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The role of genetics in regulation of weight loss and food intake

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Abstract

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While obesity is a world leading health problem, the most efficient treatment option for severely obese patients is Roux-Y gastric bypass (RYGB) surgery. However, there are large inter-individual differences in weight loss after RYGB surgery. The reasons for this are not yet elucidated and the role of genetics in weight loss-regulation is still not fully understood. The main aim for this thesis was to investigate the effects of common obesity-associated genetic variants and their effect on weight loss and food intake.

We examined if the weight loss two years following RYGB surgery depends on the *FTO* genotype, as well as pre-surgery vitamin D status. For *FTO* AA-carriers, the surgery resulted in a 3% per-allele increased excess BMI loss (EBMIL; P=0.02). When split by vitamin D baseline status, the EBMIL of vitamin D deficient patients carrying AA exceeded that of vitamin D deficient patients carrying TT by 14% (P=0.03). No such genotypic differences were found in patients without pre-surgery vitamin D deficiency.

As the influence of individual single nucleotide polymorphisms may be small, we identified a novel method to combine SNPs into a genetic risk score (GRS). Using the random forest model, SNPs with high impact on weight loss after RYGB surgery were filtered out. An up to 11% lower EBMIL with higher risk score was estimated for the GRS model (p=0.026) composed of seven BMI-associated SNPs (closest genes: *MC4R, TMEM160, PTBP2, NUDT3, TFAP2B, ZNF608* and *MAP2K5*).

Pre-surgical hunger feelings were found to be associated with EBMIL and the SNP rs4846567. Before surgery, patients filled out the Three Factor Eating Questionnaire and were genotyped for known BMI and waist-hip ratio (WHR) associated SNPs. Patients with the lowest hunger scores had up to 32% greater EBMIL compared to the highest scoring patients (P=0.002). TT-allele carriers of rs4846567 showed a 58% lower hunger feelings. TT- carriers also showed a 51% decrease in disinhibition, but no significant impact on cognitive restraint was observed.

Due to the association of eating behaviour and weight loss, acute effects on DNA methylation in response to a food intake intervention of a standardized meal were also investigated.

After food intake, 1832 CpG sites were differentially methylated compared to the baseline after multiple testing correction. When adjusted for white blood cell fractions, 541 CpG sites remained. This may be interpreted as that the immune system is playing an active role in the response to food intake and highlights the dynamic nature of DNA-methylation.

These findings will contribute to a better care for morbidly obese patients. Post-surgical treatment may be optimized so that patients with a less favourable genetic profile may receive additional support for weight loss and weight management. This may be considered as a step in the transition towards personalized medicine.

Keywords: FTO, RYGB, LYPLAL1, TFEQ, Genetic Risk Score, methylation, food intake

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Patria et artes

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Bandstein M, Schultes B, Ernst B, Thurnheer M, Schiöth HB, Benedict C. (2015) The Role of *FTO* and Vitamin D for the Weight Loss Effect of Roux-en-Y Gastric Bypass Surgery in Obese Patients. *Obesity Surgery*, 25(11):2071–7

II Bandstein M, Voisin S, Nilsson EK, Schultes B, Ernst B, Thurnheer M, Benedict C, Mwinyi J, Schiöth HB. (2016) A Genetic Risk Score Is Associated with Weight Loss Following Roux-en Y Gastric Bypass Surgery. *Obesity Surgery*, [Epub ahead of print]

III Bandstein M, Mwinyi J, Ernst B, Thurnheer M, Schultes B, Schiöth HB. (2016) Genetic variant rs4846567 is associated with lower hunger sensation and increased weight loss following RYGB surgery. *Scandinavian Journal of Gastroenterology*, 51(9):1050–5

IV Rask-Andersen M, Bringeland N, Emil K. Nilsson, Bandstein M, Olay- Búcaro M, Vogel H, Schürmann A, Hogenkamp PS, Benedict C, Schiöth HB. (2016) Major difference in DNA methylation in blood between fasted and postprandial state; before and 160 min after meal. *American Journal of Clinical Nutrition*, [Epub ahead of print]

Additional papers

I Olivo G, Wiemerslage L, Nilsson EK, Solstrand Dahlberg L, Larsen AL, Olaya Búcaro M, Gustafsson VP, Titova OE, Bandstein M, Larsson EM, Benedict C, Brooks SJ, Schiöth HB. (2016) Resting-State Brain and the FTO Obesity Risk Allele: Default Mode, Sensorimotor, and Salience Network Connectivity Underlying Different Somatosensory Integration and Reward Processing between Genotypes. *Frontiers in Human Neuroscience*, 10:52

II Wiemerslage L, Nilsson EK, Solstrand Dahlberg L, Ence-Eriksson F, Castillo S, Larsen AL, Bylund SB, Hogenkamp PS, Olivo G, Bandstein M, Titova OE, Larsson EM, Benedict C, Brooks SJ, Schiöth HB. (2016) An obesity-associated risk allele within the FTO gene affects human brain activity for areas important for emotion, impulse control and reward in response to food images. *European Journal of Neuroscience*, 43(9):1173-80

III Ciuculete D, Bandstein M, Benedict C, Waeber G, Vollenweider P, Lind L, Schiöth HB, Mwinyi J. (2016) A Genetic Risk Score Significantly Impacts Long Time Therapy Outcome and Cognitive Abilities in Statin Users. *PLoS One*, Submitted

IV Kanders S, Bandstein M, Pisanu C, Preisig M, Schiöth HB, Mwinyi J. (2016) Pharmacogenetic evaluation of long time therapy response in major depressive disorder using polygenic risk score models. Manuscript in production.

Contents

Abbreviations

BMI	Body mass index
CpG	cytosine-phosphate-guanine
DMP	Differentially methylated probe
DNA	Deoxyribonucleic acid
DNM3	Dynamin 3
EBMIL	Excess BMI loss
FDR	False discovery rate
FTO	Fat mass and obesity-associated gene
GO	gene ontology
GRS	Genetic risk score
GWAS	Genome wide association studies
HOXC13	Homeobox C13
LYPLAL1	Lysophospholipase-like 1
MAP2K5	Mitogen-activated protein kinase kinase 5
MC4R	Melanocortin 4 receptor
miRNA	microRNA
MSE	mean squared error
NUDT3	Nudix hydrolase 3
PIGC	Phosphatidylinositol glycan anchor biosynthesis class C
PTBP2	Polypyrimidine tract binding protein 2
RNA	Ribonucleic acid
RYGB	Roux-Y gastric bypass
SD	Standard deviation
SNP	Single nucleotide polymorphisms
TFAP2B	Transcription factor AP-2 beta
TFEQ	Three Factor Eating Questionnaire
TMEM160	Transmembrane Protein 160
WBC	White blood cell
WHR	Waist-hip ratio
ZNF608	Zinc finger protein 608

Introduction

The world health organization estimates that in 2014 39% of adults worldwide were overweight and 13% were obese. The Organization for Economic Co-operation and Development (OECD) has called obesity a global epidemic[1] and co-morbidities such as diabetes, dyslipidaemia, hypertension and cardiovascular diseases continue to rise and are becoming the heaviest financial strain on public health care systems[2,3]. Due to the rapidly increasing number of morbidly obese, treatment with bariatric surgery has increased. It is the most efficient treatment option for substantial and sustainable weight loss and the most efficient surgery type is Roux-en-Y Gastric Bypass (RYGB)[4,5]. However, large inter-individual differences in weight loss are observed after RYGB surgery despite standardized surgery procedures and pre- and post-operation treatment[6]. The reasons why these differences occur is yet to be elucidated. Phenotypes, such as age and body mass index (BMI) before surgery, have been identified to influence the success in weight loss after RYGB surgery[7,8] but these still only explain a small part of the total variance[9]. Furthermore, the genetics of weight loss-regulation is reported to determine the majority of the variance[10] but the roles of specific genes are still poorly understood. If personalized medicine shall advance in this field, it is necessary to identify and understand genetic factors, biological mechanisms and lifestyle factors that may predict the RYGB treatment outcome. The knowledge of these influencing factors will have a direct effect on post-operation treatment and will be the foundation to provide the best, personalized support. In a longer perspective, understanding the mechanisms for weight loss may enable new treatments that would make invasive and irreversible surgery obsolete. Therefore, the main aim for this thesis was to investigate the effects of common obesity-associated genetic variants and their effect on weight loss and food intake. This is addressed in paper I-III.

