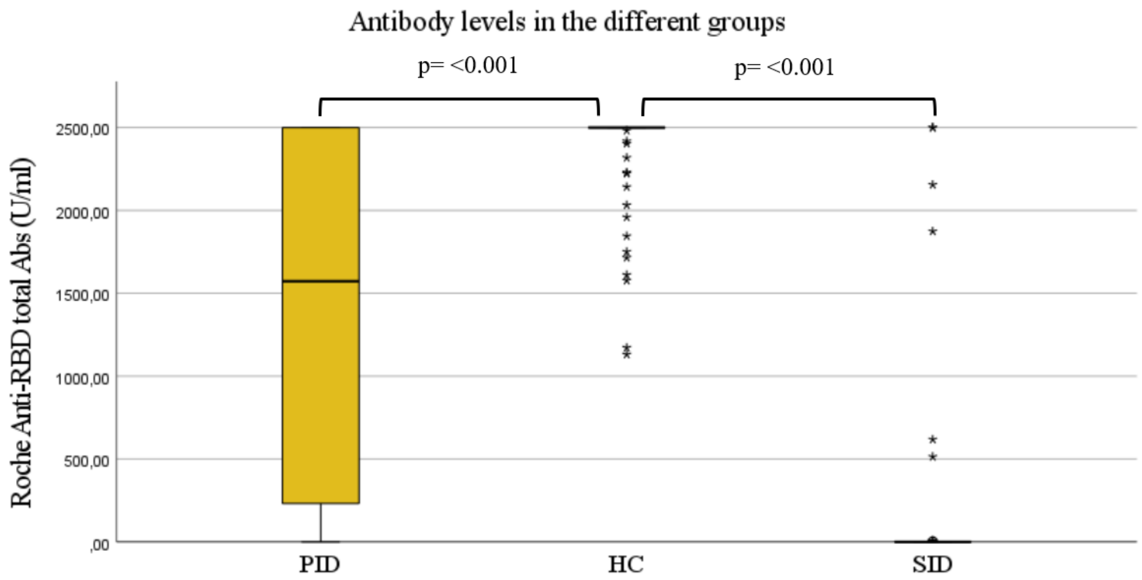


*Figure 5: Comparison of antibody levels, divided into persons with primary immunodefiencies, persons with secondary diseases and healthy control subjects. Boxplot shows a significant difference in the number of antibodies formed.*

### 3.2.1 Antibody levels were not correlated with age

In order to investigate if age does impact anti-SARS-CoV2 antibody formation in healthy individuals, the Spearman's rank correlation coefficient was used to calculate correlations between the two variables. The statistical analysis showed no significant correlation between age and antibodies formed, as showed in figure 6.

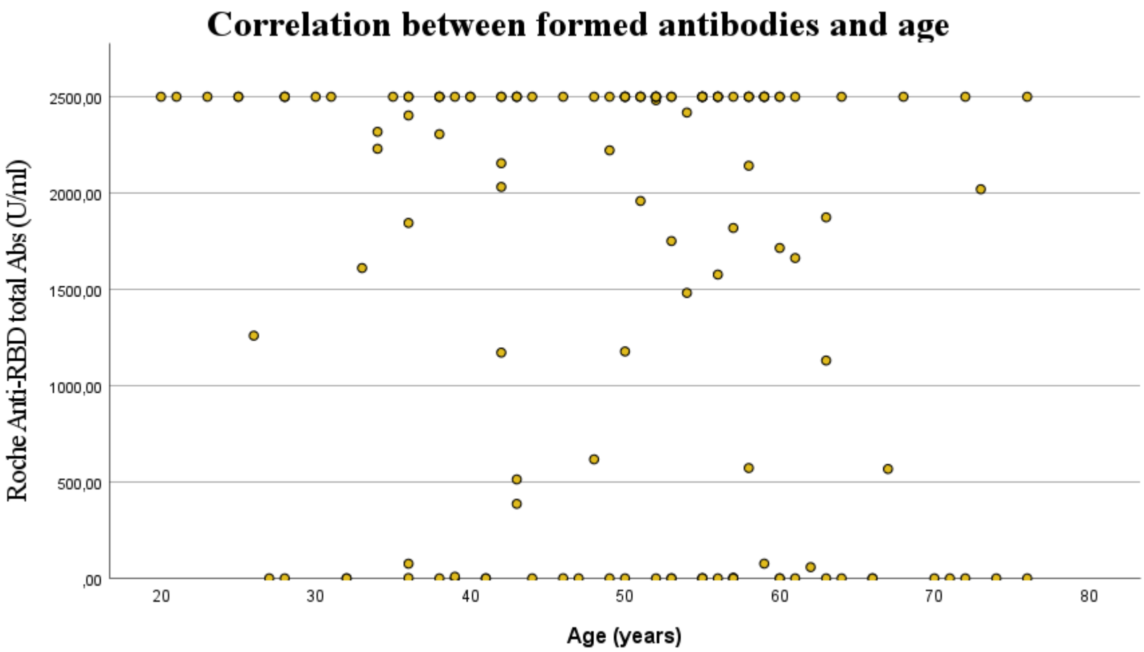


*Figure 6: Correlation between formed antibodies and age showed no significance*

### 3.2.2 Antibody level comparison between gender

Furthermore, we wanted to investigate if there is a difference in the antibodies formed between males and females. For this purpose, a group comparison was made with the Man-Whithney-U test. Figure 7 shows a comparison of the antibody levels in the different groups in a boxplot. Again, there was no significant difference found. Since only one person with the gender “diverse” participated in the study, this one person was removed for the gender analyses.

