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METABOLISM, MORPHOLOGY, AND EFFECT OF FAT GRAFTS

Studies on Fat Graft Browning and Therapeutic Use in the Prevention of Peritoneal Adhesions

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To my family

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ERIKA HOPPELA: Metabolism, Morphology, and Effect of Fat Grafts – Studies on Fat Graft Browning and Therapeutic Use in the Prevention of Peritoneal Adhesions
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ABSTRACT

BACKGROUND: Fat transfer is a basic technique in the field of plastic surgery, but there is very little information about what happens to fat grafts after transfer. The aim of this thesis was to determine whether metabolically inactive white adipose tissue (WAT) used in fat grafts is transformed in the recipient area in the direction of thermogenic brown adipose tissue (BAT), referred to as beige adipose tissue (Study I and III). Utilizing the anti-inflammatory and tissue-healing properties of fat owing to abundant adipose-derived stem cells, we aimed to determine whether a fat graft can prevent the formation of adhesions in the abdominal cavity after surgery.

METHODS: In Study I, we investigated changes in subcutaneous and intramuscular WAT grafts using PET/CT ([18F]2-fluoro-2-deoxy-D-glucose, ^{18}F-FDG, as a tracer), histology, and BAT-related *Ucp1* gene expression in a mouse model. Using a mouse model, we also performed ^{18}F-FDG-PET/CT imaging, clinical analysis, histology, and macrophage phenotyping to determine whether fat graft transfer to the injured area of the peritoneum can prevent the formation of adhesions (Study II). In Study III, we investigated the metabolic activity of the fat graft area of patients who had previously received fat grafts using ^{18}F-FDG-PET/MRI imaging (in cold and warm conditions) and by harvesting tissue samples from the fat graft area.

RESULTS: In Study I, we found that some intramuscular fat grafts had transformed in terms of morphology and gene expression in the direction of BAT, as a sign of WAT browning. In Study II, we found that fewer adhesions were formed in the fat graft group and that they were looser in structure compared to the adhesion group without fat. In addition, the inflammatory activity was lower. In Study III, a cold-induced increase in glucose metabolism, typical of BAT, was observed in the fat graft areas in patients.

CONCLUSION: Fat grafts may transform into more metabolically active beige fat as a result of browning, which is indicated by both the tissue samples and the increase in *Ucp1* gene expression (Study I) and the metabolic changes after cold exposure detected by PET imaging (Study III). Fat grafts can prevent the formation of peritoneal adhesions in a mouse model (Study II).

KEYWORDS: fat graft, fat transfer, white adipose tissue, brown adipose tissue, beige adipose tissue, browning of fat, peritoneal adhesions

TURUN YLIOPISTO
Lääketieteellinen tiedekunta, Kliininen laitos
Kirurgia
ERIKA HOPPELA: Rasvasiirteiden aineenvaihdunta, morfologia ja vaikutus – tutkimuksia rasvasiirteen ruskistumisesta ja terapeuttisesta käytöstä vatsaontelon kiinnikkeiden ehkäisyssä
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TIIVISTELMÄ

TAUSTA: Rasvansiirrot ovat plastiikkakirurgian alan perustekniikoita, mutta rasvan ominaisuuksia siirron jälkeen tunnetaan huonosti. Väitöskirjan tavoitteena oli selvittää, muuntuuko siirteissä käytetty inaktiivinen valkea rasva kohdealueella aktiivisemman, lämmönsäätelyyn osallistuvan ruskean rasvan suuntaan (ns. beige rasva) (osatyöt I ja III). Rasvan tulehdusta hillitseviä ja kudoksen paranemista edistäviä ominaisuuksia hyödyntäen pyrimme selvittämään, voidaanko rasvasiirteellä ehkäistä leikkauksen jälkeistä vatsaontelon kiinnikemuodostusta (osatyö II).

MENETELMÄT: Osatyössä I tutkittiin ihonalaiskudokseen ja lihakseen siirretyn rasvan muutoksia koe-eläinhiirillä PET/CT-kuvauksella (merkkiaineena [18F]2-fluoro-2-deoxy-D-glukoosi, ^{18}F-FDG), kudosanalyysillä ja ruskealle rasvalle tyypillisen *Ucp1*-geenin ilmentymisen avulla. Osatyössä II selvitettiin ^{18}F-FDG-PET/CT-kuvauksella, kliinisellä kiinnikkeiden arvioinnilla, kudosanalyysillä ja makrofagi-määrityksellä, voidaanko vatsakalvon vaurioalueelle siirretyllä rasvalla ehkäistä koe-eläinhiirien vatsaontelon kiinnike-muodostusta ja hillitä tulehdusreaktiota. Osatyössä III tutkimme aiemmin rasvasiirteitä saaneiden potilaiden rasvasiirre-alueen aineenvaihdunnan aktiivisuutta ^{18}F-FDG-PET/MRI-kuvauksella (sekä kyl-mässä että lämpimässä) sekä keräämällä rasvasiirrealueelta kudosnäytteitä.

TULOKSET: Osalla hiiristä lihaksen rasvasiirre oli solurakenteeltaan ja geenien ilmentymiseltään muuntautunut ruskean rasvan suuntaan merkkinä valkean rasvan ruskistumisesta (osatyö I). Kiinnikkeiden muodostus oli vähäisempää rasvasiirreryhmässä ja ne olivat löyhempiä koostumukseltaan verrattuna kiinnikeryhmään ilman rasvaa. Lisäksi tulehdusaktiviteetti oli rasvasiirreryhmissä vähäisempi (osatyö II). Potilaiden rasvasiirrealueilla todettiin kylmäaltistuksessa ruskealle rasvalle tyypillistä glukoosiaineenvaihdunnan lisääntymistä (osatyö III).

JOHTOPÄÄTÖKSET: Rasvansiirteet saattavat muuntua beigeksi rasvaksi, mihin viittaa sekä kudosnäytteet ja *Ucp1*-geenin ilmentyminen (osatyö I) että PET-kuvauksella todetut aineenvaihdunnalliset muutokset (osatyö III). Rasvasiirteillä pystytään ehkäisemään vatsaontelon kiinnikkeiden muodostumista (osatyö II).

AVAINSANAT: rasvasiirre, rasvansiirto, valkoinen rasva, ruskea rasva, beige rasva, ruskistuminen, vatsaontelon kiinnikkeet

Table of Contents

Abbreviations

ADSCs	adipose-derived stem cells
ASG	autologous fat grafting
ATMs	adipose tissue macrophages
ATP	adenosine triphosphate
BAT	brown adipose tissue
BMI	body mass index
CAL	cell-assisted lipotransfer
CT	computed tomography
^{18}F-FDG	[18F]2-fluoro-2-deoxy-D-glucose
GU	glucose uptake
H&E	hematoxylin and eosin
IF	immunofluorescence
IL	interleukin
MRI	magnetic resonance imaging
MSCs	mesenchymal stem cells
NSAID	non-steroidal anti-inflammatory drug
PET	positron emission tomography
PMCs	peritoneal mesothelial cells
PRP	platelet-rich plasma
SUV	standardized uptake value
TNF-α	tumor necrosis factor alpha
tPA	tissue-type plasminogen activator
UCP1	uncoupling protein-1
uPA	urokinase-type plasminogen
VOI	volume of interest
VEGF	vascular endothelial growth factor
WAT	white adipose tissue

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Erika Hoppela, Tove J. Grönroos, Tomi V. Tervala, Katri Kivinen, Susanna Kauhanen, Pirjo Nuutila, Anne Saarikko, Pauliina Hartiala. Fat grafting can induce browning of white adipose tissue. *Plast Reconstr Surg Glob Open* 2018;6:e1804.

II Mervi Laukka*, Erika Hoppela*, Jemiina Salo, Pia Rantakari, Tove J. Grönroos, Katri Orte, Kaisa Auvinen, Marko Salmi, Heidi Gerke, Kerstin Thol, Emilia Peuhu, Saila Kauhanen, Pirjo Merilahti, Pauliina Hartiala. Preperitoneal Fat Grafting Inhibits the Formation of Intra-abdominal Adhesions in Mice. *J Gastrointest Surg* (2020) 24:2838–2848.

III Erika Hoppela, Katri Orte, Susanna Kauhanen, Terhi Tuokkola, Kirsi A. Virtanen, Pirjo Nuutila, Anne Saarikko, Pauliina Hartiala. Glucose metabolism of free white adipose tissue fat grafts changes towards beige adipose tissue. *Manuscript submitted for publication.*

*These authors contributed equally to this work.

1 Introduction

Fat grafting is a basic procedure in plastic surgery with a history of more than hundred years. First, fat transfer was used as a method for volume replacement and for aesthetic purposes with modest equipment and large cannulas, but the understanding of graft survival, the aim for a better cosmetic result, and the development of technology have resulted in the current principles of liposuction and fat transfer. Since adipose-derived stem cells were discovered in 2001, the use of fat grafting has expanded from body contouring and volume transfer to scar treatment, wound healing, and rejuvenation (Shauly, Gould and Ghavami, 2022).

Adipose tissue is not only the body's energy storage, but also an important endocrine organ and part of the thermoregulation system. The main type of adipose tissue is white adipose tissue (WAT), which stores excess energy in the form of triglycerides, and is metabolically inactive. WAT is an abundant and accessible source of fat for grafting, particularly in the subcutaneous spaces of the abdomen and thighs. Therefore, it is widely used by plastic surgeons. Brown adipose tissue (BAT), on the other hand, is a metabolically active type of adipose tissue located e.g. in supraclavicular and paravertebral areas in small amounts. It is activated by external stimuli, such as cold exposure, causing BAT to consume energy and produce heat, thereby increasing overall energy expenditure (Cypess, 2022). Long-term exposure to similar stimuli can also lead to morphological and metabolic changes in WAT towards BAT, transforming this inactive adipose tissue into more active beige fat in a process commonly known as ‘browning’ (Harms and Seale, 2013).

The growing challenge of obesity and metabolic disorders has increased the research on the metabolic properties of fat, and during the past decade several factors that may influence WAT browning have been discovered, including cold, thyroid hormones, exercise, and diet (Cheng *et al.*, 2021). Previous experimental studies have shown that fat grafting influences the morphology and metabolism of transferred fat through ischemia, adipocyte necrosis, inflammatory reactions, and neovascularization (Qiu *et al.*, 2014, 2018; Sakamoto *et al.*, 2016; Machida *et al.*, 2018; Liu *et al.*, 2021b, 2021a). Although fat grafting is a common procedure, the resulting changes in the WAT grafts remain poorly understood.

Adipose tissue is mainly composed of adipocytes, but it also contains a variable number of other cells and structures, such as mesothelial cells, nerves, lymphatics, and vascular structures (Emont *et al.*, 2022). When these components are removed in terms of centrifugation and collagenase, the result is stromal vascular fraction (SVF), which is a mix of ADSCs, endothelial precursor cells, T regulatory cells, macrophages, smooth muscle cells, pericytes, and preadipocytes (Nguyen *et al.*, 2016). SVF, especially ADSCs, has pro-healing and immunomodulatory effects, and is used for e.g. the treatment of scars and chronic wounds (Guo *et al.*, 2016). In addition, peritoneal adhesions have a structure similar to that of a scar, but the effect of fat grafts on the prevention and treatment of adhesions has not been studied. Peritoneal adhesions are a common result of peritoneal injury due to abdominal surgery, trauma, or infection, in which the formation of fibrin increases, and the balancing effect of fibrinolysis fails, eventually leading to the formation of fibrin bands between the bowel, omentum, and peritoneal wall (Wang, Guo and Li, 2022), causing e.g. intestinal occlusions, chronic pain, or female infertility (Arung, Meurisse and Detry, 2011).

The aim of this dissertation was to investigate whether the metabolism and morphology of free adipose tissue grafts change towards beige adipose tissue as a result of browning in both mouse (study I) and human (study III) models using [18F]2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET imaging and immunohisto-chemical analysis as the main methods. We also aimed to study the effects of fat grafts on adhesion formation by examining changes in the inflammatory process, mesothelial cell healing, and adhesion structure after controlled peritoneal injury using a mouse model and comparing the results between the fat graft group and the adhesion group without fat.

2 Review of the Literature

2.1 Fat grafts in plastic surgery

Liposuction and lipotransfer are among the most popular procedures in plastic surgery worldwide and are widely used in both reconstructive and aesthetic surgery under various conditions. Similar to all surgical procedures, fat transfer has its own developmental curve, which has led to its popularity as a quick, cost-effective, and safe procedure for body contouring, volume transfer, wound healing, and scar treatment.

2.1.1 History of fat grafting

The history of fat grafting (as illustrated in **Figure 1**) began in 1893 when Gustav Neuber made the first lipotransfer recorded in literature by transferring autologous adipose tissue from the forearm into the face for orbital scar correction caused by osteomyelitis (Neuber, 1893; Shauly, Gould and Ghavami, 2022). About the same time in 1895, Vincent Czerny transplanted a large lipoma into the patient's breast to compensate for the volume loss from a unilateral partial mastectomy (Czerny, 1895; Costanzo, Romeo and Marena, 2022; Shauly, Gould and Ghavami, 2022), both of which had good aesthetic results. Fat grafts were first used for cosmetic indications in 1910 by Erich Lexer, who augmented the malar area to fill facial wrinkles (Lexer, 1910; Costanzo, Romeo and Marena, 2022). The first fat grafting using a needle and syringe was performed in 1911 by Brunning, who transferred autologous adipose tissue to the subcutaneous tissue of the nasal area for aesthetic purposes after rhinoplasty (Brunning and Broeckaert, 1914; Shauly, Gould and Ghavami, 2022). In addition, Eugene Holländer was one of the first surgeons to transplant fat via injections. To reduce fat reabsorption, he mixed human fat and hard fat from rams with a rather good outcome. The injection technique was later modified by Miller, who used metal cannulas (Miller, 1926; Shauly, Gould and Ghavami, 2022).

During the late 19th and early 20th centuries, facial contouring and fillings were mostly achieved by paraffin injections, but due to multiple complications, such as infections, chronic inflammation, drainage, and necrosis (Stein, 1904; Costanzo, Romeo and Marena, 2022), the development of autologous fat grafting was initiated.

The initial period in the discovery and introduction of fat grafting was referred to as the "the open surgery" period (1889–1977) where fat was transplanted en bloc (Varghese and Mosahebi, 2017). Already during the first procedures mentioned above, a high rate of fat resorption, oil cysts, and fibrosis formation was noted, and consequently, unreliable and asymmetrical results, which limited the use of fat in procedures.

The invention of liposuction started the second period (1978–1994) in the history of fat grafting. In 1975, Ficher father and son developed liposuction method using blunt metal cannulas (Fischer and Fischer, 1976). Using the same technique, more efficient equipment, and smaller cannulas, Illouz published a description of the method in 1977 (Illouz and Illouz, 1983), and took the development of liposuction to a new level. Illouz began using unpurified lipoaspirate for transplantation in 1983 (Illouz, 1986).

Understanding graft survival and aiming for a better cosmetic outcome drove the development forward, and more attention was paid to the gentle handling of adipose tissue at both the donor and recipient sites. The third and current period, known as "the atraumatic period and regenerative medicine" has been considered to begin in 1994 after Sidney Coleman published his description of "the Coleman technique" (Coleman, 1994), a method for harvesting adipose tissue with a blunt multihole cannula at lower pressures to reduce cell damage, processing the lipoaspirate using a centrifuge, and placement of grafts by injecting small parcels of pure fat to maximize the surface area of contact between the graft and recipient site.

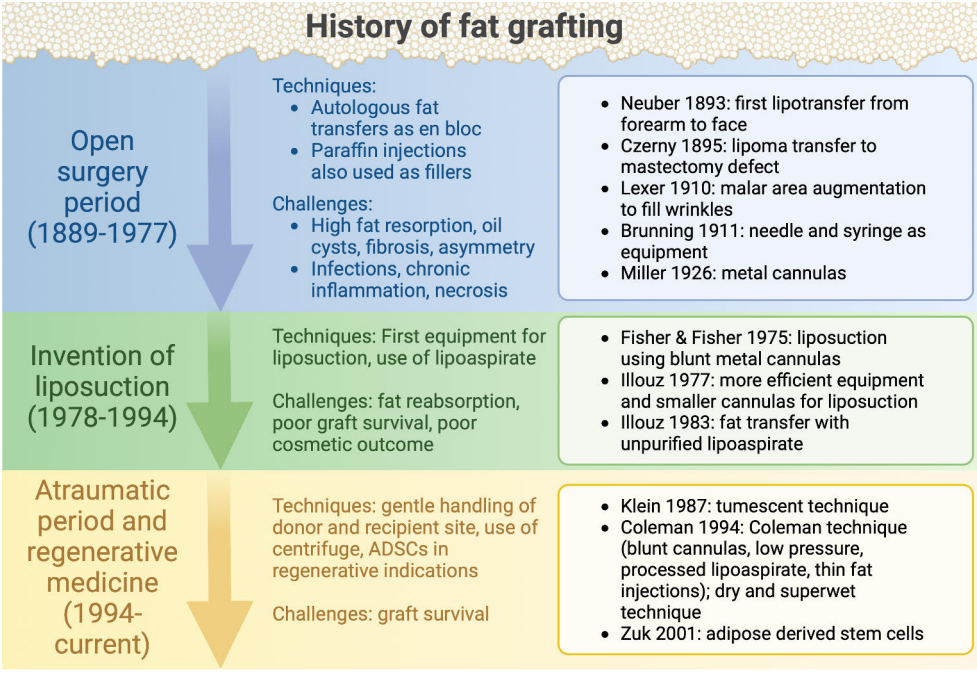

Figure 1 The history of fat grafting. *The author's own illustration, created using Biorender.com.*

The first liposuctions Coleman performed with a dry technique, but later used a superwet technique with epinephrine and lidocaine added to the solution (Egro *et al.*, 2022). The tumescent liposuction technique, which remains the most popular technique used by plastic surgeons, was first presented by Klein et al. in 1987 to minimize perioperative bleeding and postsurgical irregularities and enable safe liposuction for large volumes (Klein, 1987).

Adipose-derived stem cells (ADSCs) were first discovered by Zuk et al. in 2001 (Zuk *et al.*, 2001), which expanded the field of research on adipose tissue and regenerative medicine, and the possibility of using fat for new therapeutic indications, such as wound healing.

2.1.2 Technique: liposuction and lipotransfer

Liposuction is a surgical procedure in which the deep or intermediate layer of subcutaneous adipose tissue is removed by aspiration cannulas through small skin incisions assisted by suction. The synonyms include lipectomy, lipoplasty, fat suction, and liposculpture. It is typically performed for body contouring and/or fat harvesting. The adipose tissue commonly used for harvesting is located in the subcutaneous tissue of the abdomen, flanks, or inner thighs, which offers an easy and safe approach. Donor-site selection is based on the availability of adipose tissue and the patient's and surgeon's preferences. Other areas for liposuction include the brachial and antebrachial regions, outer thighs, knees, legs, dorsal region, neck, and the chin.