Additionally to bariatric surgery, lifestyle factors are having a direct and immediate influence on the epigenetic profile[11,12]. The secondary aim was to explore how food intake may affect epigenetic factors in healthy volunteers (Paper VI).

Roux-en-Y Gastric Bypass

Patients treated with RYGB surgery have an average relative weight loss of 32±8% (±SD) after 1-2 years[5]. This weight loss is also sustained: ten years after gastric bypass, patients maintained an average weight loss of 25±11%[5]. RYGB surgery also has large impacts on co-morbidities. As an example, after two years, 72% of type 2 diabetic RYGB patients had gone into remission. Overall, treatment results and quality of life assessments are better for bariatric surgery compared with non-surgical weight loss treatments[4,5,13].

Genetics of obesity and RYGB

Obesity is of multifactorial pathogenesis, where several genetic, epigenetic and non-genetic factors appear to play important role[14]. A genetic influence on the development of obesity and body weight distribution has been established in both hypothesis driven studies and hypothesis-free genome wide association studies (GWAS)[9,15-19].
The single nucleotide polymorphism (SNP), which has the strongest association with BMI, is located in an intron of the fat mass and obesity-associated gene (*FTO*) on chromosome 16. It was first reported by Frayling *et al*. in 2007 and has been confirmed in the largest BMI associated GWAS to date (July 2016)[20,21]. Though the mechanism for this SNP is still unclear, the gene has been well studied and has e.g. been associated with eating behavior[22,23].

It has been suggested that up to 70% of the variance in weight loss following RYGB surgery are attributed to genetic factors[10]. However, only a small part of that variance has been reported. For example, Hatoum *et al.* included 1,020 RYGB patients and identified one SNP, rs17702901, which could explain 2.8% of the variance in relative weight loss[9]. Furthermore, it is largely unknown how these genetic variants influence post-surgical weight loss. It is therefore important to identify additional associations between genes and post-surgical weight loss, and to discover the underlying mechanisms.

Vitamin D

Vitamin D is mainly synthesized in the skin or absorbed through diet. It is metabolized in the liver to 25-hydroxyvitamin D, which is used as a biomarker to determine a patient's vitamin D status. 25-hydroxyvitamin D is metabolized in the kidneys by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) to its active form, 1,25-dihydroxyvitamin D.

Several studies have reported a high level of vitamin D deficiency in the general population (i.e. <50 nmol/l or 20ng/ml[24]). Between 40-100% of U.S.

and European elderly men and women still living in the community (i.e. not in nursing homes) are vitamin D deficient[24]. Also, a study from Maine, USA showed that 48% of white, preadolescent girls, had 25-hydroxyvitamin D levels below 20ng/ml[25]. Moreover, obese patients have a reduced bioavailability of vitamin D which exposes the group for further risk of deficiency[24,26]. Vitamin D deficiency is associated with several health risks such as insulin resistance[27], osteoporosis[24] and higher all-cause mortality[28]. In combination with this, understanding the association between weight loss and vitamin D deficiency is relevant.

Genetic risk score

A risk score is a continuous numeric factor built up by several risk contributing items. In the same manner as small streams make great rivers, the purpose is to fuse several small contributors of risk into a factor that relevantly influences a trait, such as BMI. As several SNPs have been reported to influence phenotypes in a modest, but significant way, they make good candidates for a genetic risk score (GRS). However, the selection of which SNPs to include in the analysis is important. Otherwise non-relevant SNPs will dilute the accuracy of the GRS.

Most GRSs are calculated in a weighted or unweighted manner[29]. Unweighted GRSs consider only the number of risk alleles of each SNP included in the calculation[30]. Weighted GRSs take advantage of previously reported effect estimates from the discovering GWAS, which add a dimension to the GRS as not all SNPs have the same impact[31,32]. Both types are present in literature, but when compared the weighted GRS showed better accuracy[33,34].

In Paper II we investigated the impact of BMI- and WHR-associated SNPs on excessive BMI loss (EBMIL) two years after RYGB surgery. Based on a selection of genetic variants we developed a weighted GRS and estimated the post-surgery weight loss associated with the considered risk gene variants. To select SNPs which would have a relevant impact on EBMIL, the random forest statistical method was used[35]. This machine learning tool has been used previously to screen or reduce dimensions in large GWAS datasets[36-39].

The Three Factor Eating Questionnaire

Weight loss may be affected by psychological factors related to eating behaviour such as impulse control and hunger feelings[40,41]. The choice of food may further influence a person's body weight distribution. Dietary patterns and food preference are linked to both general obesity and abdominal obesity[42,43]. The Three Factor Eating Questionnaire (TFEQ) is a tool to assess

individual eating habits by evaluating three traits: hunger, disinhibition and cognitive restraint [44,45]. Several studies indicate that the TFEQ appears to be a useful instrument to explain changes in body weight based on individual food intake behaviour. Using the TFEQ, French *et al*. detected an association between BMI and the factors hunger and disinhibition[46], while Drapeau *et al.* were able to show that changes in cognitive restraint are associated with changes in body weight[47,48]. Whether pre-operatively obtained eating behaviour scores may be linked to the magnitude of weight loss after bariatric surgery has not yet been proven[40,41].

In paper III we investigate if eating behaviour, as quantified by the TFEQ, has an impact on EBMIL after RYGB surgery and if BMI- and waist-hip ratio (WHR)-associated genetic variants may influence eating behaviour.

In a study of 792 mono- and dizygotic twins, the heritability of the TFEQ scores was estimated to be 60% for hunger, 59% for cognitive restraint and 45% for disinhibition[49]. The remaining variation would therefore be attributed to the environment, but how this is influencing human biologically is still unknown. Epigenetics is one proposed mechanism.

DNA methylation

DNA methylation is one section of epigenetics. It has the characteristics of both heritable trait and dynamic process. However, the dynamic process may span over years or change during minutes[11,50]. The DNA methylation is linked to the cells' ability to respond to their environment, to adapt and to differentiate. The methylation of cytosine in cytosine-phosphate-guanine (CpG) pairs constitutes the most studied alterations of DNA molecules within human cells. Association studies have been able to link the methylation level of specific CpG methylation sites to both disease[51-53] and phenotypic traits[54,55]. The theory is that a higher level or frequency of methylated CpG sites will lead to less expression of the nearby gene(s) due to e.g. chromatin rearrangement[50,56,57].

This has made DNA methylation into a good candidate as a biomarker[58]. Measuring the genome wide methylation in DNA from a blood sample is both non-invasive and quick.