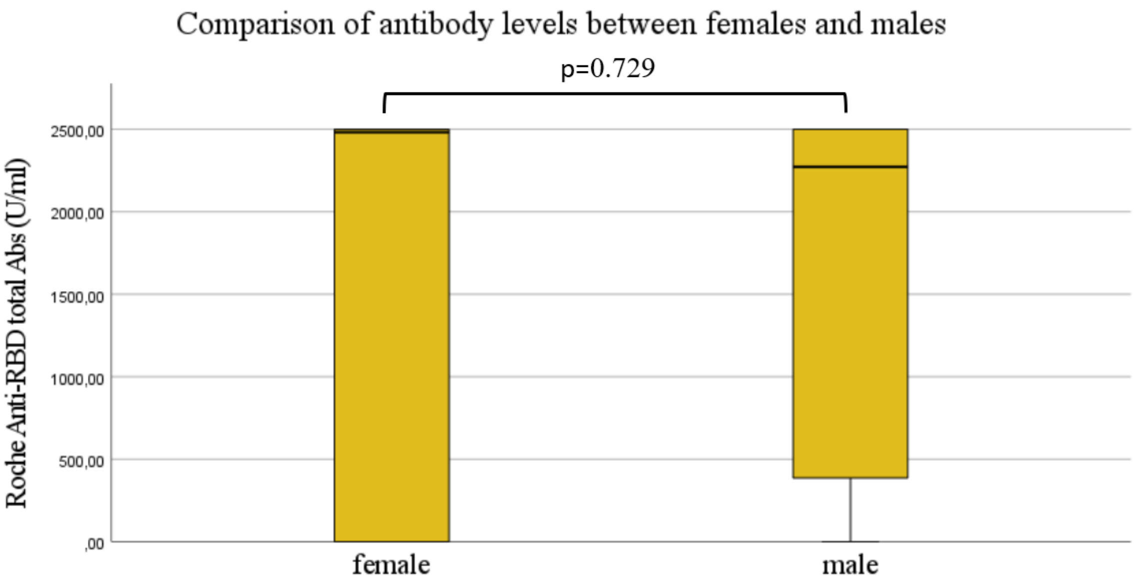


*Figure 7 boxplot show antibody levels in the groups female and male*

## *3.3 Body fat patterning in HCs/PID/SID*

### 3.3.1 Body fat levels correlated with age

To find out if there was a statistical relationship between body fat levels and age, Spearman's rank correlation test was performed between age and the various values of fat patterning. The correlations were calculated separately for the study groups PID, SID and HC.

As can be seen in figure 8, Spearman´s Test showed a significant correlation between age and BMI ($r_s(72)=0.254$, $p=0.032$) in the healthy group.

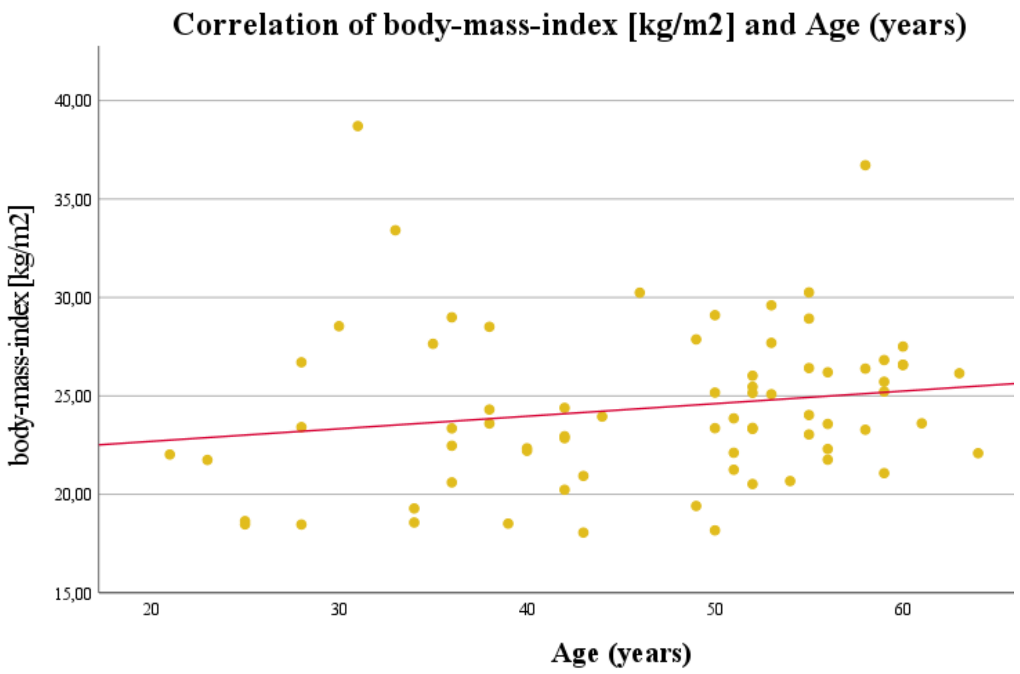


*Figure 8: Increasing BMI with age. Graph shows a correlation of BMI with age in healthy individuals.*

As figure 9 shows, we also observed a significant correlation between age and waist circumference ($r_s(72)=0.288$, $p=0.014$) in the healthy group. Both correlations suggest increasing body mass index and increasing waist circumference with increasing age. The analysis showed no significant correlations in the other groups, so the previously mentioned observations were not made analyzing the disease groups.