The technique is chosen according to the indication of the procedure and the desired volume to be removed and/or transferred. Liposuction is usually performed using the tumescent technique (developed by Klein, as mentioned earlier), which involves infiltrating tumescent fluid before liposuction, thus preparing the donor site. "Klein solution" includes crystalloid fluid containing a small amount of lidocaine and epinephrine, permitting liposuction under local anesthesia, and minimizing blood loss. The liposuction technique was further modified using suction devices that work with the assistance of power, laser, ultrasound, radiofrequency, or water; however, the tumescent technique assisted by negative-pressure suction remains the most popular among plastic surgeons (Simonacci *et al.*, 2019). The procedure may also be performed using a dry technique (often using a torniquet to reduce bleeding) under general anesthesia, which may be a viable option e.g. for upper or lower extremity liposuction in lymphedema patients (Wojnikow, Malm and Brorson, 2007). Small amounts of fat can be harvested for grafting using only a cannula and syringe, generating low negative pressure (Chia, Neinstein and Theodorou, 2017). Cannulas also play an important role in procedural success. Several studies have investigated the differences between cannulas and have shown that a larger-diameter cannula enhances cell viability (Strong *et al.*, 2015).

Refinement of the lipoaspirate depends on the recipient site and purpose of use. As Coleman suggested in his technique in 1994, the lipoaspirate is allowed to decant (meaning gravity separation) or centrifuged to separate the oil, fat, blood, and infiltered solution, after which the purified fat can be collected (Coleman, 1994; Egro *et al.*, 2022) and further processed into macro-, micro-, or nanofat, which are defined according to the size of the fat particles. Other less common processing methods for lipoaspirates include filtration, gauze rolling, and washing (Strong *et al.*, 2015). The desired fineness of fat is usually already considered when choosing liposuction cannulas with a certain diameter and hole size. The diameter of the cannulas produces fats as follows: large cannulas over 2.4 mm in diameter produce conventional macrofat, and cannulas ranging from 1.2 mm to 2.4 mm produce microfat. Nanofat is refined from microfat using a 400–600 µm emulsifier, whereas microfat is processed with a hole diameter of 1.2 mm emulsifier. Nanofat is further processed using the Tonnard technique by mechanical shuffling and filtration of microfat (Tonnard *et al.*, 2013; Ding *et al.*, 2022).

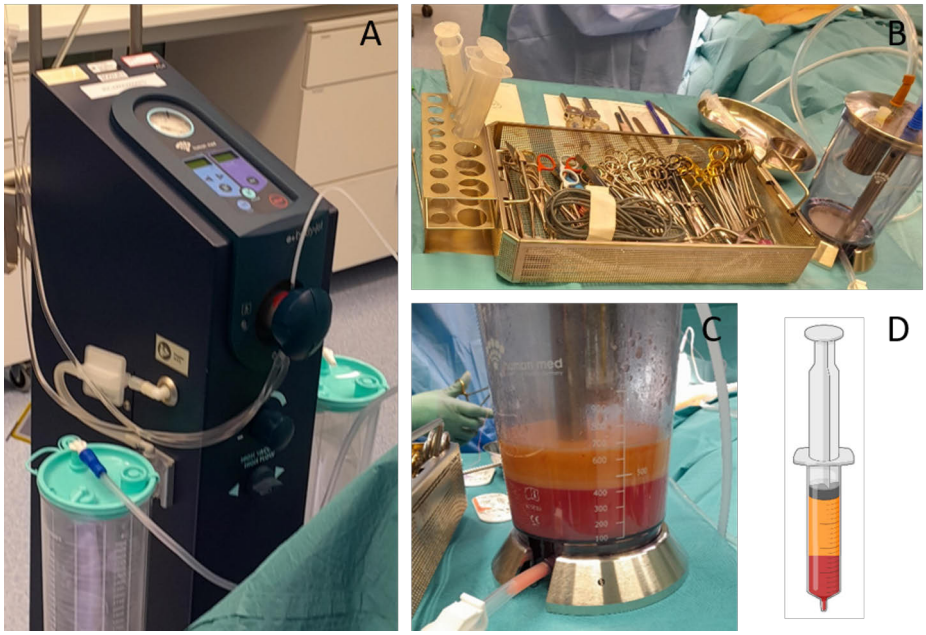

Figure 2 The liposuction protocol at Turku University Hospital. Large fat transfer volumes are harvested using a water-assisted liposuction device Body-jet® (Human Med AG, Schwerin, Germany) (**A**) and standard surgical instruments (**B**). The lipoaspirate components are separated by gravity first in a container (**C**) and later in large 100 mL syringes (**D**). Subsequently, the resulting fat layer is collected into smaller 10 mL transfer syringes. *The author's own photographs, originally taken by Oskari Kekki. Image D created using BioRender.com.*

The most revolutionary part of Coleman's technique was fat transfer, that is, a procedure (lipotransfer, lipofilling) in which harvested adipose tissue is placed in the recipient tissue through small skin incisions. By placing fat in small parcels among vascularized tissues (subcutaneous, muscle, and subdermal tissues), fat revascularization and graft survival can be improved. The basic technique for macrofat grafting is injection with a blunt needle 2 mm or more in diameter (Ding *et al.*, 2022), and the needle shape is chosen based on the surgeon's preference.

Injections are performed meticulously as diffusely as possible in three dimensions with a 0.1-ml/cm limit of graft delivery, using multiple sprinkler heads, each delivering a fine mist in all planes and directions (Khouri and Khouri, 2017). A needle of less than 1.2 mm is used for microfat grafting, and a 400–600 μm parcel diameter is used for nanofat grafting (Ding *et al.*, 2022). Macrofat is typically used in large volume transfers e.g. to breasts and buttocks, microfat for facial fat transfers and nanofat for tissue regeneration in therapeutic use and rejuvenating.

2.1.3 Graft survival

The basis of the graft survival theory is the cell survival theory created by Peer in the early 1950s, where he proposed based on his experimental study that human autologous fat grafts survived as adipose tissue after receiving blood circulation and lost an average of approximately 45% of their weight and volume through degeneration and elimination a year or more after transfer (Peer, 1950; Pu, 2016). This theory was later refined by Carbanera and Ribeiro, who reported that the percentage of graft viability depends on the thickness and geometrical shape and is inversely proportional to the graft diameter if grafts with a diameter greater than 3 mm are considered (Carpaneda and Ribeiro, 1994).

In practice, the survival of fat grafts is influenced by the quality of the transplanted fat, the injection technique, and the characteristics of the recipient site. As described earlier, fat should be harvested as atraumatically as possible to obtain viable fat cells, and then transferred into small parcels. However, preparation of the donor site has only a slight effect on fat quality. Donor location and lipoaspirate centrifugation have no significant effects on adipocyte viability or volume retention (Rohrich, Sorokin and Brown, 2004; Strong *et al.*, 2015). The tumescent technique seems to improve the quality of lipoaspirate compared with the dry technique, but the difference was not statistically significant (Agostini *et al.*, 2012). Keck et al. investigated the effect of local anesthesia on the viability of adipocytes and found that epinephrine and lidocaine impaired the quality of viable preadipocytes, as determined by their ability to differentiate into mature adipocytes (Keck *et al.*, 2010).

The recipient site should be well vascularized and capable of stretching. The capacity of the receiving area for fat grafting determines the total fat transfer volume,

and the procedure balances graft survival with optimal augmentation. External volume expansion (e.g. expansion device Brava, Brava LLC, Miami, Fla.) can induce adipogenesis, enhance tissue vascularity, and increase recipient capacity and mechanical compliance (Khouri and Khouri, 2017). Khouri et al. previously showed that fat graft survival is limited by oxygen diffusion. To improve revascularization, fat should be grafted as thin microribbons or microdroplets maximum of 1.6 mm to maximize oxygen transport and avoid central necrosis (Khouri *et al.*, 2014). This supports the cell survival theories mentioned above, according to which grafts should be injected so that they are in close contact with the recipient site as much as possible to ensure neovascularization. Therefore, overfilling should be avoided.

A mouse model by Suga et al. showed that severe ischemia or hypoxia induces degenerative changes in adipose tissue and subsequent adaptive tissue remodeling. Ischemic conditions lead to adipocyte death, whereas adipose-derived stem/progenitor cells are activated and contribute to adipose tissue repair. Based on these results, this study proposes the graft replacement theory (Suga *et al.*, 2010). After transplantation, the avascular fat graft forms three zones from the periphery to the center: the surviving area (viable adipocytes), the regenerating area (non-viable adipocytes, adipose-derived stromal cells, replacing new adipocytes), and the necrotic area (no viable cells) (Eto *et al.*, 2012). Dynamic tissue remodeling occurs during the first three months after fat transfer (Kato *et al.*, 2014).

Yoshimura et al described the timeline of cellular events after grafting. Activated adipose stem/progenitor cells initialize the adipogenesis, which is finished by 3 months (‘repair’ phase). During the next 9 months (‘stabilization’ phase) dead adipocytes may form lipid droplets that are absorbed by macrophage phagocytosis or replaced with new adipocytes. The final volume is then determined. If fat graft lipid droplets and oil cysts are small and absorption is completed within 3 months, the volume will not substantially change after 3 months. (Yoshimura *et al.*, 2011).

2.1.4 Clinical applications

2.1.4.1 Volume transfer and aesthetic surgery

Liposuction and autologous fat grafting (ASG) can be used for various purposes in plastic surgery. Common indications for liposuction include body contouring by removing excess fat deposits in undesirable areas of the body in cosmetic or post-bariatric patients, enhancement of the adjacent areas, and reconstructive purposes, such as for the treatment of lymphedema, lipodystrophy, and gynecomastia. The most common areas for fat removal are the abdomen, flank, trochanteric, lumbar, and gluteal regions (Wu, Coombs and Gurunian, 2020). The lipoaspirate suctioned

from the subcutaneous tissue during the liposuction procedure can be used for lipofilling after previously described refinement.

Lipotransfer has many indications and is routinely used to improve structural changes after soft tissue damage or loss due to various causes, such as disease, trauma, or aging. It is a common procedure in breast reconstruction, where conventional macrofat can be used for breast size augmentation/reshaping after reconstruction using an implant or muscle flap, and/or for volume transfer after tissue expansion. It can also be used after implant removal for implant-to-fat conversion, where the empty space is replaced with fat transfer. Fat is applicable for breast augmentation for cosmetic purposes as well as for correcting asymmetry. Gluteal augmentation with autologous fat transfer is a well-established procedure in aesthetic surgery, and the contour of the buttock can be improved even without fat transfer by liposuction of the flanks, thighs, and waists (Khouri and Khouri, 2017; Simonacci *et al.*, 2017; Ding *et al.*, 2022). Usually, several fat transfers are needed before the desired volume is achieved, based on graft survival theories.

In aesthetic surgery, in addition to breast and buttock augmentation, fat is used for facial and hand rejuvenation and rhinoplasty. During rejuvenation, fat is most often processed into nanofat, which is intradermally injected into the target area. Nanofats do not contain viable adipocytes after emulsification but can promote skin regeneration because of the presence of adipose-derived stem cells (ADSCs) and growth factors (Tonnard *et al.*, 2013). Due to the lack of adipocytes, nanofat is unable to enhance volume, and therefore, microfat is used for facial volume restoration caused by aging or lipodystrophy (for example, HIV-associated) or for nasal dorsum profile correction. Macrofat is also applicable but may increase the risk of irregular fat accumulation, fat necrosis, and visible lumpiness, which may be avoided by deep implantation (Simonacci *et al.*, 2017).

Complications are relatively rare with liposuction and fat transfer, making the procedure safe and usable for all indications. Donor-site complications are often related to the liposuction technique, including bruising, swelling, hematoma formation, paresthesia or donor-site pain, infection, hypertrophic scarring, contour irregularities, and damage to the underlying structures, for example, by intraperitoneal or intramuscular penetration of the cannula. At the recipient site, possible complications may include fat reabsorption, fat necrosis, oil cyst formation, and calcification if large volumes of fat are injected into a single area or into poorly vascularized areas (Simonacci *et al.*, 2017).

2.1.4.2 Regeneration, wound healing, and scar modulation

Because of ADSCs, fat transfer can be used to fill atrophic scars and reduce scar contracture as a regenerative alternative to other surgical techniques. Fat improves

dermal and dermohypodermic quality in scar areas, neosynthesis of collagen, and local neoangiogenesis, resulting in improved scar elasticity, aesthetics, and function. It can also release nerve entrapment, thereby reducing neuropathic pain (Simonacci *et al.*, 2017). Randomized controlled trials and quantitative analysis support the efficacy of fat and adipose-derived stem cells in burns and the ability to modify scar tissue; however, there is currently no significant evidence that fat grafting in acute burn wounds facilitates wound healing and ameliorates subsequent scarring, suggesting that AFG does not have a significant impact on acute wound healing (Condé-Green *et al.*, 2016). However, there is more evidence of the efficacy of AFG in the treatment of chronic wounds. In animal studies, there is in vivo evidence of the efficacy of ADSC and platelet-rich plasma (PRP) in wound healing, and clinical application may improve the management of chronic wounds, such as diabetic foot ulcers (Nolan *et al.*, 2021). Fat also has similar effects on fibrosis, radial damage, Dupuytren contracture, and scleroderma (Khouri and Khouri, 2017).

In scar treatment and wound healing, the effect of fat grafting is based on ADSCs that remodel the microenvironment of the target tissue through cytokines, growth factors, angiogenic factors, enzymes, and cellular components stored in the lipoaspirate, leading to neoangiogenesis in fibrotic tissue (Klinger *et al.*, 2020). Fat transfer is immunologically active with multiple anti- and pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), IL-6, and IL-8, which have immunomodulatory effects that participate in tissue healing. Although AFG has regenerative capabilities, numerous retrospective clinical trials comparing outcomes in breast cancer patients with breast-conserving therapy followed by AFG have found no increased risk of recurrence or new disease, making it a safe procedure even with a prior history of cancer (Shamoun, Asaad and Hanson, 2021).

The function and effects of ADSCs are discussed in more detail in chapter 2.2.4 (page 24).

2.2 Adipose tissue

Humans and other mammals have two basic types of adipose tissue, white and brown, both of which have unique roles in energy homeostasis and endocrine function. In recent years, adipose tissue has been extensively studied owing to its influence on the onset of obesity and type 2 diabetes, and in the search for therapeutic means to reduce obesity-related metabolic disorders. Adipose tissue also contains adipose-derived stem cells (ADSCs), which promote tissue healing and remodeling.

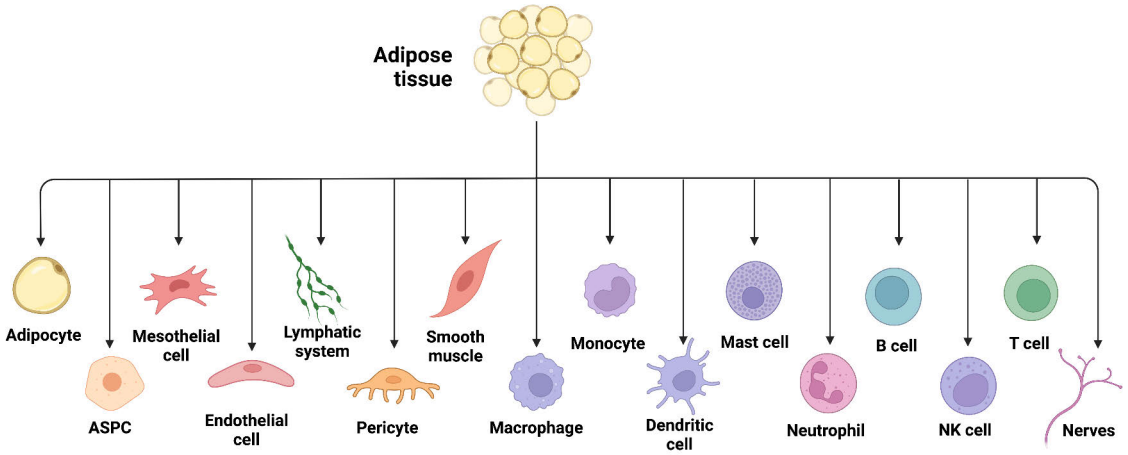

Figure 3 Adipose tissue consists of various cells and structures, including adipocytes, adipose stem and progenitor cells, vascular cells, lymphatic vessels, smooth muscle, inflammatory cells, and nerve ends (particularly the sympathetic nervous system). ASPC=adipose stem and progenitor cells, NK=natural killer. *Modified from Emont et al 2022. The author's own illustration, created using BioRender.com.*

Adipose tissue consists of a wide variety of cells and structures (**Figure 3**), including adipocytes, adipose-derived stem and progenitor cells, mesothelial cells, vascular structures, the lymphatic system, smooth muscle, inflammatory cells, and nerve endings (Emont *et al.*, 2022). Adipose tissue function is regulated by the sympathetic nervous system, which releases norepinephrine when activated by β-adrenoceptor stimulation, thus initiating lipolysis (Bartness *et al.*, 2014).

2.2.1 White adipose tissue

The adipose tissue used by plastic surgeons in fat transfer procedures is called white adipose tissue (WAT). It is the most abundant type of fat and is mostly found in the subcutaneous and visceral (including omental, mesenteric, retroperitoneal, gonadal, and pericardial fat) spaces. WAT produces serum factors (e.g., leptin and adiponectin) for energy homeostasis, stores energy in the form of triglycerides, and acts as a source of circulating free fatty acids during fasting. It also protects delicate organs, cushions body parts exposed to high levels of mechanical stress, and provides insulation against cold (Rosen and Spiegelman, 2014; Cypess, 2022). Moreover, WAT expresses enzymes for the activation, interconversion, and inactivation of steroid hormones such as aromatase, which mediates the conversion of androgens to estrogens (Kershaw and Flier, 2004). WAT also participates in immunomodulation by releasing WAT-derived proinflammatory cytokines that activate and recruit macrophages, particularly in obesity (Weisberg *et al.*, 2003).

Adipocyte hypertrophy causes hypoxia in cells, resulting in local resistance to both insulin and adrenergic signaling, increased inflammation, and cellular damage. Failure of adipose tissue to continue expanding leads to overflow of triglycerides and ectopic accumulation in the liver and skeletal muscle. This also leads to hepatic gluconeogenesis and hyperglycemia. Lipotoxicity is associated with the development of metabolic dysregulation, type 2 diabetes, and cardiovascular disease. (Shulman, 2014; Perry *et al.*, 2015; Cypess, 2022). Abdominal liposuction performed by plastic surgeons does not improve obesity-associated metabolic abnormalities and decreasing adipose tissue mass alone does not achieve the metabolic benefit of weight loss (Klein *et al.*, 2004).

Under a microscope, WAT is composed of adipocytes containing large unilocular lipid droplets filling almost the entire cell volume and few mitochondria, fibroblasts, blood and blood vessels, macrophages, and other immune cells, preadipocytes, and nerve tissue (Cypess, 2022) (**Figure 4a**). Adipogenesis, that is, the formation of new adipocytes, continues throughout life with a median turnover rate of 8% per year. Obese individuals have a significantly greater number of adipocytes, the amount being often set in childhood and adolescence (Spalding *et al.*, 2008). Hyperplastic growth from adipogenesis can include the proliferation of adipocyte differentiation into mature adipocytes (Ying and Simmons, 2021).