As a very common and integrated event of daily life, food intake activates several large metabolic processes such as endocrinological pathways, modulation of the immune response and hormonal secretion. An imbalance of factors regulating food intake would directly influence weight loss and make it either easier or more difficult. The regulation of methylation patterns governing gene expression may be one of these influencing factors. Still, the dynamic changes in the methylation are poorly understood.

Aims

The aim of this thesis was to investigate the effects of common obesity-associated genetic variants and their effect on weight loss. Furthermore, to explore factors affecting food intake and their association with weight loss and genetic variances.

Secondarily, we aimed to explore how food intake may affect, or be affected by, genetic variants and epigenetic factors in RYGB patients and in healthy volunteers.

These aims were addressed in the included articles. More precisely, Paper I aimed to investigate if the common obesity associated genetic variant *FTO* A-allele predicts the magnitude of the two-year weight loss following RYGB surgery and if this depends on a patient's vitamin D status.

In Paper II, we aimed to create a GRS, which would be associated with the weight loss following RYGB surgery, and in the process indicate the most important gene variants for weight loss.

The third study aimed to investigate to which extent pre-surgery eating behaviour had an impact on weight loss following RYGB surgery, and if BMI- and WHR-associated genetic variants may have the ability to influence eating behaviour. And finally to investigate if the factors of eating behaviour are associated with weight loss after RYGB surgery.

Paper IV aimed to identify differentially methylated CpG sites following a standardized meal in healthy volunteers from the general population.

Materials and methods

Subjects and measurements

Roux-en-Y Gastric bypass patients

In the studies presented in Paper I-III, patients were included, treated and followed-up at the Interdisciplinary Obesity Center, St. Gallen, Switzerland. Included patients all underwent Roux-en-Y Gastric Bypass surgery and none had undergone any kind of bariatric surgery procedure (e.g. gastric banding) before. Patients were mainly of Caucasian descent and remitted form various parts of Switzerland. The included 251 patients had on average a pre-surgery BMI of 44.8±6.0kg/m^2. At baseline and 24 months after RYGB surgery, height and weight were measured with patients wearing light clothing and no shoes. BMI was defined as weight (kg) divided by height squared (m^2). BMI was utilized to calculate relative EBMIL (cut-off for normal-weight BMI = 25 kg/m^2) by the following equation: 100 - [(final BMI - 25 / initial BMI - 25) * 100] [59]. Since the goal is a return to normal weight, rather than the maximal weight loss, the advantage of using EBMIL compared to relative BMI loss (100 – [final BMI / initial BMI] * 100) is that it takes into account the normal body weight. A super obese patient may show the same relative weight loss as a less obese, but still be in the weight category associated with health risks. As an example, if assuming a relative weight reduction of 25%, a patient with BMI 33 kg/m^2 would become normal weight. An average patient in this cohort would have a BMI 49 kg/m^2, and this patient would have a resulting BMI 34 kg/m^2, which is still associated with higher mortality, cardiovascular disease and type 2 diabetes.

Detailed pre-operative preparations and post-operative follow-up procedures have been described by Thurnheer *et al*.[7]. In brief, patients were instructed how to adjust their diet post-surgery. Cooking- and shopping courses were also offered. Participants were advised to lose some weight before surgery and to improve their physical condition. Besides regular follow-up visits, patients received nutritional counselling and nutritional supplements e.g. iron, calcium carbonate, vitamin D and vitamin B.

The amount of patients included in each analysis varied depending on the data availability. An overview of the availability of the studied parameters, used in Paper I – III, is presented in Figure 1. This shows the completeness of the clinical data from the included 251 participants.

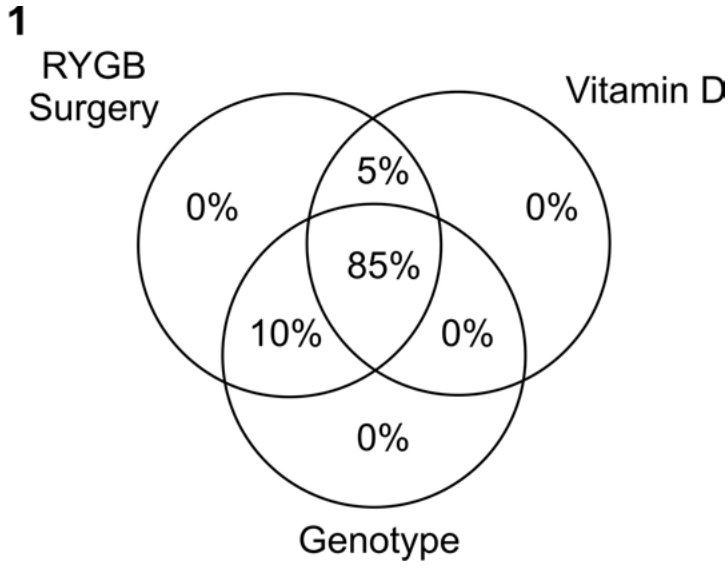

Figure 1. The Venn diagram describes the presence of key variables in the bariatric surgery cohort. The majority of the treated patients (85%) were both genotyped and had measurements for vitamin D.

Healthy volunteers with food intervention

Healthy volunteers (n=26) were included in the study presented in Paper IV. The participants were recruited from university campus areas in Uppsala, Sweden, between October 2013 and October 2014. All were male, had a BMI of 25.6±3.9 kg/m^2 and were aged 26.3±3.2 years. To be included, the volunteers needed to have normal dietary habits, be of age 18-40 and of Nordic decent. Exclusion criteria were vegetarian/vegan diet, lactose intolerance, gluten intolerance, concomitant medical therapy or smoking. The timeline of the study visit is described in Figure 2a. The participants arrived at 8 am after an overnight fast. The fasting state was confirmed with a peripheral blood glucose measurement using an Accu-Chek® Aviva, Roche. Biometric data such as height, weight was measured before the first venipuncture blood samples were drawn. For DNA extraction, and subsequent analysis, EDTA-coated tubes were used. Blood samples dedicated for RNA analysis were taken with PAXgene tubes (Qiagen) to stabilize the single stranded molecules and prevent degradation. Blood samples for DNA extraction were kept on ice before freezing in -80°C and the PAXgene tubes were treated according to the manufacturer's instructions.