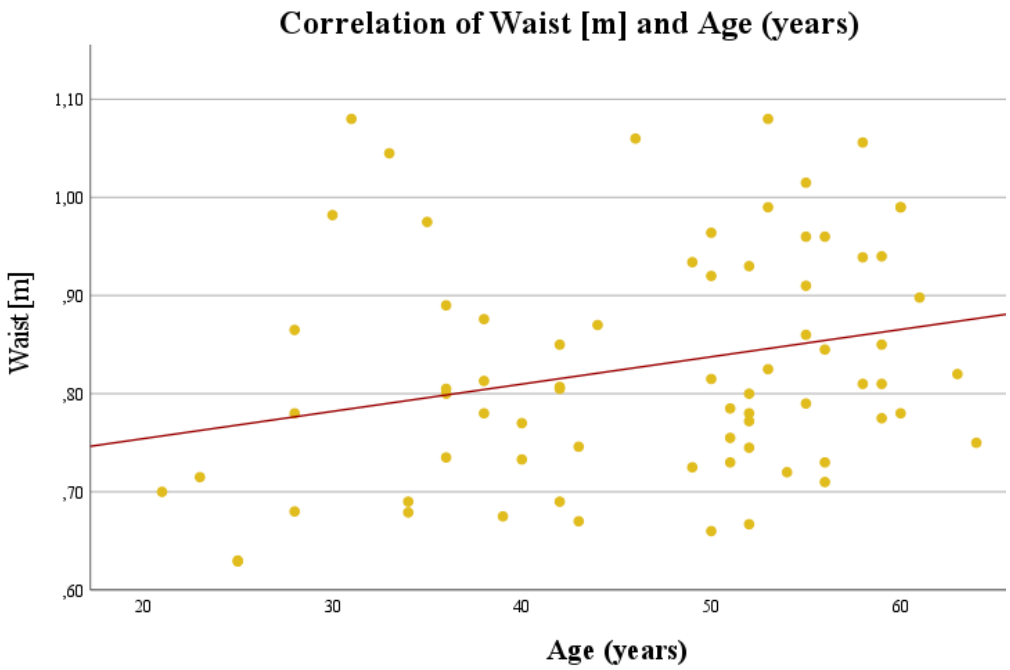


*Figure 9: Increasing waist circumference with age. Graph shows a correlation of waist circumference with age in healthy individuals.*

### 3.3.2 Comparison of body fat levels between genders

Next, we investigated if there is a difference in body fat levels between the genders. Gender was divided into male and female, then each group was compared to each other with the different values of fat patterning.

The test showed in the PID group a significant difference between the groups in the subcutaneous adipose tissue (SAT) extrapolated to body weight (p=0.008) and the measuring points Front thigh (p= 0.005), Lateral thigh (p=0.011), Medial (p= 0.017), Distal triceps (p= 0.030) and Brachio radialis (p=0.018). Figure 10 shows an example of a boxplot illustrating the difference in SAT between women and men.

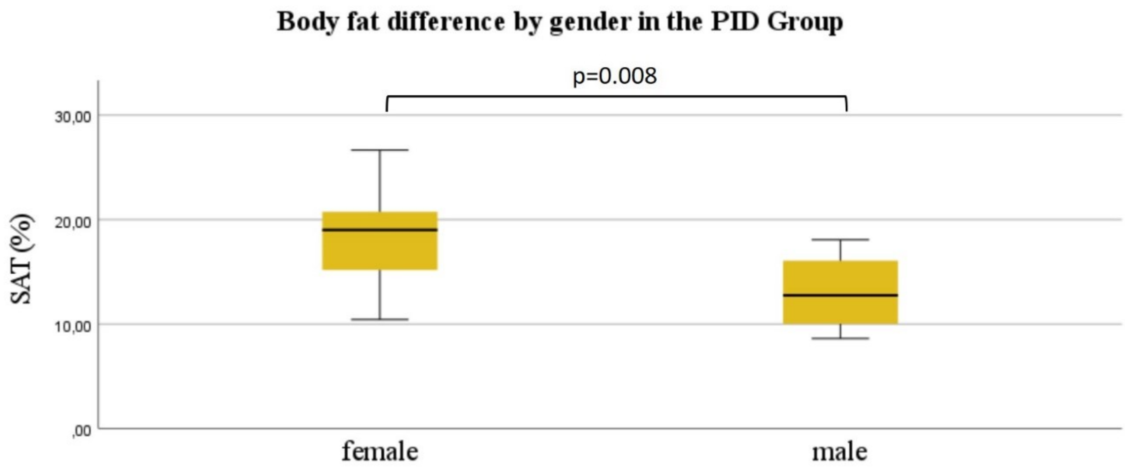


*Figure 10: Boxplot shows the difference in Bodyfat between females and males in the PID group*

In the SID Group, the test showed a significant difference between the gender-groups in Dincl (sum of the measured SAT distances at the eight sites including embedded structures) (p= 0.01), Dexcl (measured distance without embedded structures) (p=0.009), SAT extrapolated to body weight (p=<0.001) and the measuring points Front thigh (p= <0.001), Lateral thigh (p=<0.001), Medial calf (p= <0.001), Erector spinae (p= 0.007) and Distal triceps (p= <0.001). An Example is illustrated in figure 11, showing the difference in Dincl between males and females in a boxplot.