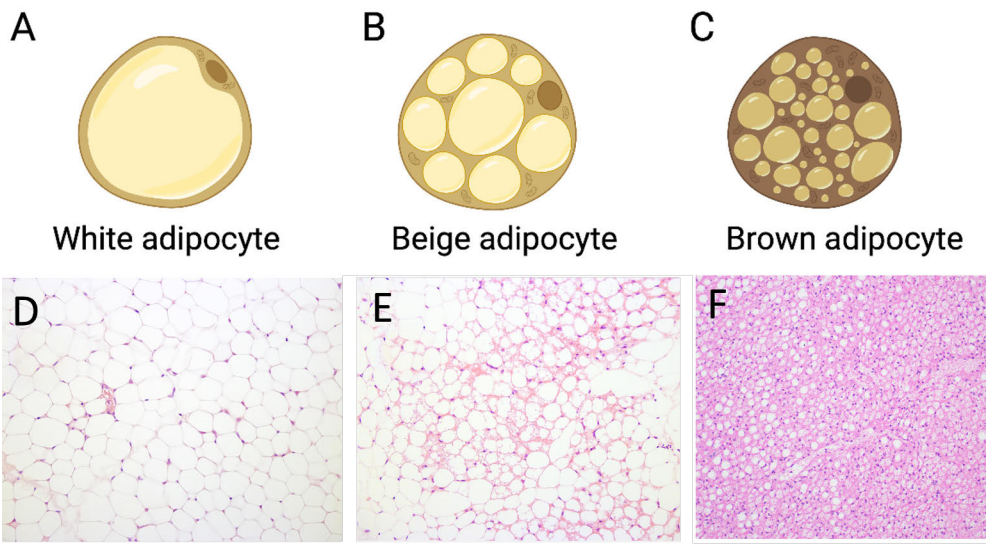

Figure 4 **Adipocytes.** White adipocytes (A) are characterized by large unilocular fat droplets, whereas beige (B) and brown (C) adipocytes contain small multilocular fat droplets and several mitochondria. The images below show hematoxylin and eosin-stained white (D), beige (E), and brown (F) adipose tissues in mice. Typical beige adipose tissue consists of both beige and white adipocytes with fat droplets of various sizes. *The author's own illustration. A-C created using BioRender.com, photographs D-F originally taken by Katri Orte.*

2.2.2 Brown adipose tissue

A second type of adipose tissue, but clearly present in a smaller volume than WAT, is brown adipose tissue (BAT). BAT can be found as small depots in e.g. supraclavicular, paravertebral, perivascular, epicardial, and supra-adrenal locations (**Figure 5**), and the amount is decreased in obese and aging individuals (Cypess, 2022). According to the study of Leitner et al, the amount of activated BAT in healthy young men (n=20) varies according to body mass index; the volume is on average 130 ml for obese men (BMI 34.8 ± 3.3 kg/m^2) and 334 ml for lean men (BMI 23.2 ± 1.9 kg/m^2). However, in obese people, the size of BAT depots can be larger than that of lean people (1646 ml vs. 855 ml), but it remains mostly inactivated during cold exposure (Leitner *et al.*, 2017). BAT activity and mass are typically higher in females (Pfannenberg *et al.*, 2010). BAT differs from WAT in several aspects. White adipocytes mainly originate from myogenic factor 5 negative (Myf5−) mesenchymal stem cells, whereas brown adipocytes originate from myogenic factor 5 positive cells (Myf5+) in the myogenic lineage, which is also the origin of skeletal myoblasts (Seale *et al.*, 2008). BAT contributes to non-shivering thermogenesis by burning glucose and free triglycerides to produce heat, thereby increasing the energy expenditure. Until 2009, when several study groups demonstrated the existence of functional BAT in adults, it was thought to be related only to thermoregulation in infants (Cypess *et al.*, 2009; Saito *et al.*, 2009; van Marken Lichtenbelt *et al.*, 2009; Virtanen *et al.*, 2009). The presence of BAT is correlated with lower odds of type 2 diabetes, dyslipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure and hypertension, when comparing ^{18}F-FDG(fluorodeoxyglucose) PET/CT imaging scans with health records in a wide retrospective study of over 52,000 patients (Becher *et al.*, 2021).

From a histological perspective, brown adipocytes are cells with small multilocular lipid droplets containing a high density of mitochondria, a high degree of sympathetic innervation and vascularization, and a high expression of mitochondrial UCP1 (Lidell *et al.*, 2013) (**Figure 4b)**. UCP1 (uncoupling protein-1) is a unique transport protein on the inner surface of the mitochondria that activates and uncouples electron transport in the respiratory chain from the generation of adenosine triphosphate (ATP), thus releasing energy in the form of heat (Zafrir, 2013). Brown adipocytes are innervated by sympathetic fibers, which release norepinephrine to activate thermogenesis. The sympathetic nervous system is also involved in the regulation of BAT growth (Kajimura, Spiegelman and Seale, 2015).

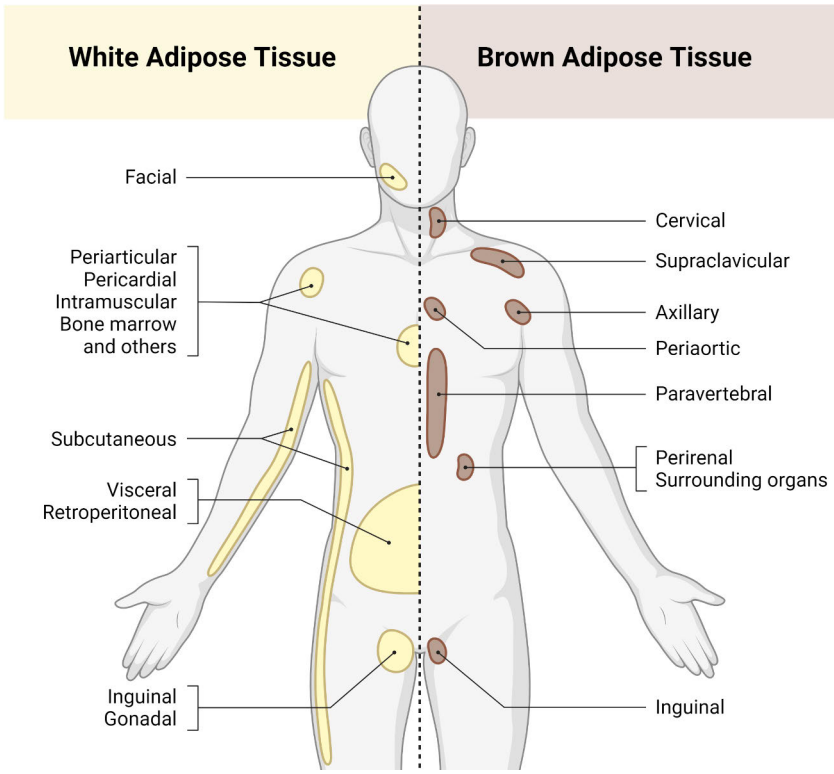

Figure 5 Locations of white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is most abundant in subcutaneous tissue and intra-abdominally in visceral fat. BAT can be found in small depots, especially in supraclavicular areas. *Adapted from "Adipose tissue depots" template, by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.*

2.2.3 Beige adipose tissue

In addition to the two basic adipose tissue types mentioned above, also a mixed form of adipose tissue exists: beige adipose tissue (sometimes also referred as brite, "brown in white"). It is located among WAT but expresses UCP1 like BAT. Under normal conditions, the expression of UCP1 is much lower, but under the influence of external stimuli such as cold or β-adrenergic stimulation, UCP1 expression increases, and beige adipose tissue resembles classical BAT both morphologically and functionally (by generating heat and secreting batokins) (Ahmad *et al.*, 2021).

Beige adipocytes may arise from three different origins: (1) through the transformation of mature white adipocytes, (2) from a distinct beige adipocyte precursor, or (3) from de novo differentiation from tissue-resident progenitors (Wu *et al.*, 2012; Cheng *et al.*, 2021). The biogenesis of beige adipocytes is induced by external environmental conditions such as exercise, chronic cold acclimation, β3 adrenergic receptors agonists, and long-term treatment with PPARγ agonists. Under

normal conditions, beige adipocytes show similar phenotypes to white adipocytes (unilocular large lipid droplets and lack of expression of thermogenic genes such as UCP1), but upon external stimuli mentioned above, they acquire the characteristics of brown adipocytes (such as multilocular lipid droplets and densely packed mitochondria) (Ahmad *et al.*, 2021).

The abundant presence of WAT and its potential to be converted into beige adipose tissue in a process called 'browning' (see Chapter 2.3, page 26) was also an inspiration for this dissertation. Our research team began to investigate the morphological and metabolic changes in free WAT grafts from a plastic surgeon's perspective: does fat grafting also induce browning?

2.2.4 Adipose derived stem cells

Many of the previously mentioned healing effects of fat grafts are based on the presence of adipose-derived stem cells (ADSCs). If the lipoaspirate is processed using centrifugation combined with collagenase and by removing all adipocytes, connective tissue, and blood, the result is a stromal vascular fraction (SVF), a mix of ADSCs, endothelial precursor cells, T regulatory cells, macrophages, smooth muscle cells, pericytes, and preadipocytes (Nguyen *et al.*, 2016). SVF can then be added to the fat graft before application using cell-assisted lipotransfer (CAL, i.e., the method presented by Matsumoto et al. in 2006), or alternatively, the SVF or isolated ADSCs may be injected without reconstitution. Matsumoto et al. reported that fat grafts supplemented with SVF resulted in 35% greater graft retention and exhibited more prominent microvasculature than non-SVF fat, suggesting their clinical potential (Matsumoto *et al.*, 2006; Nguyen *et al.*, 2016; Malik *et al.*, 2020).

Unlike bone marrow–derived stem cells, ADSCs can be harvested with minimal donor site morbidity and used without culturing or expansion, making the use of ADSCs easy, safe, and cost-effective. As mentioned earlier, ADSCs can be used under various conditions for therapeutic purposes or rejuvenation. ADSCs and SVF enhance peripheral nerve regeneration, reduce the demyelination and breakdown products seen in experimental autoimmune encephalitis, improve burn healing when grafted on the wound bed (also by secreting growth factors and extracellular matrices), increase proliferation and collagen synthesis in diabetic foot ulcers, enhance neovascularization in radio-damaged tissue, repair fistulas in Crohn's disease patients (the most frequent application of SVF cells for clinical therapy), and induce bone regeneration (Nguyen *et al.*, 2016).

ADSCs have been proposed to promote wound healing by (1) regulating inflammatory cells to reduce inflammation, (2) secreting VEGF to promote angiogenesis, (3) promoting the proliferation and differentiation of fibroblasts and keratinocyte-forming cells, (4) producing anti-fibrotic cytokines, and (5)

transforming into microvascular endothelial cells and keratinocytes (An *et al.*, 2021). One factor behind the function of stem cells is ADSC-derived exosomes, which are small nanovesicles that mediate intercellular communication (Zhao *et al.*, 2018). We took advantage of the healing effects of fat grafts on tissue damage in our peritoneal adhesion study (Study II).

2.2.5 Imaging of adipose tissue

The metabolic function of adipose tissue is most often examined using positron emission tomography (PET) with [18F]2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) as a tracer, which is a glucose analog that accumulates in all metabolically active tissues and is used routinely e.g. for tumor and metastasis imaging. PET is combined with computed tomography (CT) or magnetic resonance imaging (MRI), which provides anatomical references for the images. MRI expresses soft tissues more accurately than CT, and thus may be a better option for localizing adipose tissue depots and differentiating soft tissues (Virtanen, 2022). The activation of adipose tissue is also examined with perfusion imaging, which is conducted using oxygen-15 labeled water (^{15}O-H_2O, radiowater) as a tracer and defined as the volume of blood flowing through a certain mass (or volume) of tissue per unit time (Holstila, 2018). Perfusion is found higher when the tissue is metabolically active.

^{18}F-FDG-PET has proven to be an excellent method for BAT imaging. By changing the imaging conditions and pre-exposing the study subjects to cold, it was demonstrated that cold activates BAT. Orava et al. showed that both glucose uptake (GU) and perfusion are increased in human BAT in response to cold: GU increased up to 12-fold and perfusion doubled, indicating active thermogenesis (Orava *et al.*, 2011). A recent study by Chen et al. investigated the imaging of beige adipose tissue in mice after 14 days of cold acclimation using ^{18}F-FDG PET/CT and found that glucose uptake increases not only in BAT but also in suprascapular and inguinal WAT depots. H&E staining of tissue samples from these WAT depots showed that WAT morphology changed to beige after 14 days of cold exposure (Chen *et al.*, 2023). A similar increase in ^{18}F-FDG uptake in mice after daily β3 agonist stimulation was found by Park et al., who investigated the potential of ^{18}F-FDG uptake as an imaging biomarker that can monitor the process of WAT browning (Park *et al.*, 2015). However, the imaging of human beige adipose tissue has not been previously investigated (Study III).

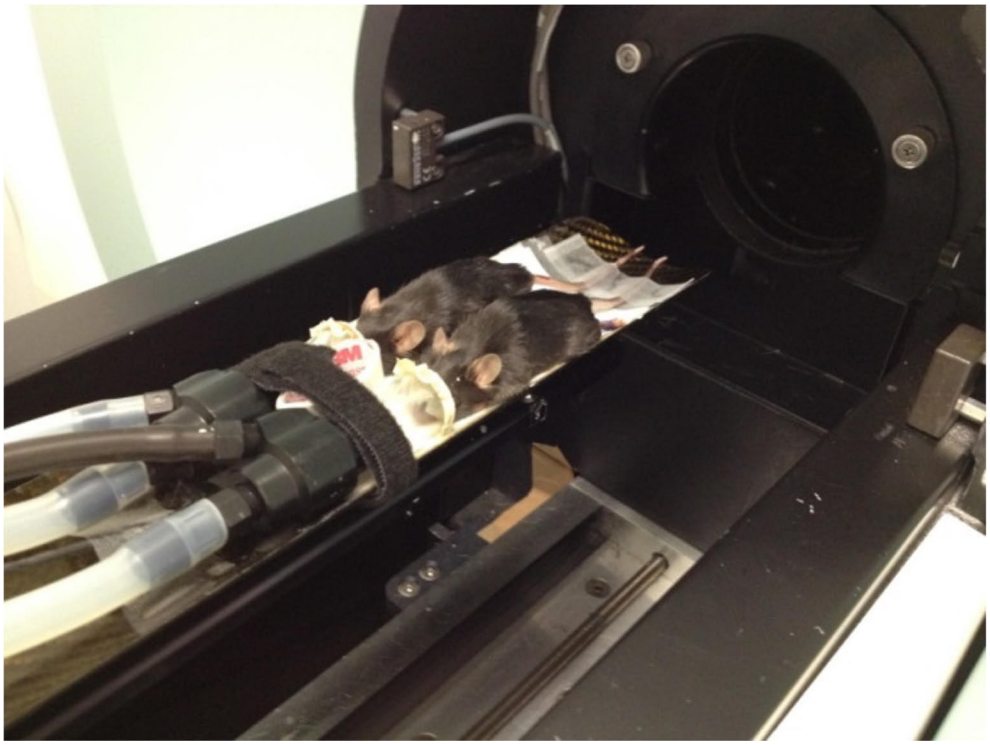

Figure 6 In animal studies (Studies I and II), imaging by PET/CT (Siemens Medical Solutions USA, Knoxville, Tenn., USA) was performed under isoflurane gas anesthesia, animals were placed in pairs in the prone position, and the ^{18}F-FDG tracer was injected into the tail vein. *The author's own photograph, originally taken by Pauliina Hartiala*

2.3 Browning of white adipose tissue

2.3.1 The stimuli of adipocyte browning

The browning phenomenon (i.e., the conversion of WAT to beige adipose tissue or the differentiation of white precursor cells to beige) has been widely investigated in animal and human studies in search for remedies for obesity and metabolic disorders. According to these studies, browning of WAT is induced by stimuli similar to those that activate BAT, including cold exposure, β-3 adrenergic receptor agonists, short-chain fatty acids, dietary factors (e.g. fish oil, capsaicin), nuclear receptors and ligands (e.g. bile acid-activated farnesoid X receptor and liver X receptors), microRNAs, drug agents (e.g. thiazolidinediones), inflammatory factors (interleukins IL-6 and IL-4), hormonal factors (thyroid hormones T3 and T4, parathyroid hormone, glucagon-like peptide 1, leptin together with insulin, melatonin, natriuretic peptides), genetic factors, batokines, exercise (e.g. via exercise adipomyokine irisin), PPAR ligands (increase UCP1 expression and BAT development), bone morphogenetic proteins, and

metabolites (e.g. lactate) (Kaisanlahti and Glumoff, 2019). These factors above show that the browning of WAT can be influenced in several ways, and that external stimuli can induce the conversion of WAT to beige (**Figure 7**; see also chapter 2.2.3 'Beige adipose tissue' page 23).

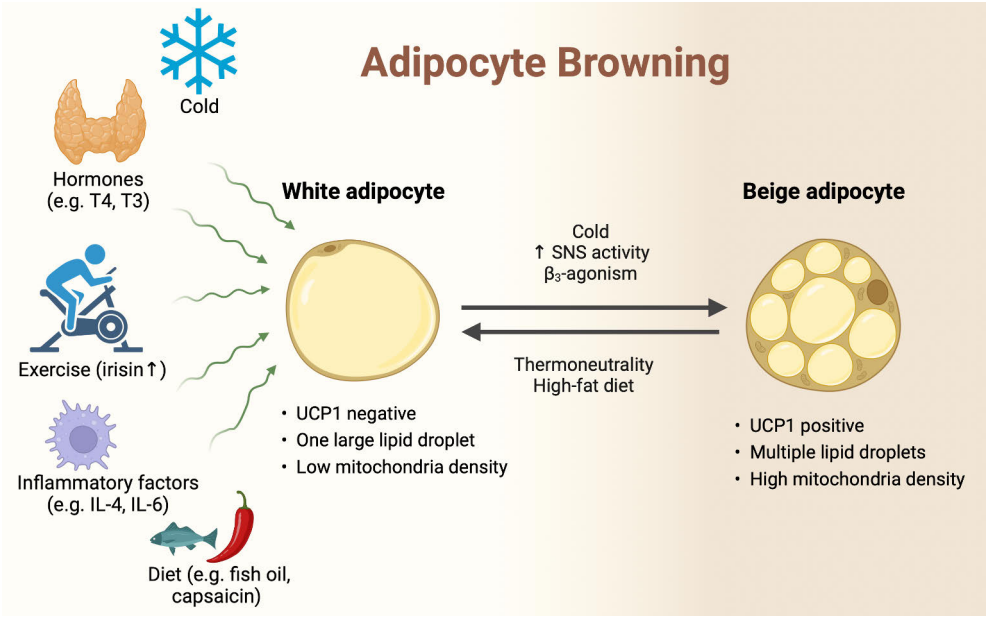

Figure 7 **Adipocyte browning.** The conversion of mature white adipocytes to beige or beige differentiation of white precursor cells is influenced by external stimuli, such as long-term exposure to cold (by stimulating the sympathetic nervous system and activating β-adrenergic receptors), thyroid hormones (by regulating body temperature, inducing UCP1), exercise (by increasing the secretion of irisin hormone), inflammatory factors, and diet. Beige adipocytes resemble brown adipocytes, with a high concentration of UCP1 required for thermogenesis, multilocular fat droplets, and abundant mitochondria. *Adapted from "Adipocyte Browning" template by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.*

2.3.2 How is fat transfer related to browning?

Tervala et al. previously investigated the vascularization, survival, and metabolic changes in free WAT grafts using a mouse model and noticed that a new environment modulated the metabolic activity of the fat grafts to resemble the situation at the recipient site (Tervala *et al.*, 2014). This led our research group to investigate whether browning of WAT is also one of the explanations behind metabolic activity using a mouse model (Study I). A similar study on graft browning was conducted by Qiu et al. in 2018 using a mouse model but with human WAT as fat grafts. Histological and immunohistochemical analyses revealed the presence of beige

adipocytes in the peripheral region of the grafts. Immunofluorescence staining demonstrated that these beige adipocytes might be derived from de novo adipogenesis from progenitors of graft origin (Qiu *et al.*, 2018). As described earlier in chapter "2.1.3 Graft survival" (page 16), ischemia in the grafted fat induces adaptive tissue remodeling, in which mature adipocytes undergo cell death, whereas adipose-derived stem/progenitor cells are activated and contribute to adipose tissue repair. This graft replacement theory combined with the activation of beige preadipocytes may be one mechanism of graft browning.