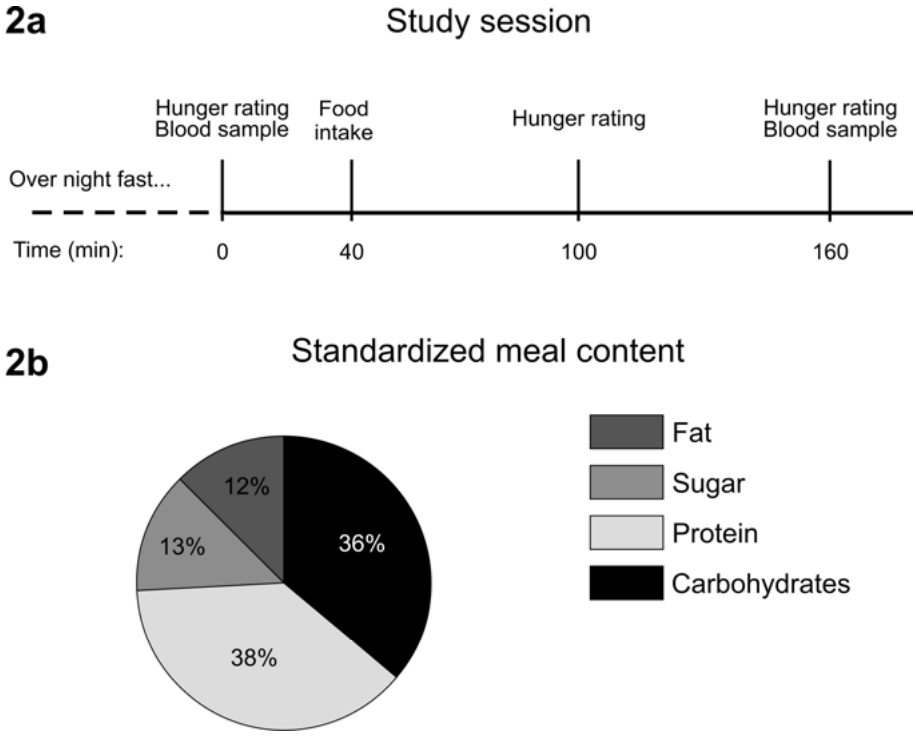

Figure 2. a) In the Food intake study healthy volunteers arrived after an overnight fast. Blood samples were taken at time 0 and 160 minutes and perceived hunger was rated by the participants at time 0, 100 and 160 minutes. During this time, a standardized meal was served. b) The standardized meal contained 490.3kcal comprising 38% protein, 36% carbohydrates, 13% sugars and 12% fat.

A food intervention was then administrated comprising a standardized mixed breakfast of whole-wheat fruitroll (Fruktkuse®, COOP) (70g) with Leerdamer cheese (30g) and 250g of Quark curd cheese (Kvarg, Arla). The meal consisted of 490.3kcal; 44.6g protein, 42.7g carbohydrates, 15.6g sugars and 14.5g fat, Figure 2b. Water (30 ml) was provided as a beverage. The meal was consumed within 15 minutes. A second blood sample was taken 160 min after the first venipuncture. This time point was selected as it was considered to be sufficient time for the meal to be digested, nutrients to have entered the blood stream and produce an effect on downstream molecular mechanisms. The volunteers also rated their perceived hunger at time 0, 100 and 160 minutes.

Bariatric surgery

Two different variants of RYGB surgery were performed on the included patients: proximal and distal RYGB [7]. In both procedures, a large part of the

stomach was transected, and a small gastric pouch of about 20–30 ml was anastomized to the proximal jejunum with the diameter of the pouch–jejunal anastomosis standardized to be about 12 mm. In the proximal RYGB procedure, the biliopancreatic limb was side to side anatomized to the jejunum 150 cm distal from the pouch–jejunal anastomosis (Roux-en Y limb length, 150 cm). In the distal RYGB procedure, the biliopancreatic limb was side to side anatomized to the ileum 60 to 100 cm proximal from Bauhin's valve (common channel, 60–100 cm). The length to the biliopancreatic limb was approximately 60 cm in the proximal and 60 to 100 cm in the distal RYGB procedure. Surgery type selection depended on clinical factors assed by a multidisciplinary team. These factors included for the distal surgery type (more malabsorptive) high BMI, type 2 diabetes, sleep apnea and unfavourable eating behaviour.

Vitamin D measurement and supplementation

Blood samples from the RYGB-patients, were drawn in the morning (8 am–11 am) after an overnight fast, and serum 25-hydroxyvitamin D3 levels were determined. Serum levels of 25-hydroxyvitamin D3 lower than 50 nmol/l were defined as vitamin D deficiency[60]. Since 25-hydroxyvitamin D3 levels exhibit seasonal variation in obese humans[61], the date when blood was collected was recorded. Post-surgery, all patients received standard oral vitamin D3 supplements (1200 IU/day). If patients' serum levels of 25-hydroxyvitamin D3 levels were below 50 nmol/l at follow-up investigations (i.e. 3, 6, 9, 12, 18, and 24 months), additional intramuscular injection of 300 000 IU were performed every 3 months.

TFEQ administration

The Three Factor Eating Questionnaire was used to assess eating behaviour prior to RYGB surgery. It was distributed to the patients at the clinic in the morning after an overnight fast. The TEFQ comprises 51 questions, which measure three dimensions of eating behaviour: hunger (14 questions), disinhibition (16 questions), and cognitive restraint (21 questions).

DNA preparation, genotyping and methylation assay

Genomic DNA was extracted from peripheral blood samples. The phenol/chloroform method was used by the Latvian Biomedical Research and Study Center in Riga, Latvia[62].

Patients from the RYGB cohort were genotyped for 35 single nucleotide polymorphisms. All has been associated with BMI or waist-hip ratio in large genome-wide association studies, and has a reported minor allele frequency of at least 15%. The analysed SNPs include *FTO* associated SNP rs9939609 and *LYPLAL1* associated SNP rs4846567[17,18]. The full list of investigated genetic variants is provided in Supplementary table 1 of Paper II. All determined SNPs were in Hardy-Weinberg equilibrium and none were in linkage disequilibrium. All participants were genotyped using a custom Illumina iSelect genotyping array (99.5% success rate).

For the genome-wide methylation analysis, the genomic DNA from the 26 participants was bisulfite converted using the EZ-96 DNA Methylation Gold Kit from Zymo Research. Product No: D5007 with 500 ng of DNA per sample. The bisulfite converted DNA was eluted in 30µl. Fifteen microliter, equivalent to approximately 200ng of bisulfite converted DNA per sample, was removed, evaporated to a volume of <4µl, and used for methylation analysis using Infinium HumanMethylation450 BeadChip array v1.2 (Illumina, San Diego, USA). Both genotyping and methylation analysis were performed at the SNP&SEQ Technology Platform in Uppsala, Sweden.

RNA preparation and RNA expression array

The frozen blood was thawed in room temperature and extracted using the PreAnalytiX PAXgene® Blood RNA Kit (Produced by QIAGEN GmbH, Cat No. 762174), in accordance with the manufacturer's protocol by the Latvian Biomedical Research and Study Center in Riga, Latvia. Before further analysis, the amount of RNA fragmentation was evaluated using the Agilent 2100 Bioanalyzer system (Agilent Technologies Inc, Palo Alto, CA). The extracted RNA was analyzed on the GeneChip® Human Gene 2.1 ST Array (Affymetrix Inc., Santa Clara, CA) which measures >40,000 coding and non-coding human transcripts. The experiments were done at the Array and Analysis Facility, Science for Life Laboratory at Uppsala Biomedical Center (BMC), Uppsala, Sweden.

Micro array data processing

Methylation

Data processing was performed in R-Studio[63]. Raw data files were background-corrected using *methylumi*-package[64], normalized using the *asmn*-package[65] and adjusted for probe type using Beta Mixture Quantile normalization (BMIQ)[66]. Due to the main source of DNA in the samples originates from leucocytes, the methylation values were adjusted for White blood cell

(WBC) fractions according to the method by Jones *et al.*[67]. WBC fractions were estimated from the methylation data using the estimateCellCounts function of the FlowSorted.Blood.450k package[68,69].

RNA expression

The array raw data was normalized using robust multi-array average (RMA)[70] in Expression Console, provided by Affymetrix (http://www.affymetrix.com). The WBC fractions adjustment method by Jones *et al.* was modified and applied for the RNA output data.

Ghrelin measurement

Plasma samples were collected from the healthy volunteers. The blood samples were stored on ice before they were centrifuged at 1,300g at 4°C for 10 min. Plasma were extracted and then frozen in -80°C.