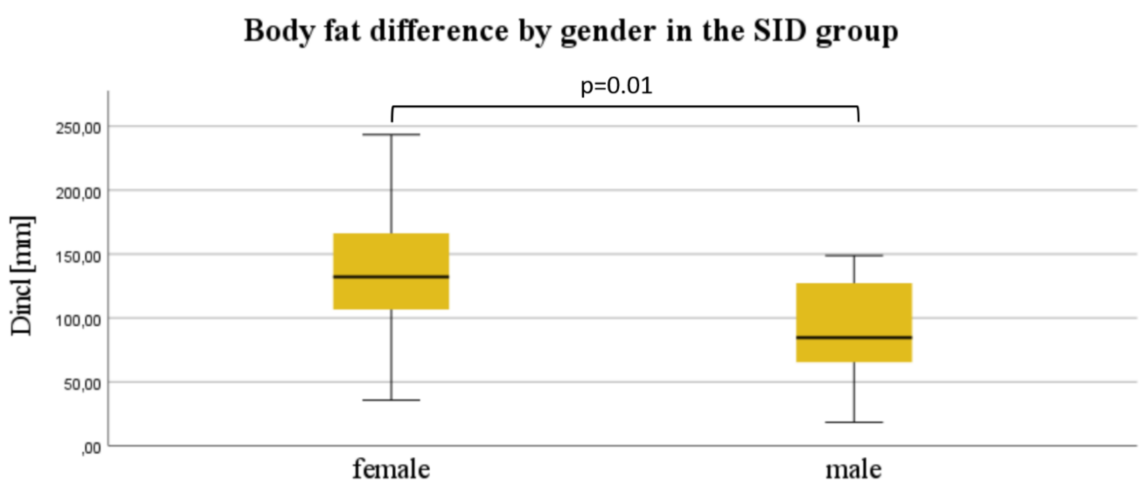


*Figure 11: Boxplot shows the difference in Bodyfat between females and males in the AID group*

In the HC Group, the gender difference in body fat levels was observed in Dincl (p=0.003) as shown in figure 12, but also in Dexcl (p=0.002), SAT (p=<0.001) and in the measuring points Front thigh (p= <0.001), Lateral thigh (p=<0.001), Medial calf (p= <0.001), Distal triceps (p= <0.001) and Brachio radialis (p=0.006).

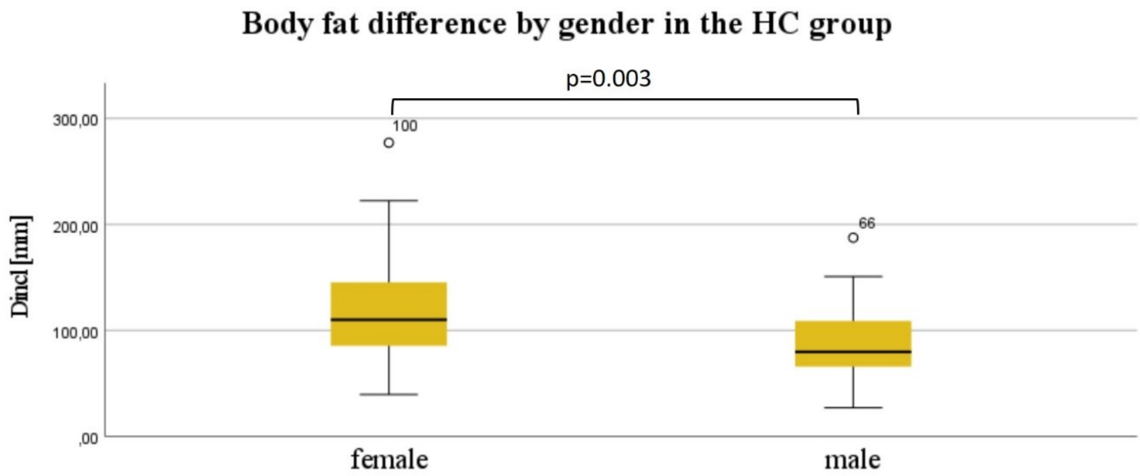


*Figure 12: Boxplot shows the difference in Bodyfat between females and males in the HC group*

### 3.3.3 Body fat patterning correlated with antibody levels

To find out if there is a correlation between antibodies formed and body fat distribution, the Spearman correlation test was used. The test showed that there is a significant correlation between antibodies formed and SAT ($r_s(22)= 0.433$, $p=0.044$) in the group of participants with a primary immunodeficiency. As figures 13 show, the number of antibodies formed increases with increasing SAT.