Another theory behind the browning of WAT after lipotransfer is related to fat necrosis. In 2021, Liu et al. noted that patients with a previous fat grafting had remarkably higher concentrations of UCP1 expression and demonstrable browning of adipocytes in necrotic adipose grafts compared to grafts with no necrosis (Liu *et al.*, 2021b). The same group also investigated the histology of human fat grafts (harvested from subcutaneous tissue from the abdominal or thigh region) placed into the dorsal flank of nude mice at 2, 4, 8, and 12 weeks after transplantation. They noticed that beige transformation occurred in large graft volumes (500 vs. 100 μL) at all time points and was associated with a higher degree of fat necrosis. The group also observed an elevation in M2 cytokines (e.g. TGF-β and IL-10) and pro-browning inflammatory cytokines (e.g. IL-6) in relation to the beige samples (Liu *et al.*, 2021a). The M2 (anti-inflammatory, tissue repairing) inflammatory response and M2 macrophages are known to promote browning by inducing increased UCP1 levels in adipocytes and mimicking the sympathetic signaling pathway (Li, Yun and Mu, 2020). Qiu et al. have previously reported that the cold-induced remodeling of subcutaneous WAT into beige fat is dependent on eosinophils (that produce IL-4), type 2 cytokines (IL-4 and IL-13), macrophages, and myeloid cell-derived catecholamines (Qiu *et al.*, 2014). In addition, two previous studies in mice observed that the presence of proinflammatory M1 macrophages inhibited browning (Sakamoto *et al.*, 2016; Machida *et al.*, 2018), supporting the M2 response-related browning theory. In conclusion, fat necrosis and the associated M2 immune response may explain browning in fat grafts.

The browning of WAT in fat transfer patients is still poorly investigated, even though this procedure is very common and widely used. Most research projects investigating WAT graft browning are carried out using a mouse model, as we did in Study I, but we also wanted to find out in our human study (Study III) what kind of histological and metabolic changes can be observed in clinical patients.

2.4 Peritoneal adhesions

The peritoneum is a thin inner layer of the abdominal wall, a serous membrane consisting of the mesothelium (i.e., a layer of mesothelial cells), a basal lamina, and the submesothelial stroma consisting of mesenchymal cells, including fibroblasts,

endothelial cells, and immune cells (**Figure 8**), followed by the outer layers of the abdominal wall. The parietal peritoneum lines the inner surface of the abdominal cavity, and the visceral peritoneum covers the visceral organs. The main functions of the peritoneum are regulation of inflammatory responses, exchange of peritoneal fluid, and prevention of fibrosis in the abdominal cavity (van Baal *et al.*, 2017; Herrick and Wilm, 2021).

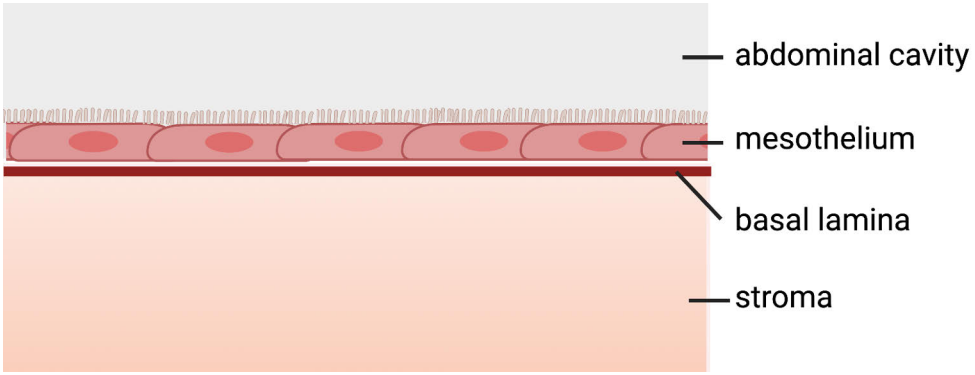

Figure 8 The peritoneum consists of three layers (from inner to outer): (1) the mesothelium, (2) a basal lamina, and (3) the submesothelial stroma. *Modified from van Baal et al 2017. The author's own illustration, created using BioRender.com.*

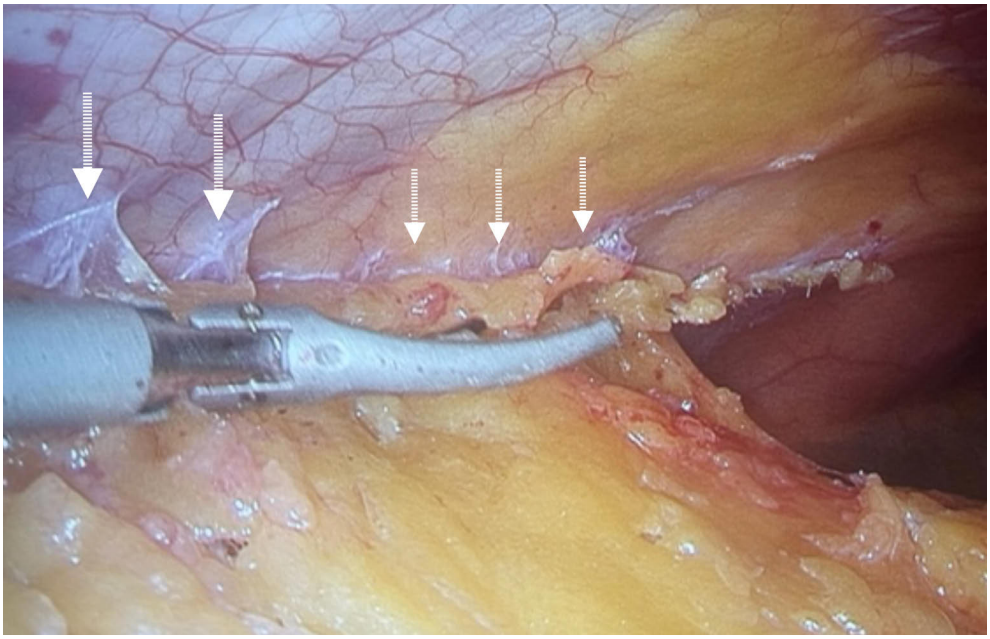

Figure 9 Post-surgical peritoneal adhesions between the inner abdominal wall and the omentum in a patient with previous intra-abdominal surgery. *The author's own photograph, originally taken by Saila Kauhanen.*

Peritoneal adhesions are a common consequence in patients after intra-abdominal surgery (**Figure 9**). They are caused by peritoneal injury (including abdominal surgery, infections, trauma, endometriosis, and peritoneal dialysis), leading to the formation of fibrin bands between the bowel, omentum, and peritoneal wall (Wang, Guo and Li, 2022). Adhesions can cause e.g. intestinal occlusions, chronic pain, or female infertility (Arung, Meurisse and Detry, 2011), thus increasing the risk of re-operations, causing long-term harm to patients and increasing costs to society. Although the formation of adhesions and the significance of their disadvantages to patients have been known for decades, the prevention and treatment of adhesions remain unresolved.

2.4.1 Formation of peritoneal adhesions

Adhesiogenesis is a process associated with an imbalance between fibrin formation and fibrinolysis (**Figure 10**). Peritoneal injury initiates an inflammatory response and activates peritoneal mesothelial cells (PMCs), which leads to the activation of the coagulation cascade, where prothrombin is converted to thrombin, which in turn triggers the release of fibrin from its storage form fibrinogen. Fibrin formation also activates the fibrinolytic system, in which plasminogen is converted to plasmin, an enzyme that degrades fibrin. The activation of fibrinolysis is enhanced by tissue-type plasminogen activator (tPA) and urokinase-type plasminogen (uPA), which are plasminogen activators expressed in endothelial cells, PMCs, and macrophages. Plasminogen activation is inhibited by plasminogen-activating inhibitors (PAI)-1 and 2 produced by endothelial cells, PMCs, monocytes, macrophages and fibroblasts (Holmdahl, 1997; Arung, Meurisse and Detry, 2011; Wang, Guo and Li, 2022). Fibrin acts as an extracellular matrix for fibroblasts and together they form a base for fibrocollagenous tissue. During this process, PMCs also undergo mesothelial-mesenchymal transition and convert to myofibroblasts, which contribute to the fibrinous matrix (Wang, Guo and Li, 2022). In normal healing, the fibrin matrix is degraded by the fibrinolysis system and matrix metalloproteinases within 5-7 days of injury; however, if this process is impaired, adhesion formation may occur. Thus, fibrinolysis and the balance between plasminogen activators and inhibitors play an important role in normal healing and the formation of adhesions; in particular, PAI-1 concentration is often elevated in patients with extensive adhesions (Arung, Meurisse and Detry, 2011), leading to a reduction in peritoneal fibrinolytic activity, which is thought to be the main reason for adhesions (Hellebrekers and Kooistra, 2011). In small peritoneal injuries, fibrin formation and fibrinolysis are balanced, leading to normal healing, but large injuries, such as laparotomy, result in adhesions by disturbing the balance between tPA and PAI-1, resulting in decreased fibrinolytic activity (van der Wal and Jeekel, 2007).

Herrick et al. investigated the composition of human peritoneal adhesions, and found that mature adhesions consist of macrophages, fibroblasts, adipocytes, and vascular endothelial cells. Histologically, adhesions are dominated by vascularized bands of collagen fibers surrounded by varying amounts of adipose tissue. Fibrin-rich granulation tissue was detectable only in recently (< 6 months) operated patients. (Herrick *et al.*, 2000). The overall collagen content and collagen type I/III ratio are higher in dense adhesions than in soft adhesions (Binnebösel *et al.* 2007). A higher collagen type I/III ratio is also associated with more visible and hypertrophic skin scarring after surgery (Kim *et al.*, 2023), thus resembling the composition of peritoneal adhesions.

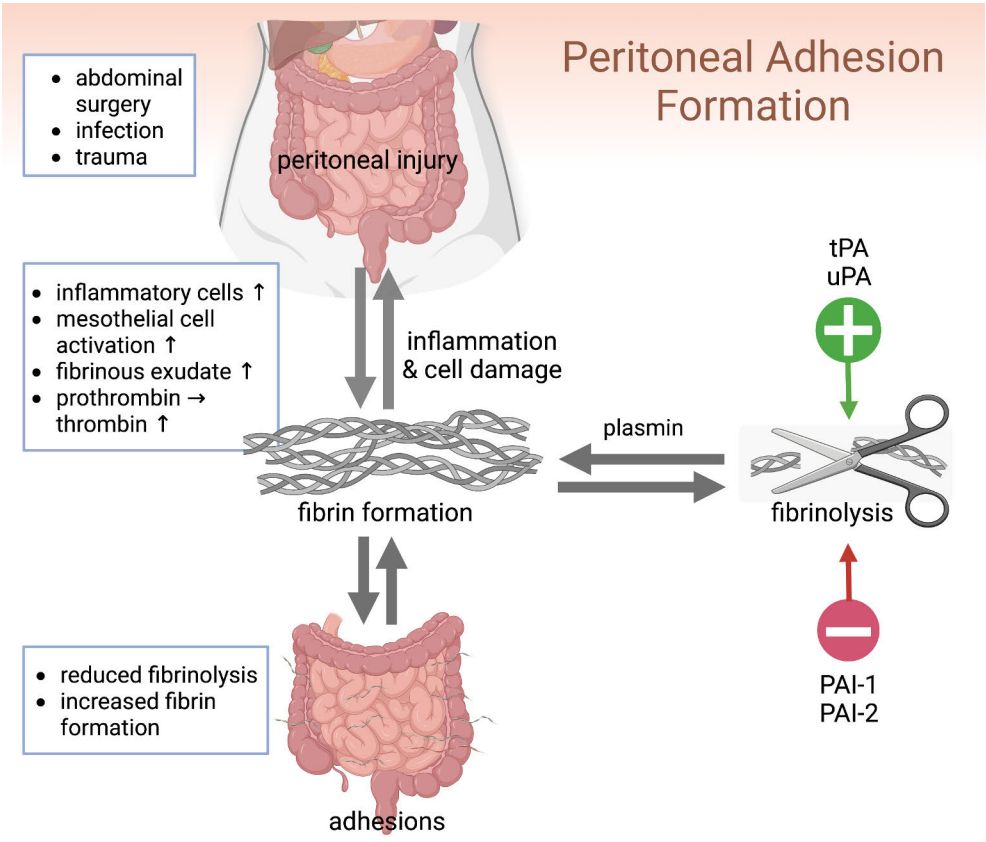

Figure 10 The formation of peritoneal adhesions. Peritoneal injury (e.g. surgery, trauma, infection) activates peritoneal mesothelial cells, macrophages, and neutrophils, which induces coagulation cascade and inflammatory response. This results in fibrin formation. To balance the formation of fibrin matrix, also fibrinolytic system activates. Tissue-type plasminogen activator (tPA) and urokinase-type plasminogen (uPA) increase the conversion of plasminogen to plasmin that degrade fibrin but plasminogen-activating inhibitor (PAI)-1 and 2 inhibit plasmin release. The peritoneal adhesions are often result of an imbalance between fibrin formation and fibrinolysis and related to high concentration of PAI-1. *The author's own illustration, created using BioRender.com.*

2.4.2 The effect of inflammatory response

Damaged PMCs induce inflammation by producing inflammatory molecules, adhesion molecules, and pro-fibrotic factors, and by recruiting neutrophils and monocytes (Wang, Guo and Li, 2022). First, M1 (pro-inflammatory) macrophages accumulate at the injury site, releasing IL-1, TNF-α, IL-6, and other cytokines involved in early wound healing (Tang *et al.*, 2020). This inflammatory response activates the extrinsic pathway of the coagulation cascade, eventually leading to the formation of a transient fibrinous matrix and a subsequent fibrin band formation (van der Wal and Jeekel, 2007). As the coagulation cascade is initiated, prothrombin is converted to thrombin, which promotes platelet aggregation. Platelets secrete cytokines that attract more macrophages, neutrophils, T cells, mast cells, and mesothelial cells. The balance between coagulation and fibrinolysis is also manifested in the inflammatory response, as macrophages also secrete tPA (Tang *et al.*, 2020).

2.4.3 Prevention and treatment of peritoneal adhesions

Peritoneal adhesions are related to coagulation, inflammation, and fibrinolysis, all of which play important roles in normal healing. Prevention and treatment of adhesions should be performed in such a way that normal healing is not compromised. According to previous studies, currently the best methods for adhesion prevention are (1) reducing peritoneal damage through improved surgical procedures, (2) blocking fibrin formation with chemical agents, and (3) reducing the contact between organs in the abdominal cavity and the damaged peritoneum by using physical barriers (Tang *et al.*, 2020). During intra-abdominal operations, e.g. avoiding unnecessary tissue damage, atraumatic tissue handling, and meticulous hemostasis may reduce the formation of adhesions. In addition, the laparoscopic approach is associated with a significantly lower incidence of postoperative peritoneal adhesions than the open technique (laparotomy) (Arung, Meurisse and Detry, 2011). Drug agents, including NSAIDs, corticosteroids, calcium channel blockers, and histamine antagonists, inhibit fibroblast proliferation and therefore prevent fibrin matrix organization. Anticoagulants (e.g., heparin) and fibrinolytic agents (e.g., Actilyse®) have shown promising results in animal models, but have not been tested in clinical trials. They may also increase the risk of postoperative hemorrhage. Some drugs (e.g., local anesthetics) have anti-inflammatory effects related to the inhibition of neutrophils and the activation of fibrinolysis (Arung, Meurisse and Detry, 2011). Mechanical barriers may prevent postoperative peritoneal adhesion formation by separating the peritoneal surfaces during the first 5-7 days that is required for peritoneal re-epithelialization. Barriers may be liquid (e.g., crystalloids, hyaluronic acid), non-absorbable or bio-absorbable film, gel, or solid membrane. Liquid barriers

tend to absorb quicky and solid barriers, on the other hand, may require a secondary operation to be removed (Arung, Meurisse and Detry, 2011).

The M1/M2 macrophage polarization balance is significant for peritoneal adhesion formation, as M1 macrophages activate the coagulation cascade. In chronic wounds, persistent and prolonged M1 (proinflammatory) macrophage polarization is often observed, whereas in normal wounds, transition to M2 (pro-healing) macrophages is observed around day three after injury (Louiselle *et al.*, 2021). Previous studies have shown that the SVF of lipoaspirate promotes M2-type macrophages to repair tissue damage (Bowles *et al.*, 2017); therefore, the use of SVF (or fat grafts) may increase the migration of M2 macrophages to the injury site. In addition, M2 macrophages from fat grafts may migrate to the recipient site and affect inflammatory response and healing (Li *et al.*, 2022).

Fat grafts (with ADSCs and variable numbers of M2 macrophages and other inflammatory cells, as previously described) have anti-inflammatory effects and promote wound healing. Based on these immunomodulatory effects, our study group wanted to investigate how free fat transfer placed preperitoneal at the injury site affects the formation of peritoneal adhesions (Study II).

3 Aims

The aim of this dissertation was to investigate the metabolic and morphological changes of white adipose tissue fat grafts at the recipient site, especially towards beige adipose tissue, and the therapeutic effects of fat grafts on peritoneal injury and formation of peritoneal adhesions. The specific aims were:

I To investigate glucose metabolism in fat graft areas with ^{18}F-FDG-PET imaging in both cold and warm conditions and to find out whether cold activation typical of brown adipose tissue occurs at the recipient site (Study I and III)

II To find out whether the histology and gene expression of the grafted fat resembles white adipose tissue or beige adipose tissue as a result of white adipose tissue browning (Study I and III)

III To explore the therapeutic potential of free fat grafts in the prevention and treatment of peritoneal adhesions in a mouse model (Study II)

4 Materials and Methods

4.1 Study I

4.1.1 Mouse model

C57BL/6 mice (C57BL/6NCrl, Harlan, The Netherlands) were used as donor mice for harvesting white adipose tissue grafts. Grafts were obtained from either epididymal, visceral or subcutaneous fat and transferred to athymic nude mice (Hsd:Athymic Nude-Foxn1nu, Harlan, The Netherlands) with inhibited immune system to avoid graft rejection. Three groups of graft mice were formed according to the three fat donor sites: epididymal (n = 6), visceral (n = 5), and subcutaneous (n = 5). The fat grafts were placed in two different recipient areas, which differed in their metabolic activity: a metabolically inactive subcutaneous space in the forehead and a metabolically active muscle in the leg. A control group (n = 5) was used as a reference to determine basic muscle, BAT, and WAT metabolism.