Ghrelin was measured by enzyme linked immunosorbent assay (ELISA) using a commercially available kit (EZGRT-89K; Millipore Billerica, MA, USA).

Statistical analysis

Paper I

Multiple linear regression model was used to perform the regression analysis with EBMIL as the dependent variable. Predictors of interest were the three-level *FTO* rs9939609 genotype (assuming an additive model; TT=0, AT/TA=1, AA=2), baseline serum vitamin D levels, and their interaction term. For this analysis 210 patients had available data. Linear-mixed effect model, which may utilize repeated measures, were used to investigate if *FTO* genotype groups, split by baseline vitamin D status, would exhibit post-surgery differences in the time course of plasma levels of this micronutrient (i.e. comprising measurements at 3, 6, 12, 18, to 24 month). All analyses were adjusted for age, sex, BMI at baseline, and surgery type (distal or proximal RYGB), unless otherwise specified. In addition, in models where EBMIL was the dependent variable, the model was controlled for the seasonal time point, i.e. when the patient's baseline session took place. These analyses were performed in SPSS Statistics for Windows, Version 21.0 (IBM Corp. Released 2013. IBM. Armonk, NY: IBM Corp.).

Paper II

The genotype of each SNP was coded based on the amount of effect alleles, i.e. 0 for no effect alleles, 1 for heterozygote carriers and 2 for individuals carrying two effect alleles. Subsequently, SNP-associated beta-values, as published by Speliotes *et al*. resp. Heid *et al*., were multiplied with the amount of alleles to obtain weighted SNP scores. Weighted scores were included in the random forest model[35] as predictors and EBMIL as the dependent variable. Ten thousand random decision trees were created. The random forest model result for the weighted BMI-associated SNPs showed, when plotted, a clear change in mean squared error (MSE) around 10% (Supplementary figure 1 in Paper II). SNPs above 10% MSE were considered having a relevant influence on the model and were chosen for inclusion in the genetic risk score. The change in MSE was also seen in the random forest results for the weighted WHR SNPs, although not as clear. The cut off for 10% was therefore used in that model as well. The trajectory direction for each SNP with a MSE >10% (i.e. if the SNP increased or decreased EBMIL) was tested by performing a preliminary linear regression analysis. The coding of a BMI- or WHR-associated SNP was inverted in case an association with EBMIL-increase was detected.

GRS were calculated by summing up the weighted SNP scores for all variants that induced a MSE >10% in the random forest model according to the formula: $\sum n\ effect\ alleles_{SNPi}\ x\ beta\ value_{SNPi}$ (i = number of included SNPs in the model, n = number of risk alleles). The GRSs were used as continuous variables in a multiple linear regression with EBMIL as the dependent variable, adjusting for age, sex, initial BMI and surgery type. Student's t-test was used in the post-hoc analysis to compare weight loss between GRS quartiles. P-values<0.05 were considered significant and, if necessary, adjusted for multiple testing according to Benjamini-Hochberg (BH) with the Q-level of 5%. Analyses were performed using the CRAN package *Rattle* in R studio [63,71] and SPSS Statistics for Windows, Version 22.0 (IBM Corp. Released 2013. IBM. Armonk, NY: IBM Corp.).

Paper III

To determine which of the BMI- or WHR-associated SNPs had a significant impact on the TFEQ factor outcome, the genetic variants were included as covariates in a multiple linear regression model, besides age, sex and preoperative BMI. Initially, a genetic additive effect model was assumed and included in the analyses, coding the genotypes as 0 (two major alleles), 1 (heterozygote) and 2 (two minor alleles), respectively. In case that the plotted result clearly indicated a recessive or dominant relationship (i.e. the score from two genotypes were visually similar and different from the third), the SNP was recoded accordingly (i.e. 0 for homozygous and heterozygous ma-

jor allele carriers, 1 for homozygous minor allele carriers) and considered in the analyses.

Genetic variants that were shown to have a significant impact on TFEQ outcome were further investigated and included as a covariate in a multiple linear regression model to study their impact on relative weight loss two years after surgery (EBMIL). The model was adjusted for age, sex, initial BMI and surgery type and included 235 patients. The relative BMI loss was calculated according to Deitel and Greenstein and was used as a confirmatory dependent variable in genetic association analyses[59]. The impact of the preoperative TFEQ factor scores on EBMIL was analysed using a multiple linear regression model, adjusting for age, sex, pre-surgery BMI and surgery type. Post-hoc t-tests were performed to compare if the quartiles of TFEQ scores were associated with EBMIL. Bonferroni-adjusted P-values<0.05 were considered significant and calculated using the p.adjust-function in R[63]. Analyses were otherwise performed using SPSS Statistics (version 22.0 for Windows, IBM, Chicago, IL, USA).

Paper IV

Linear models for microarray data were utilized via the limma R-package[72,73] to analyse the change in methylation for each probe. Our study design allowed accounting for variation between technical replicates by including correlation between technical duplicates in our linear models utilizing the "duplicateCorrelation" functions included in the limma package[74]. Empirical bayesian statistics was used to calculate moderated t-statistics for each analysed probe.

As a quality control step, a hierarchal cluster plot was made assuming that the paired intra-individual variance should be less than the inter-individual variance. One subject was therefore excluded for further analyses due to deviation from this.

Differentially methylated probes (DMPs) were tested for correlation with gene expression of related mRNA transcripts as measured on the GeneChip® Human Gene 2.1 ST Array. Transcripts corresponding to DMPs were determined from annotation provided by Illumina[75] or by manual query of methylation probes using the UCSC genome browser at https://genome.ucsc.edu/. Tests for correlation between methylation and gene expression were performed for both unadjusted and WBC-adjusted DMPs by calculating Pearson product-moment correlation coefficients and testing against the null hypothesis of no correlation using the cor.test function included in the stats package in R.

In order to search for differentially expressed transcripts between the fasted and postprandial state, a robust version of the empirical Bayes moderated paired t-test was applied using the limma package[73,76]. To address the prob-

lem with multiple testing, the p-values were adjusted using a 5% false discovery rate[77].

Ethics statements

All studies adhere to the Declaration of Helsinki and its later amendments. Study participants provided written informed consent to the use of their clinical data and blood samples for genetic analyses. The studies on Roux-en-Y Gastric bypass patients were approved by the Cantonal Ethic committee St. Gallen (Kantonal hospital St. Gallen, Flurhof 7, 9007 St. Gallen, Switzerland). The regional ethic committee in Uppsala also approved the analysis performed in Sweden (DNR:2016/181). The food intervention study was approved by the regional ethical review board in Uppsala, Sweden (DNR:2010/201).

Results and discussion

Results of Roux-en-Y Gastric Bypass

On average, the studied RYGB patients had lost more than 80% of their excess body weight (83.4±17.9 (mean±SD)) two years after their surgery. It was also confirmed that younger age and lower initial BMI are beneficial factors for RYGB surgery weight loss (β=-0.23, P=0.031 resp. β=-0.73, P<0.001)[30]. However, gender and surgery type did not significantly impact changes in excess weight loss in the studied cohort.

Paper I

In Paper I we examined if the *FTO* SNP is associated to weight loss after RYGB and if vitamin D levels may impact this association. We report that the minor allele of *FTO* SNP rs9939609 (A) was significantly associated with higher excess BMI loss two years after RYGB surgery, as revealed by a multiple linear regression model analysis (dependent variable coefficient B(AA)=20.7, B(TA/AT)=13.3, B(TT)=0 (reference value), P=0.02). The per-allele effect was 3% EBMIL for each *FTO* A-allele (EBMIL, AA, 86.1±2.3%; TA/AT, 83.0±1.7%; TT, 81.5±2.3%; P=0.02).