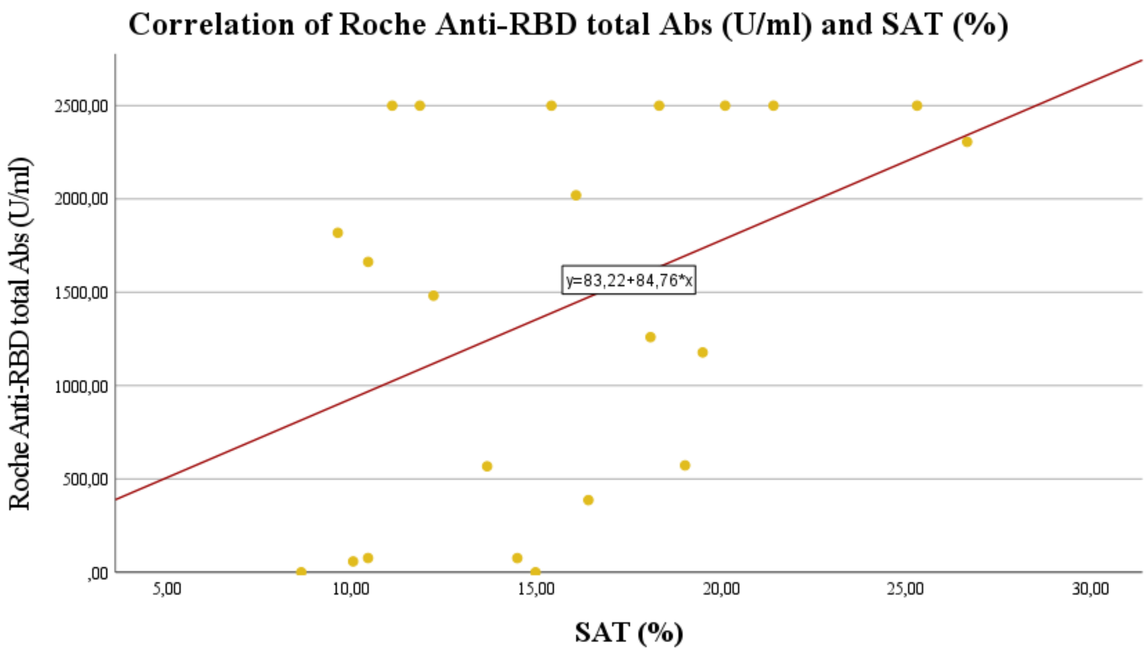


*Figure 13: Correlation between formed antibodies and SAT shows increasing antibodies with increasing SAT in PID*

The Spearman correlation test also showed a significant correlation between antibodies formed and Medial Calf (rs(22)=0.462, p=0.030) in the group of participants with a primary immunodeficiency. In figure 14 it can be seen that the number of antibodies formed increases with increasing medial calf.

In the remaining groups, there were no significant correlations between values of body fat patterning and antibodies formed.

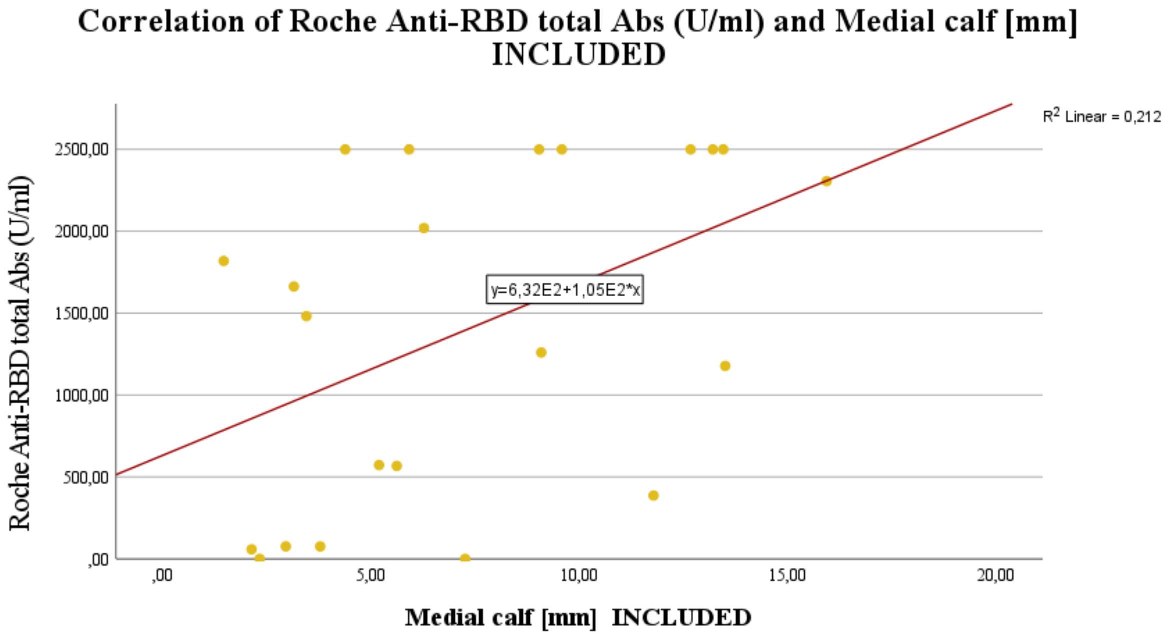


*Figure 14: Correlation between formed antibodies and medial calf (mm) included shows increasing antibodies with increasing medial calf (mm) included in the PID group*

## 3.4 Correlation of ACE2 Levels

### 3.4.1 ACE 2 levels are not correlated with age

In order to evaluate if there is a correlation between formed ACE 2 levels and age, the Spearman test was again used. As illustrated in figure 15, the test showed no significant correlation between age and ACE2 levels.