4.1.2 ^{18}F-FDG-PET/CT imaging

Glucose metabolism and the overall metabolic activity of the graft areas and reference tissues were examined using PET/CT (Siemens Medical Solutions USA, Knoxville, Tenn. USA) using [18F]2-fluoro-2-deoxy-D-glucose (^{18}F-FDG), a glucose analog that accumulates in all metabolically active tissues, as a tracer. The scans were performed at 4- and 12-week time points on two separate days with different temperatures (to stimulate BAT): first with cold exposure (26–28°C) and second with warm exposure (36–38°C). The volume of interest was drawn on all target tissues to determine the ^{18}F-FDG uptake in tissues (Bq/mL) and to calculate the standardized uptake value (SUV), which is the ratio of ^{18}F-FDG radioactivity (Bq) per milliliter (mL) of tissue to the radioactivity in the injected dose corrected by decay and animal body weight. The SUV of fat grafts was compared with that of the liver, and the results were expressed as fat-to-liver uptake ratios (SUV of fat/SUV of liver).

4.1.3 Histology and immunohistochemistry

At the 12-week time point, the mice were sacrificed, and graft samples were harvested for analysis. The morphology of adipocytes and the presence of beige/brown adipose tissue were evaluated by hematoxylin and eosin (H&E) staining and immunohistochemical analysis using UCP1 staining. The degree of multilocular brown adipocytes, inflammation, cystic degeneration, and fibrosis was determined using a scale of 0-5.

4.1.4 *Ucp1* expression

Separate tissue samples were collected for *Ucp1* gene expression analysis using QIAzol Lysis Reagent (Qiagen) for RNA extraction and the First Strand cDNA Synthesis Kit for real-time polymerase chain reaction (PCR) (AMV) (Roche). Quantitative real-time PCR was performed on a ViiA7 instrument (Applied Biosystems) using the Power SYBR Green PCR Master Mix (Applied Biosystems). The standard curve method with *Rplp0* as the normalizing gene was used to determine the relative *Ucp1* gene expression levels.

4.2 Study II

4.2.1 Study design (mouse model)

C57BL/6N male mice (aged 8–12 weeks) and CAG-DsRed mice (expressing DsRed in all cells) were used as study subjects. A laparotomy (open incision through the abdominal wall) was performed in all mice, and a controlled peritoneal injury was induced by tangentially scraping the surface of the cecum and adjacent peritoneal wall with a scalpel 30 times. Two groups were formed according to the injury, as illustrated in **Figure 11**: a group with a large injury area of 20 × 10 mm to induce extensive adhesions (group ADHE) and a group with a small injury area of 10 × 5 mm to induce moderate adhesions (group adhe). Injured surfaces were approximated using a single interrupted suture. The effect of epididymal white adipose tissue graft injected preperitoneal at the injury site (volume 0.2 mL) was examined in three groups as follows: (1) adhe + fat, (2) ADHE + fat, and (3) fat. The fat graft groups were compared to the adhesion and control groups without fat grafts. Each group included six mice.

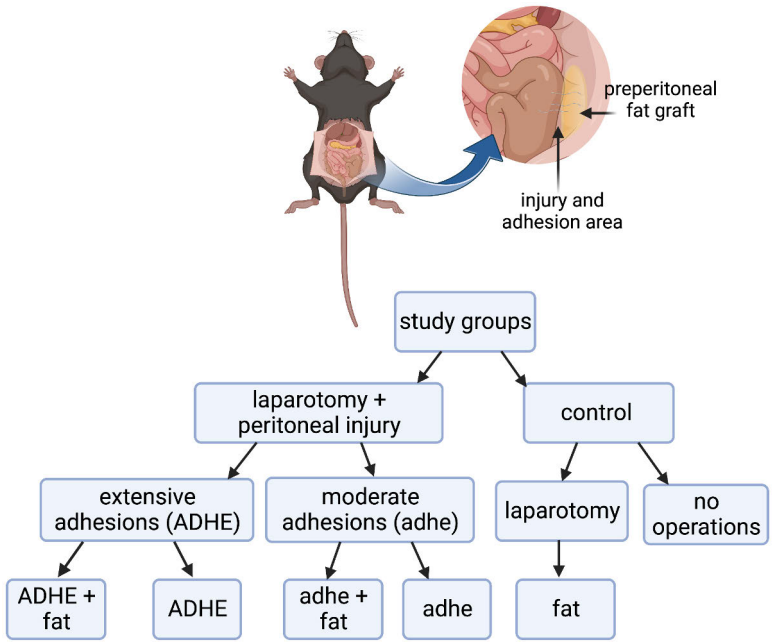

Figure 11 Study II design. ADHE=extensive adhesion group, ADHE =moderate adhesion group. *The author's own illustration, created using BioRender.com.*

4.2.2 In vivo ^{18}F-FDG-PET/CT imaging and ex vivo ^{18}F-radioactivity

All mice were examined using ^{18}F-FDG PET/CT (Siemens Medical Solutions USA, Knoxville, Tenn.) at 30-day time-point to determine glucose uptake and metabolic activity at the injury site. In addition, ex vivo tissue samples were analyzed for ^{18}F-radioactivity in a single-well counter of an isotope calibrator and expressed as the percentage of the injected dose per gram (% ID/g) of tissue of ^{18}F-FDG-derived radioactive compounds.

4.2.3 Adhesion score

At 30 days postoperatively, adhesion length and tenacity were evaluated by a gastrointestinal surgeon blinded to the experiment. The evaluation results for moderate adhesions were scaled as follows: length in millimeters (0 = no adhesions, 1=1–2 mm, 2=3–5 mm, 3= > 5 mm) and tenacity when handled with or without surgical instruments (0 = no adhesion, 1 = release with gentle pulling with forceps or by just touching, 2 = release with blunt dissection, 3 = need to be cut with scissors). Adhesion length and tenacity were added to generate a total score. For extensive adhesions, the total score was calculated based on the following scaling: the length

of the adhesion area (0 = no adhesions, 1=less than 10 mm, 2=10–15 mm, 3=over 15 mm) and the adhesion tenacity using the same scale as above.

4.2.4 Histological analysis

H&E and Wright van Gieson staining were performed to visualize the connective tissue at the adhesion site and peritoneum. Samples were analyzed for thickness of the connective tissue layer of the peritoneum and inflammation (score 0–5:0 = absence, 1 = minimal presence, 2 = minimal to moderate presence, 3 = moderate presence, 4 = moderate to extensive presence, and 5 = extensive presence).

4.2.5 Evaluation of the injured peritoneal surface and inflammation response

Immunofluorescence staining of collagen 1, Acta2 (smooth muscle actin marker), Keratin 8 (mesothelial marker), and DAPI (nuclear marker) was performed 7 days postoperatively to examine the injured peritoneal surfaces. The integrity of the peritoneal mesothelial layer was calculated as the percentage of Keratin 8–positive mesothelium on the whole peritoneal surface of the samples. The inflammatory response was examined by quantitative real-time PCR with primers for GAPDH, IL-10, TGF-b1, IL-1b, TNF-a, IL-2, IL-4, IL-12b, IL-13, and IL-17a.

4.2.6 Macrophage phenotyping

CAG-DsRed mice were used as fat graft donors (only for macrophage phenotyping), and C57BL/6N mice were used as recipients. Macrophage analysis was performed at the 7-day time point using an extensive adhesion model (ADHE). Snap-frozen peritoneal samples were stained with F4/80 to detect the macrophages. For analysis, peritoneal cells were harvested by flushing the peritoneal cavity. Antibody staining and flow cytometry was performed for macrophage type and amount evaluation (macrophage score 0–5, see chapter 4.2.4).

4.3 Study III

4.3.1 Study subjects

Four patients with a history of fat grafting were recruited for the PET/MRI examination (**Table 1**). Three patients had a history of breast cancer-associated mastectomy and secondary unilateral or bilateral breast reconstruction with fat grafts, and one patient was diagnosed with hemifacial microsomia (with

symmetrizing fat grafting to the affected side). The total volume transferred to the recipient area was 437 ± 191 mL (range 180–660 mL). The histological analysis included seven patients with a prior unilateral or bilateral breast reconstruction using fat grafts alone in three or four fat transfer operations, mean volume 168 mL ± 12 mL (range 123-228 mL) per fat transfer, and mean total volume in the grafted areas 544 mL ± 147 mL (range 369–824 mL). Fat transfers were performed approximately 7.5 months (± 3.1, range 3–13 months) before the tissue samples were obtained. As shown in **Figure 12**, the samples were harvested during nipple reconstruction (scGraft=subcutaneous fat samples, n=9) or another repeated fat grafting procedure (mcGraft=intramuscular pectoral muscle region fat samples, n=2).

Table 1 PET/MRI patient details. GRAFT=fat transfer recipient area. *Modified from the original manuscript.*

Patient ID	Age	BMI	Cold blanket temperature	Number of fat transfers	Volume per transfer		Total transfer volume		Time of procedures before scan (range)
					GRAFT 1	GRAFT 2	GRAFT 1	GRAFT 2	
1	31	29.3	12 °C	3	60 ± 0 mL	-	180 mL	-	44–58 months
2	57	25.2	15 °C	3	220 ± 62 mL	-	660 mL	-	30–39 months
3	65	27.1	16.5 °C	3	200 ± 46 mL	200 ± 10 mL	600 mL	480 mL	8–15 months
4	54	26	10 °C	3	160 ± 23 mL	153 ± 18 mL	459 mL	241 mL	13–28 months

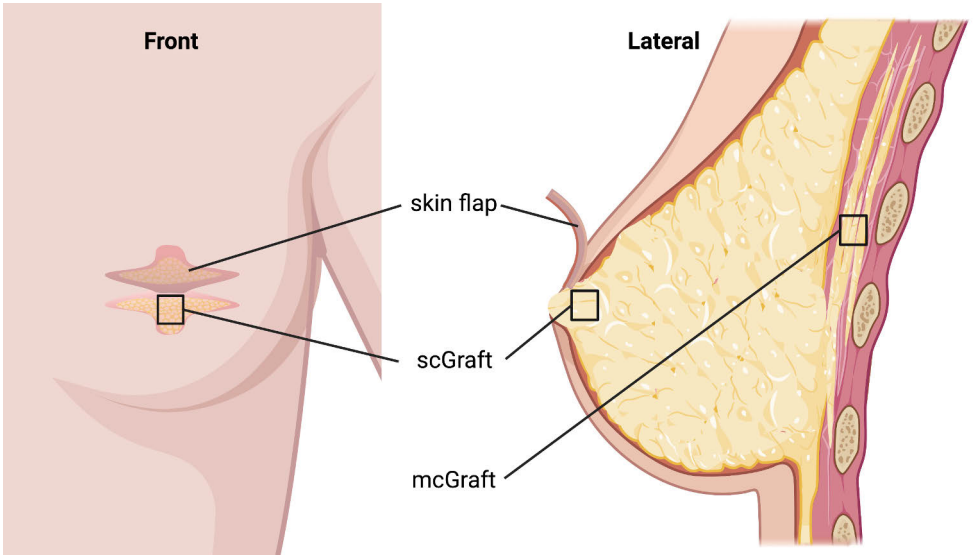

Figure 12 The fat graft open biopsy samples were harvested from either subcutaneous space (scGraft) during nipple reconstruction or from muscle tissue (mcGraft) during the fat transfer procedure. *Modified from the original manuscript. The author's own illustration, created using BioRender.com.*

4.3.2 ^{18}F-FDG-PET/MRI-imaging

The grafted areas, control WAT and BAT depots were examined for glucose uptake and metabolic activity using ^{18}F-FDG PET/MRI (3T Philips Ingenuity TF PET/MR scanner, Philips Healthcare, Cleveland, OH, USA) on two separate days: the first scan with a 2-hour cold exposure under cooling blankets prior to the scan to activate brown/beige adipose tissue and the second scan under warm conditions (normal room temperature). Carimas 2.10 software (Turku PET Centre, Turku, Finland) was used to analyze all acquired PET/MRI images. The volumes of interest (VOIs) were drawn manually on fat depots (fat grafted area, reference WAT, and BAT, if visible) and reference skeletal muscle on the fused PET/MRI images. Subsequently, the mean glucose uptake (GU) was calculated for each area as previously described by Honka et al. (Honka *et al.*, 2018).

4.3.3 Histology and immunohistochemistry

Subcutaneous fat graft (scGraft, n=9) and intramuscular fat graft (mcGraft, n=2) samples were examined in separate groups, and subcutaneous WAT samples taken from abdominoplasty patients (n=5) were used as references. H&E staining and immunohistochemical staining with UCP1 were used to evaluate the adipocyte types in grafted areas and fat quality, as well as the degree of inflammation, fibrosis, and cystic degeneration (same scaling as in Study I). CD31 staining was used to detect vascularization, Ki67 staining was used to evaluate adipose tissue proliferation, and CD68 staining was used for macrophage staining.

4.4 Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 7 and 9) software (Study II-III) and SPSS (Study I). In Study I, groups were compared using 1-way ANOVA, and pairwise comparisons were made using the Kruskal-Wallis test or Student's t-test. In Study II, One-way ANOVA and Tukey's multiple comparison test, Mann-Whitney U test, or Kruskal-Wallis analysis of variance test followed by Dunn's multiple comparison test were used. The Wilcoxon matched-pairs signed rank test or Mann-Whitney *U* test was used to calculate statistical differences between groups in the human study (Study III). $p < 0.05$ was considered statistically significant.

4.5 Ethics

All animal experiments (Study I–II) were approved by the National Animal Experiment Board in Finland and adhered to the rules and regulations of the Finnish Act on Animal Experimentation.

The human study (Study III) was approved by the ethics committee of the Hospital District of Southwest Finland and conducted according to the principles of the Declaration of Helsinki.

5 Results

5.1 Study I: Fat grafting can induce browning of white adipose tissue

5.1.1 Histology and immunohistochemistry

A total of 16 mice were included in the study. In all mice, the subcutaneous fat grafts (scGraft) were preserved during the 12-week follow-up, but only in 15 mice was the intramuscular fat graft (mcGraft) found and harvested for histological analysis. H&E and UCP1 staining showed typical signs of beige adipose tissue in four intramuscular samples, including small multilocular fat droplets and high intensity of UCP1 (**Figure 13** and **Figure 14**). Two had fat grafts of epididymal origin, one had visceral fat, and one had subcutaneous fat.

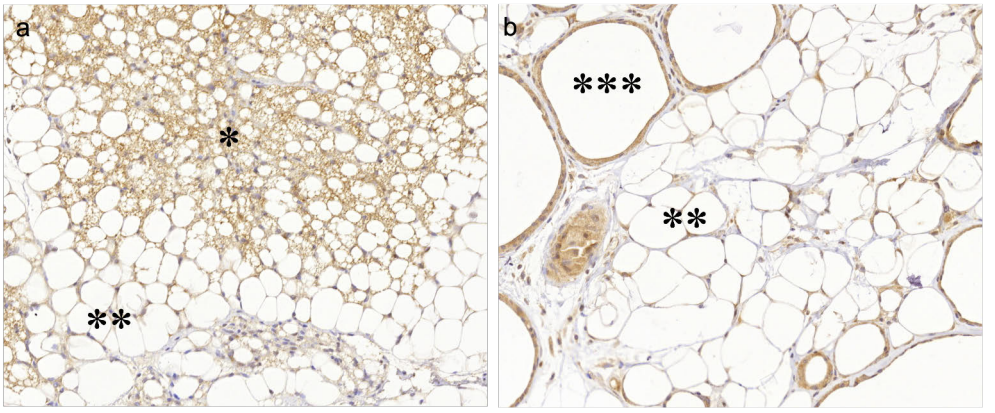

Figure 13 UCP1 staining of beige intramuscular fat graft (**a**) and subcutaneous fat graft (**b**) with dominant WAT morphology shows differences in UCP1 intensity (brown staining) in beige adipose (*) and WAT (**). In beige grafts, the fat is of good quality, but other grafts show a higher degree of cystic degeneration and associated oil cysts (***). *The author's own photographs, originally taken by Katri Orte.*

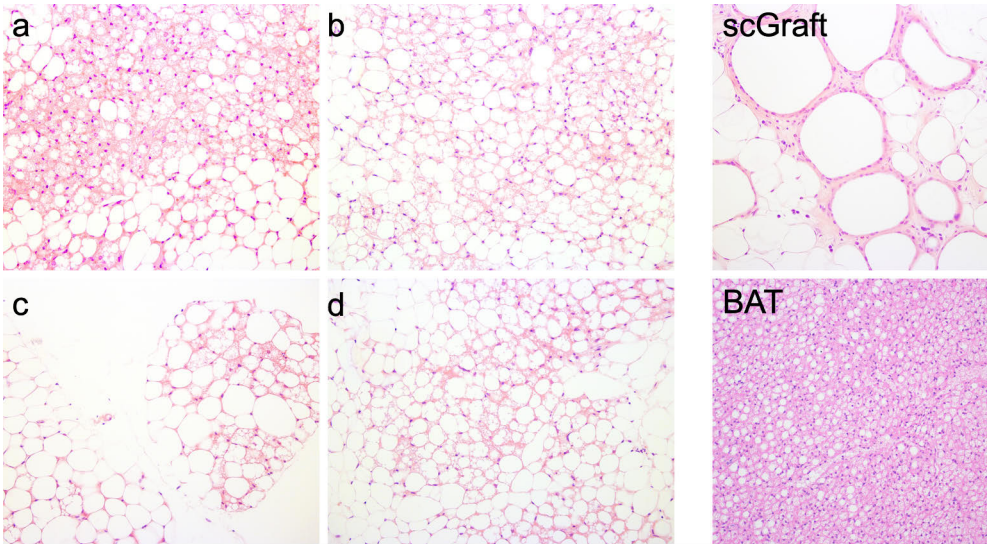

Figure 14 Histological analysis (hematoxylin and eosin staining) showed beige adipose tissue (**a–d**) in four intramuscular samples, regardless of the transferred WAT type. Figures **a–b** consist of epididymal fat graft, **c** visceral fat graft, and **d** subcutaneous fat graft. Other fat grafts showed unilocular large fat droplets typical of WAT and oil cysts (e.g., in figure **scGraft**), but beige adipose tissue had characteristics similar to those of **BAT**, showing small multilocular fat droplets. BAT=brown adipose tissue, WAT=white adipose tissue. *The author's own photographs, originally captured by Katri Orte.*

The fat in the beige samples was well preserved (fat cells 76±8 % vs. 15±18 % in other fat grafts, $p < 0.001$) and of good quality with a lower degree of inflammation, fibrosis, and cystic degeneration compared to other mcGrafts or scGrafts. **Figure 15** shows the degree of evaluated fat graft characteristics (beige graft versus other grafts on a scale 0–5): inflammation 0.25±0.5 in beige and 2.96±1.3 in other grafts ($p < 0.001$), fibrosis 0.5±0.6 and 3.1±1.2 ($p < 0.001$), and cystic degeneration 0 and 2.0±1.7 ($p = 0.03$), respectively. Other grafts (scGraft and mcGraft) showed dominant WAT morphology with large unilocular fat droplets and large oil cysts (**Figure 13**). Necrosis in these poorly preserved fat grafts was also evident.