Large cross-sectional genome-wide association studies have demonstrated that humans who carry the rs9939609 A allele have higher BMI values than non-carriers with an average per-allele explained variance of 0.17kg/m^2 [20,78-80]. Interestingly, patients in our study who carried the rs9939609 A-allele exhibited the highest EBMIL after RYGB surgery. This may be counterintuitive at first glance, but our findings are supported by a number of previous observations. For instance, in a study involving 520 obese patients, bariatric surgery-induced weight loss was largest in those who carried the rs9939609 A-allele[81-83], although there are conflicting reports[84]. It has also been proposed that AA-carriers have more initial weight to lose, and therefore have higher EBMIL-values. Conversely, this is not supported by our findings or the literature since lower initial BMI were shown to be beneficial for EBMIL[30].

In 2013, Louchenço demonstrated that children who carried the *FTO* A-allele showed a greater weight gain over five years compared to non-carriers; but only in those who were vitamin D deficient [85]. Due to the reported findings by Lourenço, serum levels of 25-hydroxyvitamin D3 were included in

the model as a covariate. This confirmed that the strength of this association in the linear model was influenced by pre-surgery levels of serum 25-hydroxyvitamin D3 (P=0.04 for the interaction term '*FTO**vitamin D level'). When the cohort were split by patients' baseline vitamin D status, AA-carriers who were vitamin D deficient exhibited a surgery-induced EBMIL that was 14% higher than that of vitamin D deficient TT carriers (P=0.03). In contrast, no such genotypic differences could be observed in patients without pre-surgery vitamin D deficiency. The pre-surgery vitamin D status was not independently linked to EBMIL (P=0.81) but only in the interaction term with *FTO*. This finding of an interaction between the pre-operative vitamin D status and the *FTO* A-allele in regard to weight loss, further reveals new aspects of the association between *FTO* and body weight regulation in humans. It suggests that vitamin D may possess biological properties that can regulate the magnitude by which *FTO*-associated genetic risk factors impact body weight[86,87].

Baseline serum levels of 25-hydroxyvitamin D3 revealed that 49.5% of the patients in the cohort were vitamin D deficient, compared to 9.1% who were vitamin D deficient two years after surgery (P<0.01). A Fisher's Exact Test showed that serum 25-hydroxyvitamin D3 levels after surgery did not differ between *FTO* genotype groups (P>0.05 at all time-points post-surgery). The mean vitamin D level rose quickly after surgery (and initiation of vitamin D supplementation). Among the pre-surgery deficient patients, only 30% remained deficient at the first follow-up visit (three months post-surgery) and 11% after 24 months. Some patients with baseline levels above the deficiency cut off were deficient 24 months after surgery.

A major part of the vitamin D available in the body is due to the synthesis by the skin after sun exposure. Vitamin D deficiency may therefore partly be a result of a lifestyle predominantly spent indoors and in sedentarism[88-91]. Moreover, a previous study involving patients who underwent bariatric surgery has shown that patients significantly increase the time spent in leisure activities after the surgery[92]. With this in mind, it could be argued that RYGB surgery treatment may have led to increased time spent in leisure activities especially in those patients who were most sedentary before the surgery, i.e. obese patients who were vitamin D-deficient. Of note, physical activity has been shown to counteract the impact of *FTO* on body weight in humans[93]. In the same way, physical activity may boost the weight loss after RYGB surgery

Paper II

This is the first study that developed GRSs to investigate the impact of common BMI and WHR-associated SNPs on post-operative EBMIL after RYGB surgery using the random forest method as a novel selection approach for relevant SNPs. The EBMIL estimates differed up to 11% depend-

ing on the SNP configuration in both the BMI-associated and the WHR-associated model.

To be able to take advantage of the SNPs' weights reported in previous genome-wide association studies, two separate models were made. The first included 23 SNPs associated with BMI as identified by Speliotes *et al.*[18]. The second model considered 12 WHR-associated SNPs [17]. Seven genetic variants associated with BMI and three variants associated with WHR induced a >10% MSE and were considered in the subsequent calculation of GRS scores.

The first random forest model comprised the BMI-associated SNPs within or close to the genes *MC4R, TMEM160, PTBP2, NUDT3, TFAP2B, ZNF608* and *MAP2K5*. The weight value of these multiplied with the number of effect alleles, were summed up generating a individual weighted genetic risk score, GRS_{wBMI}. The score range was 3 to 37. The GRS_{wBMI} was significantly negatively associated with EBMIL (β=-0.32, P=0.026) in the multiple linear regression model adjusting for age, sex, initial BMI and surgery type, indicating a 0.32% decrease of EBMIL per score unit. Maximum and minimum GRS_{wBMI}-score were associated with 83% and 89% EBMIL, respectively.

In the same way as GRS_{wBMI} was generated, WHR-associated SNPs were included in the random forest model. SNPs close to the genes *HOXC13*, *LYPLAL1* and *DNM3-PIGC* had a MSE >10% and were used to create GRS_{wWHR}. The score ranged from 0 to 19 and was associated with EBMIL when using multiple linear regression analysis (β=-0.59, P=0.021). Maximum and minimum GRS_{wWHR}-scores were associated with 78% and 89% EBMIL, respectively.

These findings suggest a relevant and additive effect of genetic variants on EBMIL, translating into about 1.9 kg/m^2 average weight loss. Speliotes *et al.*[18] reported a per-allele impact of 0.17kg/m^2 for the strongest BMI-associated SNP, *FTO*. Our observations strengthen the hypothesis that the consideration of several risk SNPs helps to estimate post-operative weight response more accurately than isolated genetic variants, and can be considered as a complement to the predictive clinical factors such as age and initial BMI. A previously reported generic risk score developed from all 32 SNPs reported by Speliotes *et al*. was associated with BMI but not with weigh loss[94]. It may be speculated that the random forest model is necessary to select the influencing SNP for the particular trait i.e. excess weight loss compared to BMI.

Not all previously reported effect alleles linked to BMI and WHR in GWAS corresponded to be the effect allele reported in this study (Paper II, table 2). The observed differences in effect may be attributed to the difference in the investigated trait, i.e. BMI and WHR were investigated in other studies compared to EBMIL investigated in our study. Furthermore, external factors, such as exercise, may impact the strength of association between SNP and trait, as e.g. observed for the gene *FTO*[93].

When the patients were divided into quartiles depending on their genetic risk score, the quartile with lowest score, quartile 1, lost more weight than patients in quartile 2-4. Interestingly, there were no differences between the quartiles 2-4 regarding EBMIL and suggest that that the risk alleles do not follow a strictly linear effect pattern. Moreover, this was seen in both the GRS_{wBMI}-model and the GRS_{wWHR}-model.

Paper III

In Paper III, we investigated pre-operative appetite sensations and BMI- and WHR-related genetic variants, and their relation to the weight loss response after RYGB surgery. The SNP rs4846567, located on chromosome 1 with the *LYPLAL1* as the closest gene[17] was associated with hunger in the TFEQ. Rs4846567 was the only SNP out of 32 BMI-, and WHR-associated genetic variances that remained significant after Bonferroni-correction (adjusted P=0.045). TT-allele carriers of the variant rs4846567 showed a 58% lower hunger-associated score compared to GG+GT carriers (TT: 4.2±3.6, GG+GT: 7.3±3.2, adjusted P=0.022). Furthermore, TT-allele carriers showed a 51% decrease in disinhibition (TT=5.7±2.9, GG+GT=8.7±3.2, adjusted P=0.048) but no significant impact on cognitive restraint was observed.