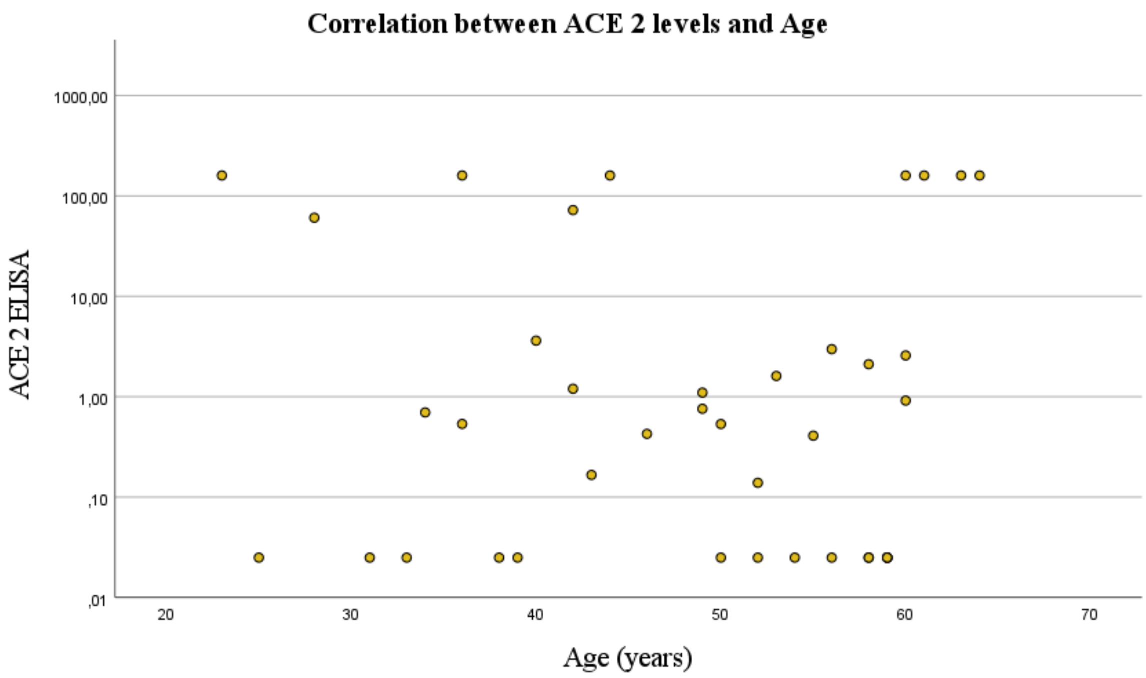


*Figure 15: Correlation between ace2 levels and age shows no significant correlation*

### 3.4.2 ACE2 levels compared between gender groups

In order to examine a potential link of ACE2 and age, we measured ACE2 levels in a subcohort of 40 healthy individuals. In contrast to the lack of correlation of ACE 2 levels and age, the Mann-Whitney U test revealed a significant difference between the ACE2 level of males and females (p=0.039). As the boxplot in figure 16 show, the ACE 2 levels are significantly higher in males than in females.

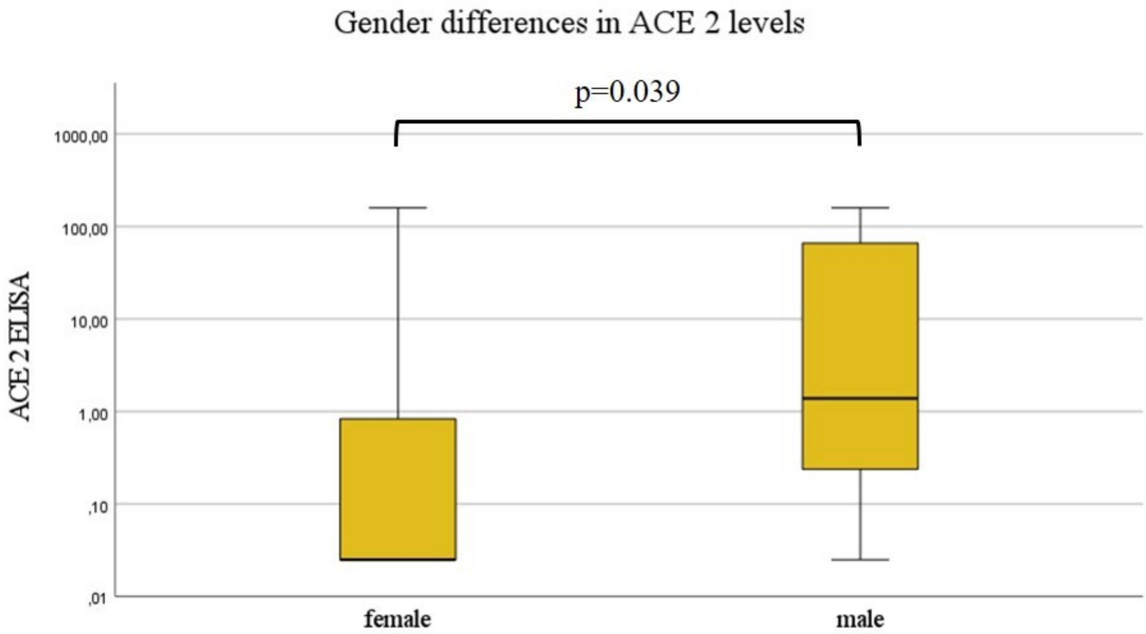


*Figure 16: Boxplot showing higher ACE2 levels in males than in females*

### 3.4.3 ACE2 levels correlated with body fat patterning

A connection of ACE2 levels and body mass was recently reported. (Freuer et al, 2021) To evaluate if there is a correlation between ACE2 levels and body fat distribution in our cohort, the Spearman test was applied to all measured values of body fat patterning with ACE2 levels. It showed, that there was no significant correlation between any value of body fat patterning and ACE 2 levels. As an example, the not significant correlation between ACE2 levels and Dincl (mm) is shown graphically in figure 17.

