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Search for single nucleotide polymorphisms (SNPs) for weight loss and lifestyle factors associated with body mass index

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Summary

Genome-wide association studies provided evidence for an association of single nucleotide polymorphisms (SNPs) with body mass index (BMI), and gene expression analyses indicated a hypothalamic role for some of the associated genes. Thus, it was hypothesized that the BMI associations might be due to a modulation of nutritional intake and energy expenditure. Furthermore, some candidate gene studies showed a relationship between genetic loci and lifestyle-induced weight loss. The present study investigated whether genetic factors are associated with anthropometric changes during lifestyle intervention using a candidate gene approach. Furthermore, this work addressed whether genetic factors are associated with lifestyle parameters and whether lifestyle factors are mediators within the gene-BMI association in a population-based study.

Therefore, 653 adults from a randomized clinical weight loss trial comparing the efficacy of the Weight Watchers (WW) programme with “usual care” performed by general practitioners, and additionally 358 children from a short-term in-patient weight loss trial (LOGIC) were analyzed for the association between SNPs and weight loss success. In the lifestyle mediator analysis 12,462 adults from the population-based MONICA/KORA study were included. SNP selection and statistical analyses are based on a systematic approach.

The WW study showed a mean (± standard deviation) twelve months weight loss of -4.98±5.98 kilograms (kg) in persons finishing the study (N=434). Children from the LOGIC study had a mean weight loss of -8.19±2.84 kg or -10.88±3.66 kg after four or six weeks, respectively. Some of the 40 (44) investigated SNPs in the WW (LOGIC) study showed significant associations with weight changes without adjustment for multiple testing. The associations between *ADRB2* and *MC4R* locus and delta weight remained borderline significant (p=0.002) in the WW study and the association between *HTR2C* locus and weight loss (p<0.001) in the LOGIC study after adjustment for multiple testing. For three (*TMEM18*, *FTO*, *SH2B1*) of the seven analyzed loci in the MONICA/KORA study an association with BMI ($p=1.22x10^{-8}$; $p=2.85x10^{-7}$; $p=9.83x10^{-3}$) observed in previous studies could be replicated. None of the loci was significantly associated with lifestyle factors nor were lifestyle factors mediators within the gene-BMI associations.

This is the first study investigating the effect of almost all BMI-related loci identified by genome-wide association studies for an association with changes of anthropometric traits during intervention. The analysis was extended by loci from candidate gene studies. The investigated genetic factors seem to have a weak effect if any in modulating weight changes induced by lifestyle intervention in adults or children. There were no associations of SNPs with lifestyle factors nor were lifestyle factors mediators within the gene-BMI association in the MONICA/KORA study. The evaluation of the findings from this work in larger studies is required.

Zusammenfassung

Genomweite Assoziationsstudien zeigten signifikante Assoziationen zwischen Einzelnukleotidaustauschen („single nucleotide polymorphisms“ (SNPs)) und dem Body Mass Index (BMI). Genexpressionsanalysen lassen auf eine hypothalamische Rolle für einige dieser Gene schließen, was eine Modulation von Energieaufnahme und –verbrauch vermuten lässt. Kandidatengenstudien zeigten eine Assoziation zwischen SNPs und Gewichtsabnahme. Die vorliegende Arbeit untersuchte in einem Kandidatengenansatz die Assoziation zwischen Genvarianten und anthropometrischen Veränderungen während einer Lebensstilintervention. In einer populationsbasierten Studie wurde untersucht, ob die SNPs neben dem BMI auch mit Lebensstilfaktoren assoziiert sind und ob die Lebensstilfaktoren als Mediatoren in der Gen-BMI-Assoziation fungieren.

Daten von 653 Erwachsenen aus einer randomisierten klinischen Studie, in der die Effektivität des Weight Watchers (WW) Programms mit der leitliniengerechten Betreuung durch den Hausarzt verglichen wurde, sowie von 358 Kindern aus einer Kurzzeitstudie mit stationärer Adipositastherapie (LOGIC) wurden für die Assoziation zwischen SNPs und Gewichtsabnahme ausgewertet. In die Mediatoranalyse wurden 12,462 Erwachsene aus der MONICA/KORA Studie eingeschlossen. Die SNP-Auswahl sowie die statistische Auswertung basierten auf einem systematischen Ansatz.

Die WW Studie zeigte eine mittlere (± Standardabweichung) Gewichtsreduktion von -4,98±5,98 Kilogramm (kg) für Personen, die die Studie nach 12 Monaten abschlossen (N=434), und die LOGIC Studie von -8,19±2,84 kg oder -10,88±3,66 kg nach vier oder sechs Wochen. Einige der 40 (44) untersuchten SNPs in der WW (LOGIC) Studie zeigten ohne Adjustierung für multiples Testen signifikante Assoziationen mit der Gewichtsabnahme. Nach Adjustierung für multiples Testen blieben in der WW Studie die Assoziationen zwischen dem *ADRB2* und dem *MC4R* Lokus und der Gewichtsabnahme grenzwertig signifikant (p=0,002) und in der LOGIC Studie die Assoziation zwischen dem *HTR2C* Lokus und Gewichtsabnahme (p=0,001). Für drei (*TMEM18*, *FTO*, *SH2B1*) der sieben untersuchten SNPs in der MONICA/KORA Studie wurde eine BMI-Assoziation repliziert ($p=1{,}22\times10^{-8}$; $p=2{,}85\times10^{-7}$; $p=9{,}83\times10^{-3}$). Keiner der Lozi war signifikant mit Lebensstilfaktoren assoziiert noch waren die Lebensstilfaktoren Mediatoren in der Gen-BMI-Assoziation.