Eating behaviour and its relation to BMI have been described earlier[48]. Appetite, or hunger feelings, may be considered as a summation of psychological drivers and metabolic processes. This would partly explain why some individuals have a stronger predisposition to accumulate weight than others. Many genes associated with BMI are mainly expressed in the central nervous system[21] which may further support the presence of genetic drivers which increase appetite which leads to obesity. This would also explain some of the large inter-individual differences in weight loss observed after RYGB surgery. Unsurprisingly, eating behaviours change after a RYGB surgery[95-97].

To investigate if rs4846567 had an impact on weight loss, the SNP was inserted as a factor in a linear regression model with EBMIL as the dependent variable. Homozygous carriers of the SNP showed a 7% higher EBMIL two years after RYGB surgery (90±15%) compared to GG- and GT-allele carriers (83±18%; P=0.031). When using the relative BMI loss as dependent variable, the TT-allele carriers had also a significant higher weight loss compared to the GG- and GT-allele group.

An inverse association was observed between per-surgery hunger scores and EBMIL after surgery (β=-2.52, P<0.001). Especially individuals with low hunger scores lost weight after surgery. Patients in the lowest hunger score quartile lost 32% more excess weight than individuals in the quartile of patients with highest scores. Similar results were obtained when using rela-

tive BMI loss as the dependent variable. No significant associations were observed between EBMIL and disinhibition resp. cognitive restraint.

Results of food intervention

Paper IV

After food intake 140,828 probes were differentially methylated compared to the baseline i.e. when the participants were fasted. After Bonferroni correction for multiple testing 1832 probes remained significant. When further adjusting for WBC fraction 541 probes were significantly methylated differently. Adjusting the methylation data to account for changes in WBC fractions between sampling allows us not only to correct for individual differences, but also to adjust for the confounding effects of alterations in WBC populations between sampling times. The loss of significant probes would indicate that the majority of the changes in methylation after food intake would to be attributed to alterations in WBC populations.

Enrichment analyses were performed via the online tool Enrichr[98], using the gene ontology (GO) Biological Process library and intracellular pathway libraries such as KEGG. The closest transcripts to the DMP, according to Price[75], were used in the analysis. The analyses were first performed including the 1832 DMPs without WBC fraction adjustment. The unadjusted set showed enrichment of genes associated to immune response, cell activation and response to wounding, while the pathway analysis showed enrichment of genes involved in T cell and B cell receptor signalling.

The 541 WBC fraction adjusted DMPs were enriched for genes involved in regulation of cell morphogenesis, as well as genes involved in glycan structure biosynthesis, axon guidance, O-linked glycosylation, P75 neurotrophin receptor-mediated signalling, O-glycosylation of TSR domain-containing proteins and spinal cord injury. This shows a clear profile shift away from factors mostly associated with the immune system.

Eleven subjects were analysed for differential gene expression. One subject was excluded due to the quality of the methylation data mentioned above. 2,252 RNA-transcripts were differentially expressed after food intake when adjusted for a false discovery rate of 5%. Out of these transcripts, 44% were upregulated. The GO biological process enrichment analysis of the upregulated transcripts showed the top terms differentiation of lymphocytes and differentiation of T cells, which are associated to the adaptive immune system. The downregulated transcripts also revealed GO terms involved in immune response but more related to innate immunity such as inflammatory response, response to wounding, cytokine secretion and response to bacterium.

The 541 differentially methylated probes adjusted for WBC fractions did not correlate to any differentially expressed transcripts. A targeted approach was also conducted for a few candidate genes as suggested by Adan *et al*.[99]. These included ghrelin, glucagon-like peptide 1, insulin receptor and neuropeptide Y. Out of the 541 WBC fraction adjusted DMPs, one was found in the promoter of neuropeptide Y and one in the promoter of the insulin receptor. As ghrelin have been reported to be expressed in leucocytes[100], the unadjusted DMP set was also investigated if the DMPs were present in the candidate genes' promoters. Of these DMPs, seven were encountered in preproghrelin, four within the promoter region of leptin and three within the promoter region of pro-opiomelanocortin.

Plasma levels of ghrelin were determined and the methylation in the preproghrelin promoter was found to be correlated with ghrelin plasma levels. However, the methylation did not correlate with ghrelin expression levels. This may suggest a feed-back system where the change in plasma level concentration may promote a change in the methylation profile.

Conclusion

Weight loss following RYGB surgery

Weight loss after RYGB surgery have been the major outcome variable in this thesis. The reported inter-individual difference in therapy success of weight reduction surgery is coupled to several factors. Some of these factors have been confirmed in our studies and other influencing factors are novel. Put together, they form an interesting network of associations (Figure 3). The well reported BMI-associated *FTO* SNP was associated to excess BMI loss in our cohort, but only if the patient was vitamin D deficient.

Using the *FTO* SNP and several other BMI and WHR associated SNPs, the most influential were identified using random forest models and combined into genetic risk scores. One GRS comprised seven BMI associated SNPs and the other one three WHR-associated SNPs, but both showed an estimated weight loss difference of up to 11%.

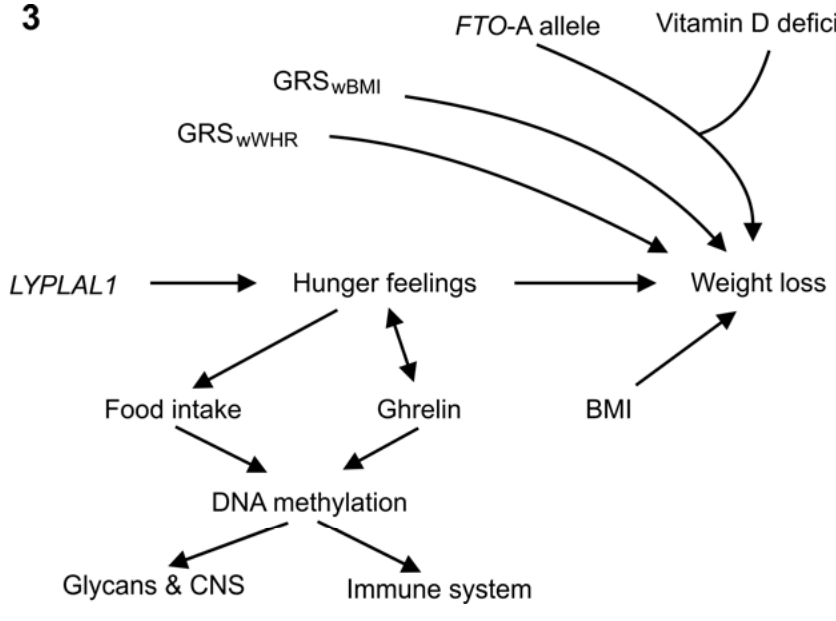

Figure 3. The figure indicates an overview of the discovered and confirmed associations between factors in the studies included in this thesis.

One of the WHR-associated SNPs (closest gene: *LYPLAL1*) was independently associated with weight loss and the same genotype also showed associations with decreased hunger feelings and disinhibition. Lower reported hunger feelings were furthermore associated with increased weight loss. These findings will contribute to a better care for morbidly obese patients. Non-invasive blood tests may provide forehand information of which patients that may benefit most from the treatment. More importantly, post-surgical treatment may be optimized so that patients with a less favourable genetic profile may receive additional support for weight loss and weight management. This may be considered as a step in the transition towards personalized medicine.