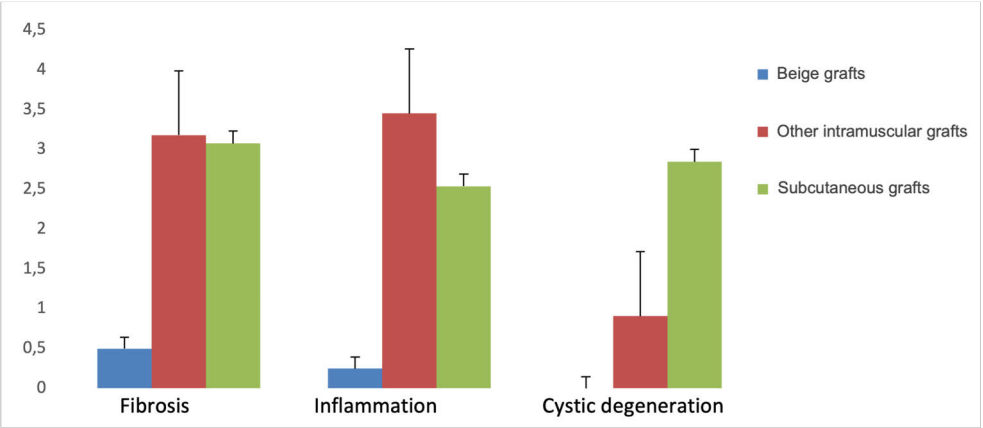

Figure 15 The degree of fibrosis, inflammation, and cystic degeneration was evaluated using a scale of 0 to 5 (0=none, 1=very low, 2=low, 3=moderate, 4=high, 5=very high). Beige grafts were generally of good quality with a low or non-existent degree of fibrosis, inflammation, and cystic degeneration compared to other grafts.

5.1.2 *Ucp1* expression

Mean *Ucp1* expression was 9.4% ± 14 % for beige grafts and 0.002% ± 0.004% for other grafts, respectively. Thus, *Ucp1* expression in beige grafts was 4,700-fold greater than that in the other grafts ($P < 0.001$), resembling that of BAT (**Figure 16**). *Ucp1* expression was also significantly higher in beige grafts than in control WAT (from 430 to 1,383-fold greater).

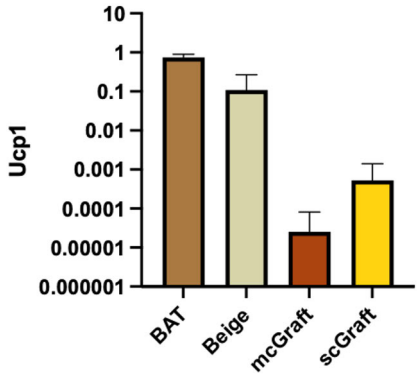

Figure 16 This logarithmic scale (log10) shows significantly increased *Ucp1* gene expression in beige adipose tissue, resembling that of BAT. BAT=brown adipose tissue, mcGraft=intramuscular graft, scGraft=subcutaneous graft. *Modified from the original publication.*

5.1.3 ^{18}F-FDG-PET/CT analysis

The mice were examined at the 4- and 12-week postoperative time points. Data are presented in **Table 2** according to the fat graft location (as fat-to-liver ratio). The 4-week scan was performed only under warm conditions, showing high ^{18}F-FDG uptake in visceral fat grafts and BAT. At 12-weeks the fat type (epididymal, visceral, subcutaneous) did not play a significant role in uptake. Instead, differences in ^{18}F-FDG activity were found in different fat graft locations: scGraft had a greater ^{18}F-FDG uptake than mcGraft. Overall, ^{18}F-FDG uptake was higher in all tissues in the warm scan than in the cold scan (**Figure 17**).

Table 2 ^{18}F-FDG-PET data according to the location of the fat deposit and the transferred fat (WAT type in grafts) at 4-week and 12-week time points postoperatively. The results are expressed as the fat-to-liver ratio. scGraft = subcutaneous fat graft, mcGraft = intramuscular fat graft, BAT = reference brown adipose tissue, Muscle = reference muscle. *Modified from the original publication.*

Location	WAT in grafts	FDG uptake at 4-week	FDG uptake at 12-week	
			cold	warm
scGraft	epididymal	1.6 ± 0.4	1.6 ± 0.4	2.8 ± 0.4
	visceral	3.1 ± 0.6	1.7	2.3
	subcutaneous	2.5 ± 1.2	1.6 ± 0.3	2.6 ± 0.5
mcGraft	epididymal	0.7 ± 0.2	0.6 ± 0.2	0.9 ± 0.3
	visceral	1.4 ± 0.2	0.9 ± 0.7	1.3 ± 1.1
	subcutaneous	1.0 ± 0.3	0.8 ± 0.5	1.2 ± 0.7
BAT		2.3 ± 0.4	1.7 ± 0.5	2.6 ± 0.6
Muscle		0.6 ± 0.1	0.5 ± 0.3	0.6 ± 0.2

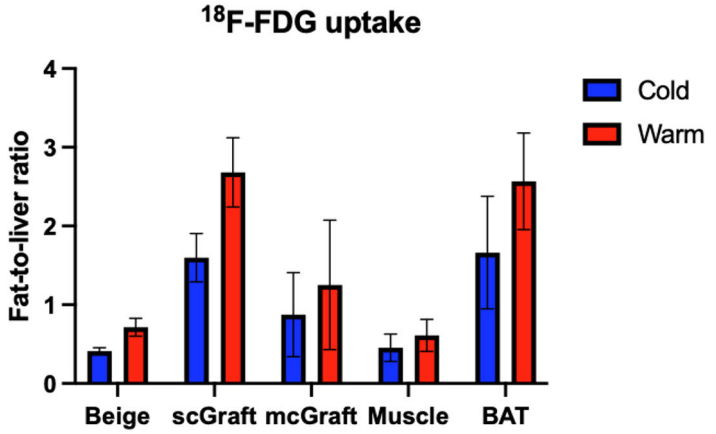

Figure 17 ^{18}F-FDG uptake in grafts (beige, subcutaneous=scGraft, intramuscular=mcGraft) and reference tissues at 12-week timepoint. *Modified from the original publication.*

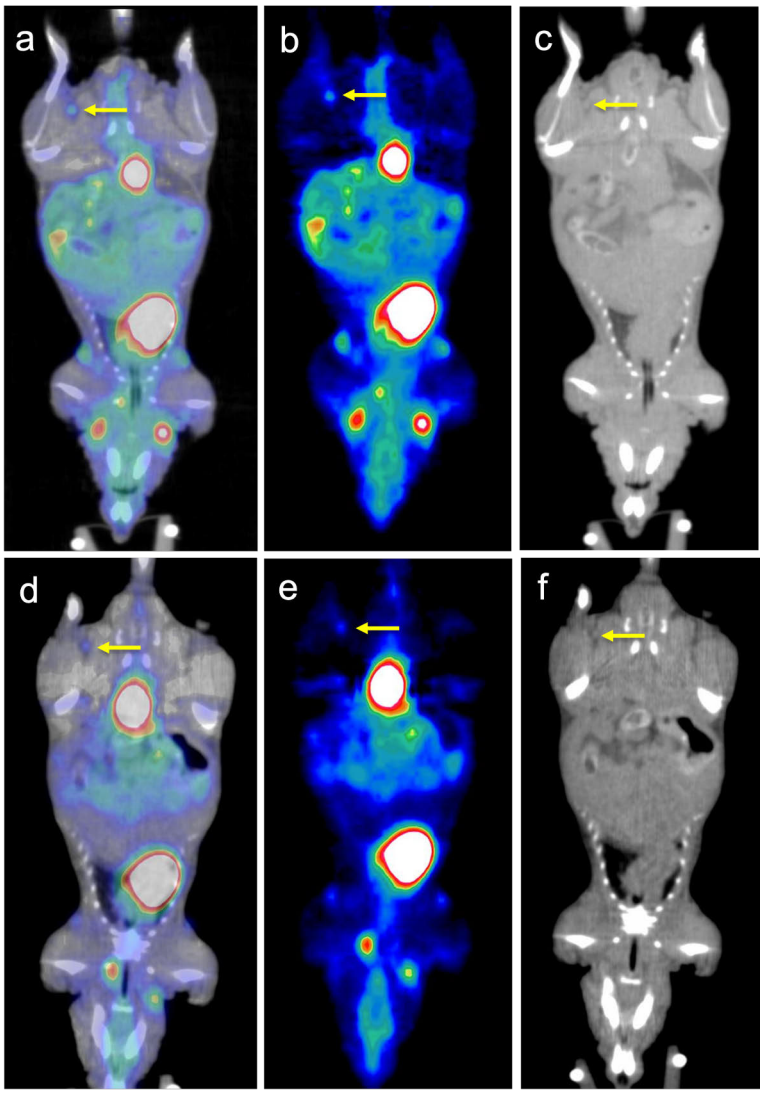

Figure 18 ^{18}F-FDG PET/CT images in the cold scan (**a-c**) and warm scan (**d-f**) at 12-week timepoint; fused PET/CT images shown on the left side, PET image in the middle, and CT on the right side. This mouse had a beige fat graft in the hind leg, and these images show a slight difference in tracer uptake intensity in the grafted area. The hot spot in the grafted area (yellow arrow) is more visible in the cold scan image than in the warm scan image, suggesting increased cold-induced glucose uptake, similar to BAT. *Images taken by the author.*

Although some beige intramuscular fat grafts showed increased cold-induced ^{18}F-FDG uptake on visual analysis (e.g., in PET image in **Figure 18**), the quantitative ^{18}F-FDG uptake in beige grafts was lower in cold than in warm exposure. Also in BAT, these mice showed higher glucose metabolism in the warm scan. In the

reference muscle, ^{18}F-FDG uptake remained low at all temperatures, and beige grafts showed similar levels of ^{18}F-FDG uptake. Considering the above histological results, accumulation of ^{18}F-FDG in fat graft areas is likely to be associated with a strong inflammatory reaction.

5.2 Study II: Fat grafting can prevent the formation of peritoneal adhesions

5.2.1 ^{18}F-FDG-PET/CT analysis

^{18}F-FDG uptake was evaluated at 30 days and expressed as tissue-to-liver ratio. No statistically significant difference between the groups was observed, but ^{18}F-FDG uptake in the ADHE groups (ADHE, ADHE + fat) was slightly higher when comparing the absolute radioactivity (**Figure 20**). Similar results were observed in the visual analysis. **Figure 19** shows an example of ^{18}F-FDG uptake in the area of peritoneal injury.

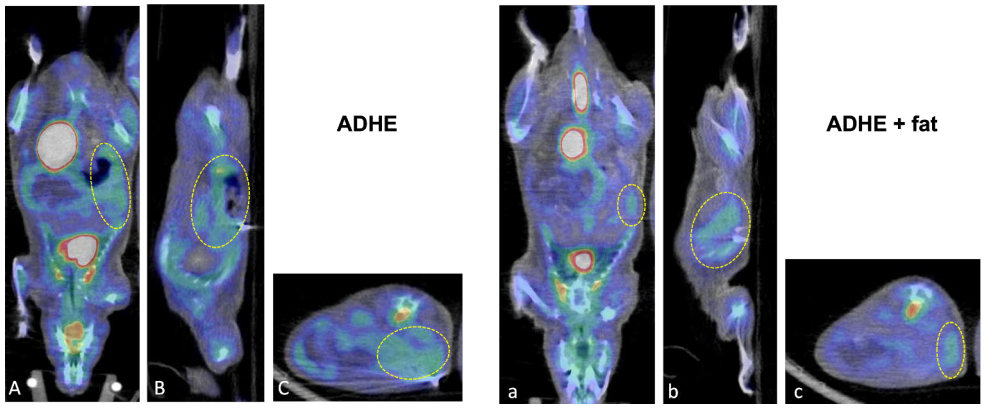

Figure 19 ^{18}F-FDG-PET/CT images in coronal (Aa), sagittal (Bb), and transaxial (Cc) planes showing moderately more intense ^{18}F-FDG uptake in the cecum/peritoneal injury area (yellow dotted circle) in group ADHE (**A-C**) compared to group ADHE + fat (**a-c**). ADHE=extensive adhesion group. *Images taken by the author.*

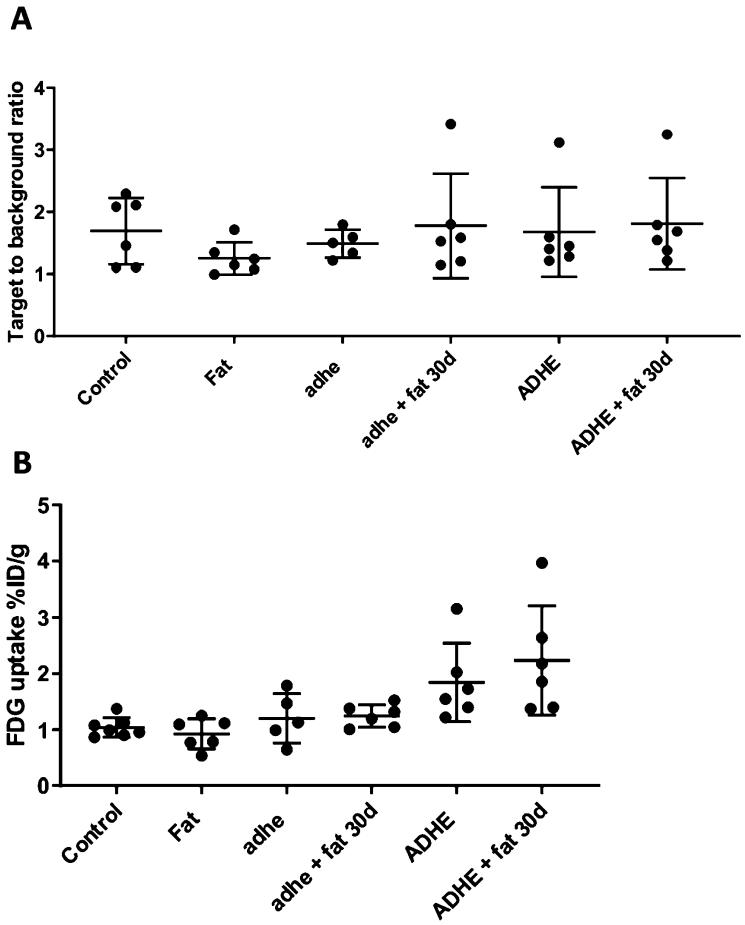

Figure 20 Quantitative analysis of in vivo ^{18}F-FDG-PET images (**A**) (expressed as the tissue-to-liver ratio) and ex vivo ^{18}F-FDG biodistribution (**B**) (^{18}F-FDG uptake as a percentage of 1 g of tissue). ADHE=extensive adhesion group, adhe=moderate adhesion group. *Obtained from the original publication* (Laukka *et al.* 2020).

5.2.2 Adhesion score

The adhesion analysis performed blinded by a gastrointestinal surgeon showed that the adhesion tenacity and width were lower in fat grafted groups at 30-day timepoint (**Figure 21**) and the adhesion score was significantly lower: 4.3 ± 1.0 in adhe group and 2.2 ± 0.4 in adhe + fat group ($p < 0.01$), and 5.2 ± 1.0 in ADHE group and 3.8 ± 1.2 in ADHE + fat group ($p < 0.05$). In the ADHE group, the effect of fat grafting was most noticeable in adhesion tenacity but not in width. In the fat graft control group (only fat grafts without adhesion induction), no adhesions were present.

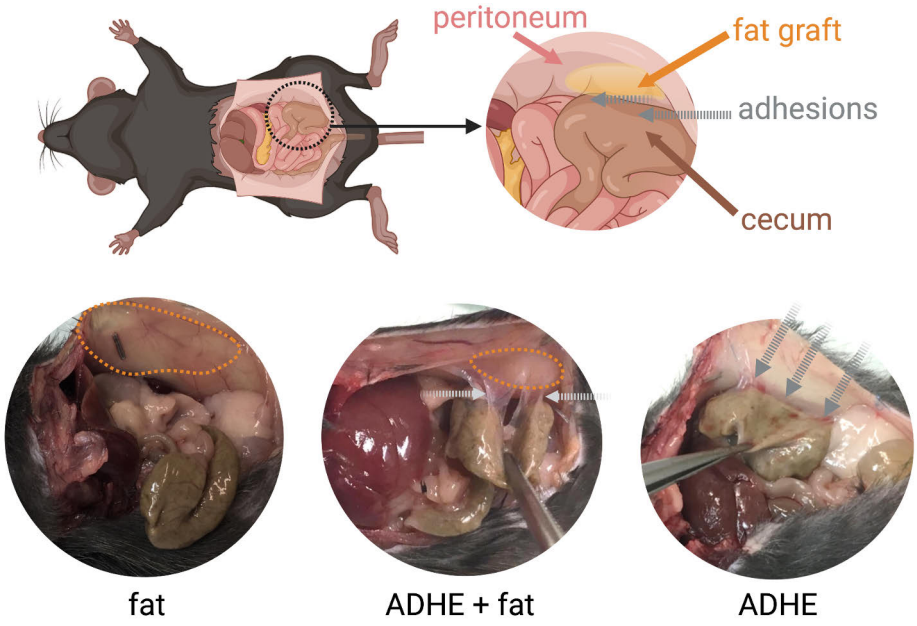

Figure 21 The adhesions in all groups (ADHE, adhe, control) were evaluated for adhesion tenacity and width (expressed as the total adhesion score). In the control group (**fat** without peritoneal injury), no adhesions were observed. In both adhesion groups, the total adhesion score was lower when the fat graft was used. The example images above show the loose structure and minor width of the adhesions in the **ADHE + fat** group, whereas in the **ADHE** group, the cecum was firmly attached to the wall of the abdominal cavity, the adhesions were more compact, and the width of the adhesions was greater. The fat grafts (yellow dotted circle) were visible through the thin and transparent peritoneum. ADHE=extensive adhesion group, adhe=moderate adhesion group. *The author's own illustration, created using BioRender.com; adhesion photographs taken by Pauliina Hartiala.*

5.2.3 Evaluation of the injured peritoneal surface

H&E and VG staining showed that peritoneal injury increased the thickness of the peritoneal connective tissue at 7 and 30 days in the ADHE group ($p < 0.05$, vs. control group). There were no significant differences between the ADHE and ADHE + fat groups. Thickening was not observed in the fat-only group. **Figure 22** shows the histological and immunofluorescence (IF) images of the ADHE + fat and ADHE groups on day 7, which demonstrate the difference in the peritoneal wall thickness and the more rapid healing of mesothelial cells. In the IF analysis of the ADHE + fat group, the Keratin 8 layer was more continuous and thicker. In quantitative analysis, Keratin 8–positive mesothelium of the whole peritoneal surface was 53 ± 14% in the ADHE + fat samples and 7 ± 9% in the ADHE samples (mean ± SD), indicating that fat grafting promotes mesothelial cell regeneration. Keratin 8 staining was also seen on the epithelial surface of the cecum lumen in ADHE + fat samples but not on ADHE samples. The adhesive tissue itself was too thin for analysis.