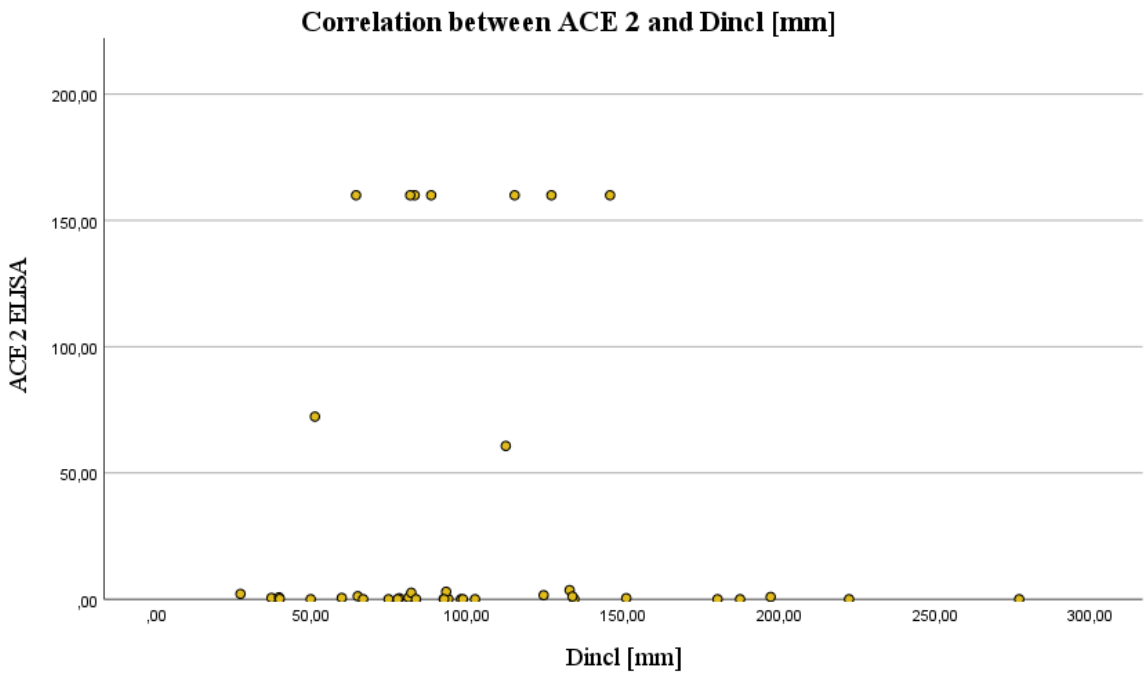


*Figure 17: Correlation between ACE2 levels and Dincl (mm) shows no significance*

### 3.4.4 ACE2 levels correlated with antibody levels

Finally, we wanted to find out if there was a correlation between the antibodies formed and the ACE2 levels measured in the healthy control group. As with ACE2 levels and body fat distribution, it showed no significant correlation between the number of antibodies formed and ACE 2 levels.

# 4 Discussion

The aim of my thesis was to investigate potential effects of elevated body fat mass on the B-cell response after Covid-19 vaccination in immunocompromised patients and healthy controls. It is already known that overweight people tend to have a more severe course of influenza infection, but have a poorer response to vaccination. (Honce, 2019) For Covid-19, more severe courses have also previously been observed in overweight individuals, but whether the immune response of overweight individuals is also altered after vaccination compared to normal weight individuals has not yet been explored. A connection between antibody formation and the ACE2 receptors, which are overexpressed in adipose tissue and through which Sars-CoV-2 enters the cell, has also not yet been investigated. In addition, a special aspect of this thesis is the comparison between individuals with immunodeficiencies and healthy controls.

## *4.1 Differences in B-cell response*

First of all, there was a significant difference in the antibodies formed between the groups. Since two out of three groups consisted of immunosuppressed people, this was to be expected. The question is whether the fat distribution or the immune deficit is the decisive reason. Since especially in the SID group, in which 100% of the participants receive B-cell depleting therapy, very few antibodies were formed, it is reasonable to assume that it was mainly the immunosuppression that affected the formation of the antibodies. There are comparative studies analyzing the immune response after Covid-19 vaccination between healthy individuals and individuals with B-cell depleting therapy, which also showed that patients with immunosuppressive therapy produced significantly fewer antibodies after vaccination than the healthy controls. (Mrak et al., 2021)
Presumably, it also makes a substantially difference in which time interval to the immunosuppressive therapy the vaccination was given, or whether immunosuppression was taken permanently, and which one. However, these points were not covered in my diploma thesis.

## 4.2 *Body fat patterning*

Obesity is a risk factor for severe progression of Covid-19, as well as many other diseases. Since preventive vaccination is particularly important for this reason, several studies have already been conducted on vaccination success in relation to BMI. The result is a lowered immune response for both hepatitis B vaccination and influenza vaccination. (Sheridan et al., 2012) Based on these previous results, the hypothesis arose to assume a correlation between body fat and immune response also for the Covid-19 vaccination.

According to the manufacturers of the mRNA vaccines Pfizer and Moderna, the vaccines show the same effectiveness in overweight people as in normal weight people. Previous studies on the Covid-19 vaccine have shown mixed results. Several studies indicate a good immune response in overweight people. (Nasr et al., 2022) At the same time, others show that obesity is associated with lower antibody production. (Pellini et al., 2021)

In this study, the hypothesis of a correlation between body fat patterning and antibody levels after Covid-19 vaccination could only be confirmed in one group, which was the PID group. Although it has previously been reported that overweight individuals produce fewer antibodies than normal weight individuals (Pellini et al., 2021), we observed in the PID group that the number of antibodies increases with increasing amount of subcutaneous adipose tissue. In all other groups we found no significant correlation between B-cell immune response and body fat patterning. Since the PID group was the only one in which there was a significant distribution of antibody levels, the very low dispersion of antibody levels in the HC and SID groups may also have been responsible for the fact that no meaningful correlations could be established with antibodies in these groups.

However, in order to examine this observed connection more precisely, it would be surely necessary to accomplish an investigation in a larger group, in which all constitution types are present.