Epigenetics of a food intervention

When analysing the consequences of food intake on healthy individuals, we could conclude that the central act of food intake is influencing the epigenome widely. While around 500 probes appear to be associated with metabolic processes, hundreds of differentially methylated probes were attributed to the change in immune cell fractions. This may be interpreted as that the immune system is playing an active role in the response to food intake. Lastly, the multitude of changed CpG sites in the promoter of the orexogenic hormone ghrelin and their correlation to ghrelin plasma levels further highlight the quick and dynamic role of the epigenome and again underline its connection to food intake and, by extension, weight management and weight loss.

Finally, a person's genetic background will largely determine the weight loss success after RYGB surgery. Our studies have elucidated some of the generic variants that have a significant impact on weight loss following RYGB surgery. Furthermore, we show that SNPs may influence emotions, such as hunger feelings, which in turn may have an impact on weight loss. However, the effect of a SNP may be dependent on other metabolites such as vitamin D.

Future perspectives

As a non-invasive treatment option to RYGB with comparable weight reduction and sustainable weight loss still is not available, there is a continued need to provide surgeons with more knowledge about the patients' background by identifying new genetic, epigenetic and clinical biomarkers. This will influence the risk-benefit-analysis and give the surgeon more information before they advise the patients on this irreversible treatment option.

During the time of writing this thesis, 65 more BMI associated SNPs have been reported. In February 2015, Locke *et al*. published the largest BMI-GWAS to date comprising 339,224 individuals[21] which indicates the research progression in this field. These additional SNPs have however not been included in the analysis since they were not available at the time of genotyping. An expansion of the study in Paper II would therefore be very intriguing. Partly to confirm the present GRSs, but also to investigate if an expansion of the GRS with relevant SNPs would strengthen the association.

Other aspects of the epigenetics regarding food intake would be a highly interesting to further investigate and broaden the understanding of the body's response to food. Primarily to perform a similar food intervention study including morbidly obese participants. Micro RNAs (miRNA) are short single stranded regulatory molecules which regulate or "fine tune" gene expression. Preliminary analyses indicate that several key miRNAs are differentially expressed after food intake. As miRNA is reported to be stable in the circulation, it may function as a biomarker for epigenetic regulation in the same manner as DNA methylation[101].

Furthermore, the observation of "healthy obese" patients is very interesting. These patients have BMI-levels that qualify them for bariatric surgery, but they do not display the comorbidities attributed to obesity, such as diabetes and hypertension. It would be interesting to identify if there are such patients in our material and identify if there are genetic factors that specially contribute the disease risk for BMI-associated co-morbidities such as cardiovascular disease and metabolic syndrome specifically in the obese.

Svensk sammanfattning

Övervikt, fetma och dess följdsjukdomar är idag en av hälsovårdens största utmaningar och kostnadsposter. Trots den prevalens fetma har i samhället är tillgången på kraftfulla och långverkande behandlingar mycket begränsad. Den effektivaste behandlingen för bibehållen viktminskning är idag en magsäcksoperation och den mest framgångsrika typen är Roux-en-Y gastric bypass (RYGB). Emellertid varierar utfallet mycket mellan individer, trots operationens generella effektivitet. Flera kliniska faktorer har identifierats, men i tvillingstudier har individens genetiska arv visats påverka 70% av den totala variationen. I denna doktorsavhandling undersöks närmare vilka genetiska variationer som påverkar viktnedgång efter magsäcksoperationer. Vidare undersöks genetiska samband mellan ätbeteenden och viktnedgång. Avslutningsvis undersöks epigenomets reaktion på födointag hos friska frivilliga.

I de tre första artiklarna gjordes studierna på en kohort bestående av sjukligt feta personer som alla genomgick en RYGB-magsäcksoperation. 251 patienter analyserades och följdes upp efter två år på det interdisciplinära fetmacentret i St. Gallen, Schweiz. Patienterna hade i snitt ett BMI på 45kg/m^2 innan operationen.

Den första artikeln visar på ett samband mellan minskning av överflödig vikt (*excess BMI loss*) och risk-allelen A för den BMI-relaterade genetiska varianten i *FTO*-genen. De patienter som hade två kopior av A allelen hade i snitt 6% större viktnedgång jämfört med de patienter som hade TT. Detta samband verkar dock vara beroende av vitamin D-nivåer vid operationstillfället, då endast de med vitamin D-brist hade detta samband.

Då en enstaka genetisk variant kan vara signifikant associerad till en variabel, så som BMI, betyder det inte att den har en stor inverkan. Den genetiska variant som rapporterats ha störst påverkan på BMI är *FTO*-genvarianten. Dock uppskattas den endast förklara 0,34% av den totala variansen. Vi utvecklade därför en ny metod för att föra samman flera relevanta genvarianter till en samlad faktor som vi kallade vi för ett genetiskt riskindex. Genom att dra nytta av den statistiska metoden random forest selekterades de genvarianter fram som hade mest påverkan på viktminskning två år efter RYGB operation, varpå dessa summerades till ett index. Hur mycket varje variant påverkade modellen berodde på den effekt som rapporterats tidigare i stora genetiska associationsstudier, s.k. GWAS. En modell gjordes för BMI-relaterade genvarianter som inkluderade *MC4R*, *TMEM160*,

PTBP2, *NUDT3*, *TFAP2B*, *ZNF608* och *MAP2K5*. Den andra gjordes för midja/höft-faktor-relaterade genvarianter och inkluderande *HOXC13*, *LYPLAL1* och *DNM3-PIGC*. Beroende på genuppsättning kunde det genetiska indexet för varje modell visa en upp till 11% skillnad i viktnedgång.

Utav de tillgängliga genvarianterna visades den genvariant i närheten av *LYPLAL1* vara länkad till ätbeteende. Detta mättes med frågeformuläret TFEQ som mäter hungerkänslor, disinhibition och kognitiv självbehärskning. Den risk-allel som tidigare varit associerad till midja-höft-kvot visades i vårt material leda till högre hungerkänslor, lägre förmåga till disinhibition och lägre kognitiv självbehärskning. Höga hungerkänslor (före operation) och denna riskgenvariant var vidare indikativa på sämre viktnedgång två år efter-RYGB operationen.

I den fjärde artikeln presenteras resultat som visar att en standardiserad måltid har direkta och snabbverkande effekter på DNA-metylering, den mest studerade av epigenetiska regleringar. Vi konstaterar att utav de drygt 1800 mätpunkterna som var signifikant ändrade efter måltiden var merparten relaterade till förändringar i fraktionerna mellan de vita blodkropparna. Endast drygt 500 visade inte detta samband. Vidare annoteringsanalyser visade att majoriteten av de 1800 mätpunkterna låg i områden relaterade till immunförsvaret, mekanismer vid sårläkning, T- och B-cellsaktivering och dylikt. Detta pekar på att immunförsvaret spelar en aktiv roll vid det vardagliga födointaget.

En människas genetiska bakgrund betingar till stor del viktnedgången efter en magsäcksoperation, och studierna i denna avhandling har påvisat vilka några av dessa genetiska varianter är. Vi har också visat att dessa varianters effekt kan vara beroende av tillgången på andra metaboliter (ex. vitamin D) eller att SNPs har en inverkan på ätbeteende som indirekt kan leda till viktminskning. Verkningsmekanismen för dessa genvarianter är dock fortfarande oklar.

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