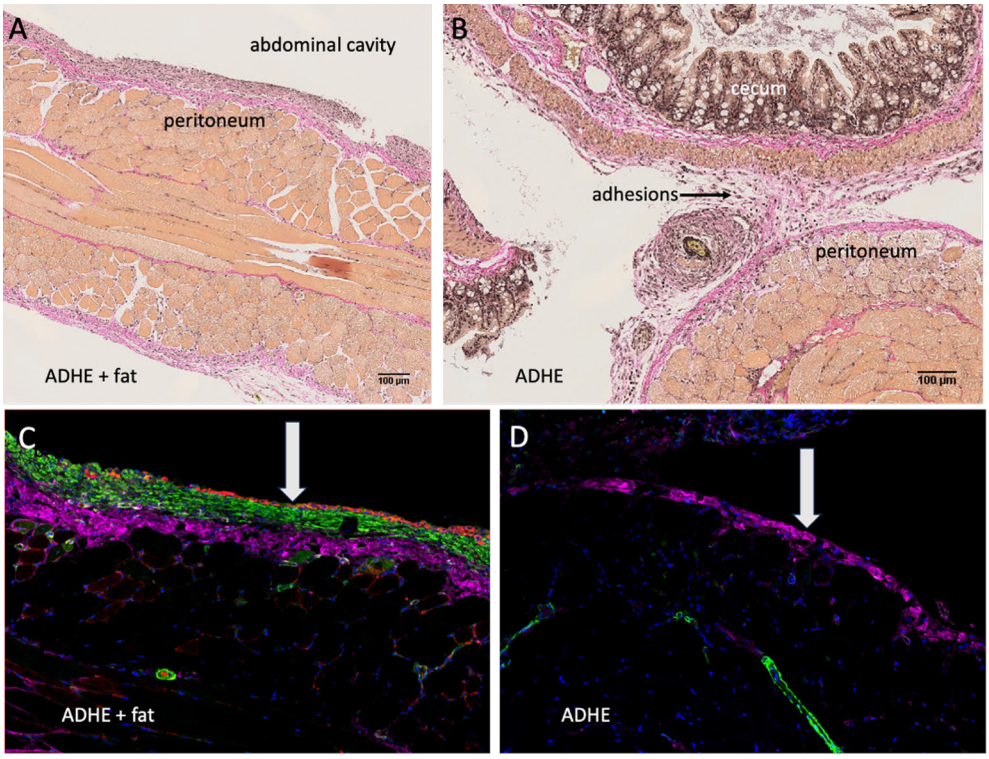

Figure 22 Histology and immunofluorescence (IF) analysis at 7-day time points. Van Gieson (VG) staining in histological samples (**A-B**) shows the peritoneal wall in both ADHE + fat (**A**) and ADHE (**B**) groups. In addition, adhesions between the peritoneum and cecum are visible in some samples (**B**). IF images of the peritoneum (**C-D**) show that the mesothelial marker Keratin 8 (red layer) on the inner surface of the peritoneum (arrow) was more continuous in the ADHE + fat group (**C**) than in the ADHE group (**D**). Colors of IF staining: Keratin 8 = red, Collagen1 = green, Acta2 = purple, DAPI = blue. ADHE = extensive adhesion group. *Adapted from the original publication.*

5.2.4 The inflammation response

In histological samples on day 7, there was no difference between the ADHE and ADHE + fat groups regarding inflammation (inflammation score ADHE 2.3 ± 2 vs. ADHE + fat 2.3 ± 0.9).

RT-PCR of peritoneal wall samples was performed at 7 and 30 days postoperatively to compare inflammatory cytokines. A trend towards lower levels of inflammatory genes was observed in the fat grafting without adhesion induction group (fat) compared with the non-operated control group. The expression of the anti-inflammatory cytokine IL-10 was significantly increased at the 7-day time point in the ADHE + fat group compared with that in the non-operated control. IL-10 was also elevated in fat grafts, but without statistical significance. TNF-α, IL-2, IL-12,

and IL-17 levels were elevated in the ADHE + fat group compared to those in the control and ADHE groups.

5.2.5 Macrophage phenotyping

Macrophage population was examined using CAG-DsRed fat grafts and peritoneal fluid samples at 7-day time point. When overall inflammation was evaluated, there were no differences in macrophage scores between groups: ADHE 2.3 ± 2 vs. ADHE + fat 2.3 ± 1.4 on a scale of 0–5. Fat grafts were compared in two groups: input (fat before grafting) and output fat (grafted fat at 7 days). The input fat had a high level of $CD11b^{high}$ $F4/80^{high}$ tissue-resident macrophages (which typically have an anti-inflammatory M2 phenotype) and $CD1lb^{int}$ $F4/80^{int}$ bone marrow–derived macrophages (which typically represent monocyte-derived pro-inflammatory M1-like cells). In the output fat, tissue-resident M2 macrophages had almost completely disappeared (input vs. output, $p < 0.001$). A high frequency of blood-derived monocytes and neutrophils was observed in the output fat. The macrophage population in the peritoneum consisted mainly of M2 macrophages; however, after peritoneal trauma, the M2 macrophage population was partly replaced by the M1 population. This effect was slightly smaller in the fat graft–treated group, although no significant difference was observed between the ADHE and ADHE + fat groups.

5.3 Study III: The cold-induced glucose metabolism of fat graft areas resembles brown adipose tissue.

5.3.1 Histology and immunohistochemistry

Tissue samples from subcutaneous (scGraft) and muscle tissue (mcGraft) showed well-preserved fat (WAT percentage 83 ± 8 % in scGraft and 85 % ± 0 % in mcGraft) with very mild inflammation, fibrosis, and cystic degeneration (see details from **Table 3**). The majority of cells in the samples consisted of white adipocytes. H&E or UCP1 staining did not show morphological findings suggestive of brown or beige adipocytes. UCP1+ staining was most detectable in macrophages related to areas with fat necrosis. CD68+ staining (macrophage marker) was also evident in the same necrotic areas. Number of CD31+ cells (CD31 = endothelial marker) was found slightly greater in grafts compared to control, but the difference was not statistically significant. CD31+ was also found in areas with a high number of macrophages and therefore CD31 staining was not reliable as an indicator of vascularization in fat grafts (McKenney, Weiss and Folpe, 2001) (**Figure 23**).

Table 3 In fat graft samples the fat was well-preserved, and the degree of inflammation, fibrosis, and cystic degeneration was low on a scale of 0–5 (0=none, 1=very mild, 2=mild, 3=moderate, 4=high, 5=very high). In grafts, CD31 (as an indicator of vascularization) was slightly higher in grafts compared to control (p=ns). Brown/beige adipocytes were not detected. scGRAFT=subcutaneous fat graft, mcGRAFT=muscular fat graft, BAT=brown adipose tissue. *Adapted from the original manuscript.*

	Adipocytes (%)	BAT (%)	Inflammation	Fibrosis	Cystic degeneration	Macrophage reaction	CD31 (n/mm2)
scGRAFT	83 ± 8	0	0.8 ± 0.4	1.1 ± 0.6	0.8 ± 0.6	1.2 ± 0.9	11.2 ± 5.4
mcGRAFT	85	0	1 ± 1.4	1.5 ± 0.7	1 ± 1.4	1.5 ± 0.7	14.1 ± 10
Control	92 ± 4	0	0	0.2 ± 0.2	0	0	10.4 ± 3.2

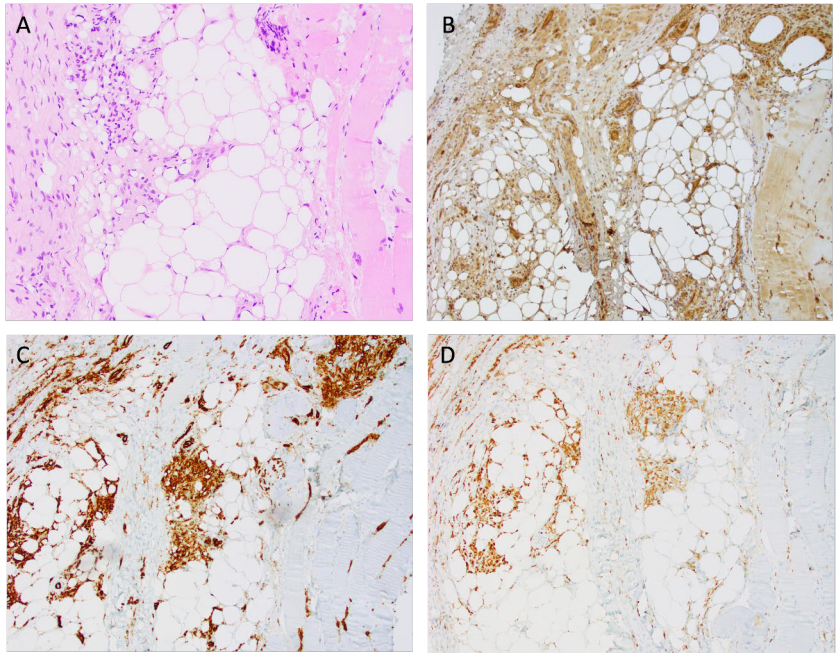

Figure 23 Figure shows examples of histological and immunohistochemical staining (10x magnification) used in fat graft analysis: **A** Hematoxylin and eosin (H&E), **B** UCP1, **C** CD31, and **D** CD68. Example images represent an intramuscular fat graft (mcGraft) with a typical white adipose tissue morphology (white areas). Inflammatory cells (macrophages and lymphocytes) can be seen in all images: in H&E staining as dark purple cells, in UCP1 as darkest brown staining, in CD31 as dark brown staining (in addition to vascular structures), and in CD68 as brown staining. *The author's own photographs, originally taken by Katri Orte.*

5.3.2 ^{18}F-FDG-PET/MRI analysis

Four patients underwent ^{18}F-FDG PET/MRI scans under cold and warm exposure. A total of 5 separate fat graft areas (all from breast regions) were included in the analysis. One PET scan of the graft area (of patient 1) was performed as a late scan

(after 40 minutes of ^{18}F-FDG injection) but the resulting GU values were not comparable with the dynamic scans performed directly after tracer injection and therefore had to be excluded. BAT, however, was comparable to the other scans and visible in this patient and was included in the group comparison. Visual analysis did not show a clear difference between cold and warm scan images and therefore the analysis was performed only with a semiquantitative method by manually drawing the volume of interest (VOI) on all three planes to cover the entire target area in 3D and subsequently calculated for glucose uptake (GU) (**Figure 24**). **Table 4** shows the average GU in tissues of interest.

Comparison of the tissue groups (GRAFT, WAT, BAT, muscle) was performed between cold and warm as well as between different tissues in both temperature scans (**Figure 25**). Active BAT was visible only in one patient. ^{18}F-FDG accumulation in BAT showed a typical cold-induced increase in the tracer uptake (**Figure 25D**). Similar GU increase in the cold scan was observed in GRAFT suggesting the presence of beige adipose tissue in these areas (**Figure 25A**). The difference in GU cold vs. warm was highest in patient 2 (+ 29 % increase) and lowest in patient 3 (+ 5 % increase). However, the difference between GRAFT scans was not statistically significant (p=0.0625). The tracer uptake in the reference tissues WAT and muscle was found higher in the warm scan. When comparing GU between GRAFT and WAT in the cold scan, the difference was statistically significant (p=0.0317), whereas difference between these areas was not observed in the warm scan (p=0.3095) (**Figure 26**).

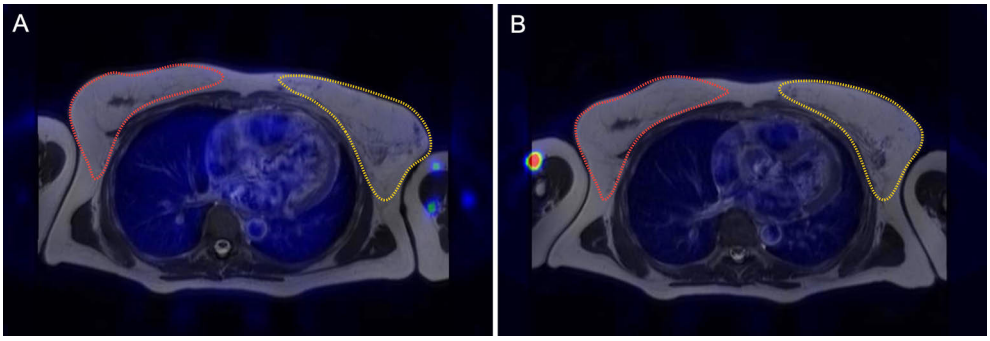

Figure 24 ^{18}F-FDG PET/MRI images of the chest of patient 2 (transaxial plane, supine position) under cold (**A**) and warm (**B**) conditions. Semiquantitative analysis of standardized uptake value (SUV) was performed by manually drawing the volume of interest (VOI) on all three planes to cover the entire target area in 3D and subsequently calculated for glucose uptake (GU). The VOI of the left reconstructed breast shown with a yellow dotted circle and the right reconstructed breast with a red dotted circle. *Pictures taken by the author.*

Table 4 Semiquantitative data from ^{18}F-FDG PET/MRI analysis showing average glucose uptake (GU, μmol/100g/min) in tissues of interest of each patient. *Modified from original manuscript.*

	GRAFT left		GRAFT right		WAT		Muscle		BAT left		BAT right	
Patient ID	**Cold**	**Warm**	**Cold**	**Warm**	**Cold**	**Warm**	**Cold**	**Warm**	**Cold**	**Warm**	**Cold**	**Warm**
1	-	-	-	-	0.316	1.013	0.225	0.55	3.268	0.789	3.127	0.452
2	1.499	1.1694	-	-	0.166	0.756	0.056	0.191	-	-	-	-
3	0.691	0.655	0.596	0.482	0.368	0.209	0.423	0.311	-	-	-	-
4	1.408	1.089	1.254	1.165	0.930	0.80	1.096	0.882	-	-	-	-

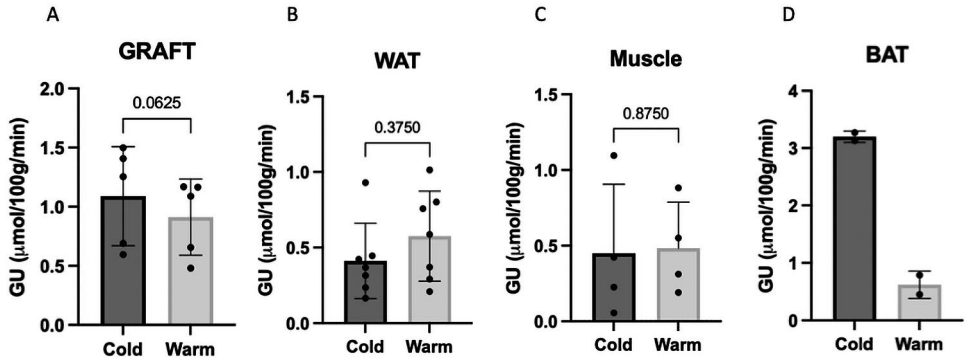

Figure 25 The mean (+ SD) uptake of ^{18}F-FDG in tissues of interest in cold and warm temperature PET scans. The glucose uptake (GU) of grafts increased in cold temperature similarly to BAT, suggesting the presence of beige fat in grafted areas. *Adapted from the original manuscript.*

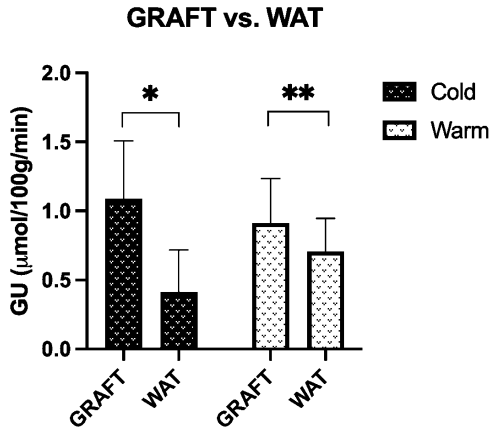

Figure 26 The difference between Graft and WAT glucose uptake (GU) was statistically significant under cold exposure, p=0.0317 (*), where glucose metabolism increased in graft areas but decreased in the reference WAT. The difference was insignificant for the warm scan, p=0.03095 (**). *Adapted from the original manuscript.*

6 Discussion

Adipose tissue has versatile properties, both in plastic surgery and in the field of other medicine, and more is learned through active research worldwide. Study II presented the novel finding that fat grafting reduces the M1 inflammatory response in acute peritoneal injury, accelerates the healing of peritoneal mesothelial cells, and prevents the formation of peritoneal adhesions. Fat grafting can also affect the morphology and metabolism of grafted WAT. Studies I and III showed that fat grafting may induce browning and modulate the metabolism of WAT towards active beige adipose tissue.

6.1 Browning of fat grafts

The metabolic and morphological changes of fat grafts in the recipient area were investigated in both mouse (Study I) and human studies (Study III). The background of the investigation was the study by Tervala et al. in 2014, in which fat grafts were noted to adapt to the metabolic properties of the recipient site (Tervala *et al.*, 2014). Our research group started to explore whether the metabolic activity of the graft was a result of browning. In Study I, we noticed that some intramuscular grafts transformed into brown-like adipose tissue (i.e., beige) in terms of histology and *ucp1* gene expression. The investigation continued in Study III, in which we examined the fat graft areas using ^{18}F-FDG PET/MRI and analyzed the histology of the fat graft samples. Although the tissue samples did not confirm the presence of beige adipose tissue, PET scans showed an increase in glucose uptake in fat graft areas after cold exposure, which is typical for BAT and beige adipose tissue.

The chapter 2.3.2 (page 27) presented the theories of fat graft browning, which relied on the existence of fat necrosis and inflammatory response. Based on our results, we suggest instead that browning is associated with graft vascularization and reduced inflammation. In Study I, we observed fat necrosis and related to this strong inflammatory reaction in subcutaneous and intramuscular fat grafts, but found no signs of brown/beige adipocytes in these grafts. Instead, beige adipose tissue was detected in well-vascularized and high-quality fat grafts, with a low secondary inflammation reaction and degeneration. In addition, considering the study by Tervala et al., these findings suggest that fat grafting can act as an external stimulus

that induces browning of WAT, possibly by increasing adipose tissue metabolism at the recipient site as a result of metabolic adaptation of the grafted WAT.