## *4.3 ACE2 Levels*

It has already been researched that high ACE2 levels are found in adipose tissue. However, our measurements did not show any correlation between ACE2 levels and fat distribution. Interestingly, in a gender comparison we found that men had significantly more ACE2 receptors than women in serum. At the same time, our measurements clearly showed that females had more adipose tissue than males. Here, one must keep in mind that the comparisons were made between body fat patterning and ACE2 levels measured in blood. It is assumed that the ACE2 levels present in the blood have been released from the tissue, but it is not known for sure if the concentrations are the same. (Oudit and Pfeffer, 2020) The fact that men have more ACE2 receptors than women has been researched before. It has already been found out that ACE2 is also increased in spermatogonia, Leydig and Sertoli cells. (Wang and Xu, 2020) This could be one reason why men have in total more ACE2 receptors than women. It is also the subject of current research whether the higher number of ACE2 receptors causes men to suffer more severe covid-19 courses than women. (Oudit and Pfeffer, 2020)

## 4.4 Age differences

Between the groups, there were significant differences in age. These differences are partly due to the official vaccination schedule of the national government in Austria that was followed. According to this plan, those persons in the population who have a high risk of a severe course of the disease should be vaccinated first, especially older persons before younger ones. At the same time, the HC group consisted largely of healthcare workers, who were vaccinated earlier because of their occupational risk. As a result, PID and SID have a higher average age than the HC group.

As discussed above, the aging body changes in many places. Related to the topics of this thesis, among other things, the quality of the B-cell immune response deteriorates due to lower antibody production and reduced antibody affinity. (Djukic et al., 2014)
Interestingly, in our research the antibody levels were not correlated with the age of the participants. Although other studies have already shown that older people produce fewer and slower antibodies (Collier et al., 2021), this could not be confirmed in our study.
One possible reason for this difference is that in the healthy group the oldest person was at the age of 64 not excessively old, and in the other groups the antibody levels were influenced by immunosuppression in all age groups.

Another change in age concerns the muscle mass and the basal metabolic rate, which decreases with age. At the same time, the body fat percentage increases. There is also a change in fat distribution, with an accumulation of abdominal fat, as well as fat in the liver, bone marrow, skeletal and cardiac muscles. In general, there is a slow weight loss with age, which accelerates after age 75. Up to the age of 75, however, there is an increase in body fat mass despite weight loss. (Al-Sofiani et al., 2019)
The exact changes with age depend on gender, ethnicity, physical activity and daily caloric intake.

Summarized, in age the muscle mass becomes less and the basal metabolic rate decreases. At the same time, this increases the body fat percentage. This is exactly what our measurements demonstrated, which showed an increase in BMI and waist circumference with age. Consequently, age could also be the reason why we found higher BMI and Waist circumferences in PID and SID.

## *4.5 Gender differences*

Our study has shown once again that women have a higher percentage of subcutaneous fat than men. A healthy percentage of body fat has been described between 8 and 20% in young men and between 21-33% in young women. For both genders, this healthy percentage increases with age up to 36 % in women and up to 25 % in men.
(Gallagher et al., 2000)
The physiologically higher body fat percentage in women is necessary, since it is used as an energy depot, which is needed during pregnancy and lactation. Also, a chronic energy deficiency would lead to an impairment of the hypothalamic-pituitary-gonadal axis, which leads to hypothalamic anovulation in women. (Boutari et al., 2020)

Earlier, it has been described that Women have more subcutaneous adipose tissue, while men have more bodyfat distributed to the visceral adipose tissue around the abdominal organs. (Chang et al., 2018) Unfortunately, the exact proportion of visceral fat could not be determined in this study.

Another point which was investigated in my diploma thesis is the question whether there is a difference in the antibodies formed after vaccination between men and women. In previous studies, women usually had a stronger vaccination reaction and consequently higher antibody titers than men. The cause of this sex difference is thought to be differences in hormones, genetics, and microbiome. (Fischinger et al., 2019)
However, a 2021 study comparing immune response after mRNA vaccine between men and women failed to demonstrate this gender difference. (Wheeler et al., 2021)
The same result was obtained in our study, which showed no significant difference in the antibodies produced between men and women. Why the typical gender difference is not observed here is a point which could be further explored in future research.

# 5 Limitations

To ensure good comparability, study groups should be as similar as possible in certain characteristics. The age difference between the healthy control group and the groups with immunodeficiencies is therefore an important limitation, since older and younger people differ in many body characteristics that are investigated in this study, for example body fat percentage and immune response. Also, the heterogeneity in the PID and SID groups reduces the comparability.

The low distribution of antibody levels in the HC and SID groups was not ideal for statistical evaluations. In the HC group all subjects reached high levels, so it was unfortunately not useful to calculate comparisons with antibody levels and amount of body fat in this group. The same applies to the SID group, with the difference that almost all subjects reached very low antibody levels.

Another limitation is the fact that although we included a very large number of people in the study, the groups still become quite small when divided into further subgroups by age and gender. For an optimal group comparison, it is also questionable whether each group included enough people with high or low BMI.

It should also be noted that only mRNA vaccines were used in this study. Unfortunately, it cannot be determined from this research whether the results also apply to other vaccine types.

# 6 Conclusion

The results of our study could neither clearly rule out nor clearly confirm that there is a relationship between fat distribution and B-cell response after the Covid-19 vaccination. However, evidence for our hypothesis is found in the well-distributed PID group, so it would make sense to further explore these hypotheses in new studies containing larger, more comparable groups.

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