According to the study by Liu et al. in which browning was associated with increased fat necrosis and inflammation (Liu *et al*., 2021b), the correct fat transfer technique should have a detrimental effect on browning. However, our results demonstrated that browning is more likely to occur when techniques that promote graft survival are used. Understanding graft survival is important for the technique used by plastic surgeons. The fat graft is placed in thin rows among the adipose tissue or muscle of the recipient area using small-caliber cannulas. Overfilling leads to graft ischemia, failure of vascularization, and eventually, fat necrosis and loss of volume, which plastic surgeons want to avoid. As shown in Study I, the presence of fat necrosis is not necessary for the development of browning, which is why it would be possible to achieve this even with proper techniques. In human fat grafts (Study III), fat was well-preserved in all samples, and the degree of inflammation, fibrosis, and cystic degeneration was low, supporting our theory of browning. In addition, Zhu et al. in their study supported the connection between neovascularization and efficient survival of fat grafts in the formation of beige adipose tissue by demonstrating that adipose-derived stem cell-derived extracellular vesicles enhance the survival of fat grafts and browning of white adipose tissue (Zhu *et al.*, 2020).

Another perspective is that the browning of WAT, as an independent factor, increases graft survival. Xia et al. used human lipoaspirate enhanced with cultured beige adipocytes or a combination of cultured beige adipocytes and ADSCs in a mouse model and noticed that in both groups fat graft retention was significantly higher and oil cyst formation was lower at 12-week time point compared to control (fat graft with phosphate-buffered saline). Induced beige adipocytes participated in adipogenesis and were prone to rewhitening into white adipocytes (Xia *et al.*, 2021). External factors, such as melatonin and tamoxifen, have been found to promote WAT browning and lead to improved graft retention (Cai *et al.*, 2018; Dang *et al.*, 2023), supporting the theory of the role of beige adipose tissue in graft survival. In post-burn mice, burn-induced browning was associated with an increase in macrophage recruitment and a type 2 macrophage profile. Same study of Abdullahi et al. observed WAT browning and increased macrophage infiltration in burn patients (Abdullahi *et al.*, 2019), suggesting that WAT browning also has immunomodulatory effects that may improve graft survival.

In Study III, histological analysis did not show any signs of beige adipose tissue, even though the increase in ^{18}F-FDG uptake in the cold exposure PET scan suggested otherwise. This may be explained by previous studies that have investigated the expression of beige fat in WAT during cold exposure. Machida et al. investigated UCP1 expression and histology in mice after 24-72 h of cold exposure (10 °C). Beige adipocytes containing multilocular lipid droplets appeared after exposure to cold

conditions for 24 h. After 72 h, the size of the droplets decreased and the area of beige adipocytes increased (Machida *et al.*, 2018). In addition, Rosenwald et al. observed that cold-induced formation of beige adipocytes in mice was reversed within five weeks of warm adaptation, but the beige adipocytes formed by cold stimulation were not eliminated. This indicates that beige adipocytes are converted into cells with the morphology and gene expression pattern of white adipocytes; however, they can be converted into beige adipocytes upon additional cold stimulation (Rosenwald *et al.*, 2013).

Interconversion between beige and white phenotypes, depending on temperature and other external stimuli, may explain the differences in histological samples observed in previous studies. Our patients in Study III did not have previous long-term cold exposure or cold exposure during tissue sample harvesting, which affects adipose tissue morphology according to Rosenwald et al. The fat graft in the area from which the tissue sample had been collected (approximately 7.5 months after the previous fat transfer, ranging 3-13 months) was in a stable stage of cellular events after the procedure (see chapter 2.1.3 'Graft survival', page 16), also with no inflammatory response in the area to maintain the stimulation needed for browning. Thus, samples collected under cold conditions may have exhibited fat with a beige phenotype.

The amount of BAT in the body is approximately 1.5 % of the BMI and 4.3 % of the total amount of fat (Leitner *et al.*, 2017), which indicates that even small amounts of BAT are relevant in terms of energy metabolism. BAT and beige fat have an inherent thermogenic capacity and the ability to improve glucose metabolism; thus, they have the potential to treat obesity and type 2 diabetes by increasing the recruitment of beige adipocytes and enhancing BAT amount and/or activity, resulting in increased UCP1 expression, energy expenditure, and thermogenesis (Cheng *et al.*, 2021). BAT has many beneficial effects on glucose and lipid homeostasis, reversing glucose intolerance and insulin resistance, lowering blood glucose levels and HbA1c, and reducing blood triglycerides and cholesterol (Klepac *et al.*, 2019). The effect on glucose homeostasis is mediated not only through glucose consumption but also through adipokines secreted by beige fat and BAT (Kaisanlahti and Glumoff, 2019). The thermogenic capacity of beige adipose tissue is lower than that of classic BAT (Shabalina *et al.*, 2013), as we also observed in Study III, where cold-induced glucose uptake increased in fat graft areas compared to WAT, but did not reach as high levels of glucose uptake as in BAT. Nevertheless, beige adipose tissue appears to play an important role in the regulation of whole-body energy expenditure and glucose and lipid homeostasis by improving glucose tolerance and lowering circulating glucose and fatty acids, similarly to BAT (Kajimura, Spiegelman and Seale, 2015). In addition, a study by Hocking et al. demonstrated using a mouse model that intra-abdominally implanted subcutaneous fat improved

glucose tolerance, prevented high-fat diet-induced hepatic triacylglycerol accumulation, reduced the levels of hepatic gluconeogenic enzymes, and decreased the levels of systemic pro-inflammatory markers (Hocking *et al.*, 2015), thus showing that fat grafting can have significant effects on metabolism.

By inducing browning, fat grafting can have beneficial effects on glucose tolerance and body weight. However, to date, this has not been investigated. As stated earlier, liposuction alone (e.g., to remove visceral fat) does not change the overall energy consumption of the body in a more favorable direction (Klein *et al.*, 2004). Giugliano et al. also investigated the metabolic effects of liposuction and observed that large-volume liposuction reduced fasting plasma insulin and circulating markers of vascular inflammation and improved insulin sensitivity in healthy obese women, suggesting that liposuction could reduce the risk of cardiovascular and metabolic disorders (Giugliano *et al.*, 2004). The development of obesity is a complex process involving an imbalance between energy intake and expenditure, ultimately leading to adipocyte growth, adipose tissue ischemia, and inflammation. A large amount of inactive fat is known to be an important factor in metabolic disorders. Possibly by reducing the excess white adipose tissue burden, liposuction could reduce the inflammatory reaction in adipose tissue and thus prevent the progression of overweight to obesity and the development of metabolic disorders; however, further studies are required.

6.2 Therapeutic use of fat grafts in the prevention of peritoneal adhesions

In Study II, we aimed to investigate the anti-inflammatory and pro-healing effects of fat grafts on the prevention of peritoneal adhesions, which remain a common complication after open abdominal surgery. Only two previous studies have investigated the effects of intra-abdominal fat grafts. Hocking et al. observed that intra-abdominally implanted subcutaneous adipose tissue improved glucose tolerance and decreased plasma concentrations of several proinflammatory cytokines (e.g. TNF-α and IL-17), thereby reducing systemic inflammation in mice. Visceral fat transplants were found to be glucose-intolerant (Hocking *et al.*, 2015), suggesting that the donor area of the transplanted fat has different properties in terms of systemic effects. Cil and Aydoghu investigated the effects of intra-abdominal fat grafting in a rat model and observed that autologous intraperitoneal fat grafting promoted mesothelial healing in serosal injury and significantly reduced adhesion formation with less fibrosis and inflammation than the control group (Cil and Aydogdu, 2018). Bresson et al. observed that autologous peritoneal grafting promotes healing of peritoneal mesenchymal cells (PMC) and prevents adhesions in rats (Bresson *et al.*, 2017). Thus, the role of PMCs in the process of adhesion formation is essential

because they participate in the initiation of both the coagulation cascade and the inflammatory response. In summary, an important step in the prevention of adhesions is to accelerate the healing of PMC damage and reduce inflammation.

Understanding the inflammatory response is the background of the study. Immediately after cell injury, macrophages are polarized to the pro-inflammatory M1 phenotype (and producing proinflammatory cytokines) to assist the host against pathogens. Subsequently, macrophages are polarized to form an anti-inflammatory response to the M2 phenotype and repair damaged tissue. Thus, improving the inflammatory environment by modulating the activation state of macrophages may be an effective method for the treatment of diseases (Yunna *et al.*, 2020). In addition, Holschneider et al. showed that modulation of the immune response to peritoneal injury may prevent postoperative adhesion formation. They created a mouse model in which post-surgical peritoneal injury was treated with intra-abdominal (anti-inflammatory) exogenous IL-10 injections, and the results showed fewer adhesions in the IL-10 treated group compared the control group (Holschneider *et al.*, 1997).

Study II showed an interesting finding in the inflammatory response: the anti-inflammatory cytokine IL-10 was significantly increased at the 7-day time point in the extensive adhesion group with fat graft (ADHE + fat) compared to the control (ADHE). The M2 macrophage population in the peritoneal wall was slightly diminished and the M1 population increased after peritoneal trauma; however, this effect was slightly smaller in the fat graft–treated group, and the M1 proinflammatory response was therefore lower in the fat graft-treated group. Thus, Study II proved that a fat graft can modulate the immune response and affect macrophage populations.

Our study also examined healing of the peritoneal wall. In the extensive adhesion group with fat graft (ADHE + fat) samples, the percentage of the continuous healed mesothelium was greater than in the control (ADHE) samples, suggesting similarly to Cil and Aydoghu that fat graft can accelerate the healing of the damaged PMCs.

As mentioned earlier in chapter 2.4.1 (‘ Formation of peritoneal adhesions’page 30) and 2.4.2 (‘The effect of inflammatory response’ page 32), the formation of peritoneal adhesions is the result of a series of events, involving both the coagulation cascade and the inflammation response. Both pathways originate in damaged PMCs. The effect of fat grafts focuses on two major events: (1) the inflammatory response, in which fat changes the M1 immune response towards the M2 immune response, and (2) mesothelial cell damage by accelerating healing. As a result, fat grafting improved the balance between fibrin formation and fibrinolysis, leading to fewer adhesions and an adhesion structure with less tenacity, as shown in Study II.

Most methods aimed at reducing adhesions focus on preventing adhesion formation, and there are few opportunities to treat existing adhesions. In Study II, we observed that the adhesion bands were too thin and translucent for detailed

histological analysis. Although peritoneal adhesions have similarities in collagen structure to the scar tissue of the skin, the local treatment of adhesions is different from that of wound scar tissue because of the differences in location and thickness. Fat grafts can be used to soften and improve the quality of scar tissue on the skin and reduce symptoms, such as pain (Negenborn *et al.*, 2016). However, there is no locally treatable target for fat transfer when thin bands of peritoneal adhesions are involved and the location is difficult to reach. The properties of fat grafting can be best utilized at an early stage of adhesion formation at the injury site.

Fischer et al. investigated the early onset of adhesiogenesis using both in vitro model (with human mesothelial cells) and a mouse model. They observed that profuse membrane bridges between mesothelial surfaces initiate adhesions within 1h after cell injury, the early signs of adhesions in terms of attachments between organs were visible 24 h post-injury, and after 3–5 days adhesions had spread to uninjured adjacent surfaces. The formation of adhesions is a rapid process, and scar tissue develops within two weeks in both models (Fischer *et al.*, 2020). Thus, the effects of fat grafts on adhesions may be modest after the formation has taken place, but these late effects of fat transfer require further investigation.

6.3 Strengths and limitations

Studies I-III comprehensively investigated the properties of adipose tissue at both metabolic and morphological levels. The strengths of this research are the experimental and prospective study design, utilization of different methods, and expertise in specialized fields, such as surgery, imaging, gene expression, and immunohistochemistry. The limitations are related to the technical implementation of the studies and the limited number of study subjects in both human and animal studies.

As the review of literature showed, a large number of fat graft studies regarding both browning and therapeutic properties have been carried out using animal models. The advantages of this research setup are predictability, ease of implementation, and comparability with other studies. In Study I, we used a similar research protocol using a mouse model as in Tervala et al. (Tervala *et al.*, 2014), which is common in other experimental studies in this field. Using a mouse model, we successfully tested different white adipose tissues (epididymal, visceral, and subcutaneous) in different recipient areas (subcutaneous and muscular locations) and studied their metabolism and histology. This mouse model also enables the use of different mouse species to provide additional information to support hypotheses that is not possible in human studies. We chose an athymic nude mouse in Study I as the recipient to prevent a graft rejection response, but choosing this type of mouse may have increased the

inflammatory reaction in the grafts. In Study II, we utilized CAG-DsRed fat grafts for IF analysis and macrophage phenotyping.

The effects of fat grafts on peritoneal adhesions have not been previously studied; therefore, in Study II, we chose a mouse model to test our hypothesis. Before final selection, the study protocol was carefully tested using different adhesion induction methods (causing damage to the peritoneal wall and cecum, number of sutures, and location of the fat graft). We used the same mouse species as both the donor and recipient, and the postoperative inflammatory reaction in the fat graft was more moderate than that in Study I.

Although studies I and II represented experimental models and basic research, the aim of our investigation was to apply our data to clinical research, and to understand and learn new characteristics of fat grafting. Our research group started to investigate the browning of fat grafts using a mouse model (Study I) and expanded that study to clinical patients (Study III). We were able to utilize the ^{18}F-FDG-PET protocols of other research groups investigating BAT metabolism, but the challenge in our study was the variability of fat graft areas; therefore, the imaging protocol had to be modified for each patient. The PET/MRI device used also did not have the same body coil typically used in clinical breast MRI, and the resulting images in the study patients with breast area fat grafting were not fully comparable to clinical imaging in terms of patient positioning. The original study protocol also included a perfusion scan using ^{15}O-radiowater, which is often used for BAT imaging. However, owing to technical difficulties, we were unable to obtain successful perfusion imaging of the patients in both cold and warm conditions, and no conclusions could be drawn from the individual results.

As discussed in Study III, this clinical study has several limitations, mostly regarding tissue samples. Tissue samples could only be collected from a limited area, they do not necessarily purely represent the fat graft, and there was no possibility of inducing and activating beige fat at the time of sample collection. Several different immunohistochemical stains were tested, but they did not provide additional information to support this hypothesis. H&E and UCP1 staining were found to be the most useful in this analysis. *Ucp1* gene expression analysis would have required a separate piece of tissue to be immediately placed in liquid nitrogen for later analysis, but this was not feasible at the time of the procedure. The small number of subjects was considered a limitation; however, the imaging studies were originally planned as a pilot study.

The time period of the studies can also be considered a limitation. In Study III, months had passed since fat transfer before the study analysis (8-58 months in PET/MRI patients, 3-13 months in tissue sample patients), and the morphology and metabolism of fat grafts could not be studied at an earlier stage, as in Study I. However, late effects appeared reliably during this study period. In contrast to Study

III, the browning of fat in Study I was studied for the first 12 weeks after surgery. This follow-up time is also common in humans; fat grafts often vascularize during this time and their final volume can be estimated. The most controversial follow-up period was in Study II. The 30-day study period was chosen to investigate the effects of fat transfer during the early stages of adhesiogenesis. As stated in the discussion, the formation of adhesions is a fast process, and the initial inflammatory response and rapid repair of mesothelial cell damage are of great importance. Study II showed that the therapeutic properties of a fat graft placed immediately on the injured area could prevent adhesion formation, indicating that the time period for testing the hypothesis was sufficient. However, a longer follow-up period could have provided more information about the late effects of the fat graft after adhesions and scars had matured.

6.4 Future prospects of fat grafts

Our studies showed that glucose metabolism in fat graft areas is different from that in normal WAT; thus, the metabolic effects of fat transfer could be studied at the systemic level. A recent review by Davis et al. summarized the metabolic effects of fat transplantation and found that fat grafting has potential for significant metabolic benefits in animal studies; however, to date, the systemic effects of autologous fat grafting in humans have not been investigated (Davis *et al.*, 2023). Using our Study III design as a background, a prospective study examining the systemic effects could be created. Glucose/lipid concentrations and systemic inflammatory markers of fat graft patients could be measured before and after the procedures to determine if there were changes in values postoperatively compared to baseline. These results could be compared to ^{18}F-FDG-PET imaging, particularly for patients whose glucose metabolism in fat-grafted areas is clearly activated by cold. Systemic effects are unlikely to be achieved with small fat transfer volumes; therefore, the study subjects would include large-volume fat transfer patients. However, external factors affecting fat browning pose a challenge to this study, and may be difficult to control. Studies I and III, as well as previous studies worldwide, indicate that browning is a multifactorial phenomenon. Fat transfer as a procedure alone or the different stages of subsequent fat graft survival are not sufficient stimuli to bring about the permanent existence of beige fat; instead, browning of WAT requires regular stimuli to maintain its activity and beige morphology. Therefore, it can be difficult to assess the systemic effects of fat transfer alone but this would be an interesting and worthwhile research to carry out in the future, and may provide interesting benefits to fat transfer procedures.

In Study II, the fat grafts prevented the formation of peritoneal adhesions in mice. Whether the same research setup can be applied in clinical trials is an interesting

question. Fat grafts can be used safely and rapidly for many indications, and are already under examination for various scar conditions and strictures. Therefore, it is both ethically and surgically possible to perform fat grafting in patients who undergo laparotomy. However, the challenge is to determine the location of the fat transfer. In abdominal operations or peritoneal infections, mesothelial injury may be too wide or difficult to define, and therefore unsuitable for treatment with fat grafting. Intra-abdominally placed grafts are quickly flushed away with peritoneal fluid, and the effect may be insufficient. Immune response polarization from M1 to M2 occurs quite soon after injury, and intra-abdominal fat grafting may have a positive influence on this event, even within a short effect time. The effect of fat transfer in patients cannot be evaluated externally or by imaging; thus, evaluating its effects would require a long follow-up period. End-point events include complications caused by adhesions that require hospitalization and/or reoperation.

The late effects of fat grafts in the prevention and treatment of peritoneal adhesions are under investigation by our research group using the same mouse model and study design as in Study II. Study II demonstrated that immediate preperitoneal fat grafts can affect the early stages of adhesiogenesis; however, we also investigated whether fat grafts can affect peritoneal adhesions four weeks after peritoneal injury and adhesion induction. The follow-up period after fat grafting was four weeks (eight weeks in total). These results could also be used when planning a clinical trial, as they provide more information about the optimal time for fat grafting.

6.5 Summary

This dissertation offers additional information on the properties of fat. Fat grafting can induce browning of WAT, as demonstrated by tissue analysis of recipient site fat graft samples and expression of the *Ucp1* gene in a mouse model (Study I), and by a cold-induced increase in glucose uptake in ^{18}F-FDG PET/MRI imaging of fat graft patients (Study III). Although the importance of single fat transfer for the browning of WAT and the formation of beige adipose tissue is small, these studies show that fat grafting has a greater effect on fat metabolism and morphology than previously known.

The wound-healing properties and anti-inflammatory effects of fat also have a lot of potential to expand into new indications, as demonstrated in Study II, where fat grafting promoted the healing of mesothelial cells and anti-inflammatory responses, thus preventing the formation of peritoneal adhesions in mice. Owing to the easy and safe implementation of fat transfer, the application could also be used in a clinical trial in the future.

7 Conclusions

I Glucose uptake increases during cold exposure at fat graft areas in humans and thus resembles the metabolism of brown adipose tissue, suggesting the presence of beige adipose tissue (Study III)

II Fat transfer can induce browning of white adipose tissue based on tissue morphology and *Ucp1* gene expression (Study I)

III Immediate fat grafting into peritoneal injury area can prevent the formation of peritoneal adhesions by promoting the healing of mesothelial cells and the anti-inflammatory response (Study II)

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