Dies ist die erste Arbeit, die fast alle durch genomweite Analysen identifizierten BMI-Lozi hinsichtlich einer Assoziation mit der Veränderung anthropometrischer Parameter während einer Lebensstilintervention in Erwachsenen und Kindern untersuchte. Die untersuchten SNPs scheinen – wenn überhaupt – einen schwachen Effekt auf die Gewichtsveränderung bei Lebensstilintervention zu haben. In der MONICA/KORA Studie gab es keine Assoziation zwischen den Genvarianten und Lebensstilfaktoren noch waren die Lebensstilfaktoren Mediatoren in der Gen-BMI Asssoziation. Eine Evaluation in größeren Studien ist nötig.

Abbreviations

A	
ADIPOQ	Adiponectin
ADRA2A	Alpha2A-adrenergic receptor
ADRB	Beta-adrenergic receptor
AGA	Arbeitsgemeinschaft Adipositas im Kindes- und Jugendalter
AgRP	Agouti-related peptide
AIF1	Allograft inflammatory factor-1
ARC	Arcuate nucleus
A+W	Anamnesis and weight control
B	
B	Blood sample
BAT2	HLA-B associated transcript-2
BCF	Baseline carried forward
BDNF	Brain derived neurotrophic factor
BIA	Bio-impedance
BMI	Body mass index
bp	Base pair
BP	Blood pressure
C	
CARD	Caspase-associated recruitment domain
Cau	Caucasian
CCK	Cholecystokinin
Chr.	Chromosome
CI	95 percent confidence intervall
ChiSq	Chi-square test
cm	Centimeter
CMA	Centrale Marketing-Gesellschaft der deutschen Agrarwirtschaft mbH i.L.
CNS	Central nervous system
CNV	Copy number variation
C18orf2	Chromosome 18 open reading frame 2
D	
Da	Dalton
DAG	German Society of Obesity
DASH	Dietary Approaches to Stop Hypertension
dATP	Deoxyadenosin triphosphate
dCTP	Deoxycytidine triphosphate
ddNTP	Dideoxyucleotide
DGE	Deutsche Gesellschaft für Ernährung
DGKG	Diacylglycerol kinase gamma
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
DNAPTP6	DNA polymerase-transactivated protein 6
dNTP	Deoxynucleotide
DPD	Dichlorophenyldiazonium
DPP	Diabetes Prevention Program
DPS	Finnish Diabetes Prevention Study
dTTP	Deoxythimidine triphosphate
E	
ECG	Electrocardiogram
ECLIA	Electrochemiluminescence immunoassay
EDTA	Ethylenediaminetetraacetic acid
e.g.	For example
EKFZ	Else Kroener-Fresenius-Centre for Nutritional Medicine
ELISA	Enzyme-linked immuno sorbent assay
EPIC	European Prospective Investigation into Cancer
ETV5	Ets variant 5
F	
FAIM2	Fas apoptotic inhibitory molecule 2
FFQ	Food frequency questionnaire
Fisher	Fisher´s exact test
FTO	Fat mass and obesity associated
G	
g	Gram
GGT	γ-glutamyltransferase
GHR	Ghrelin receptor
GLP-1	Glucagon-like peptide-1
GLUT4	Glucose transporter type 4
GNAS	Guanine nucleotide binding protein alpha stimulating activity polypeptide 1
GNB3	Guanine nucleotide-binding protein, beta-3 subunit
GNPDA2	Glucosamine-6-phosphate deaminase 2
GOT	glutamic-oxaloacetic transaminase
G protein	Guanine nucleotide-binding protein
GP	General practitioner
GPS	Genetic predispostion score
GPT	glutamic-pyruvate transaminase
H	
HbA1c	Glycosylated hemoglobin
HCl	Hydrogen chloride
HDL	High density lipoprotein
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
HERITAGE	Health, Risk Factors, Exercise Training, and Genetics
HMGU	Helmholtz Zentrum München
HOMA-B	Homeostasis model assessment of beta cell function
HOMA-IR	Homeostasis model assessment of insulin resistance
HPLC	High performance liquid chromatography
hsCRP	High-sensitivity C reactive protein
HTR2C	5-hydroxytryptamine (serotonin) receptor 2C
HWE	Hardy-Weinberg equilibrium
I	
IGF2	Insulin-like growth factor 2
IL-6	Interleukin 6
INSIG2	Insulin-induced gene 2
IPAQ-short	International Physical Activity Questionnaire – short version
IQR	Inter quartile range
IRAP	Insulin-responsive aminopeptidase
IRS-1	Insulin receptor substrate 1
ISR	Insulin receptor
IWQOL-Lite	Impact of Weight on Quality of Life-Lite
J	
JAK2	Janus kinase 2
K	
k	Kilo
kb	Kilobase
kcal	Kilocalorie
KCTD15	Potassium channel tetramerisation domain containing 15
kg	Kilogram
kg/m²	Kilogram/meter²
$KHCO_3$	Potassium bicarbonate
KORA	Cooperative Health Research in the Region of Augsburg
L	
l	liter
LD	Linkage disequilibrium
LDL	Low density lipoprotein
LEP	Leptin
LEPR	Leptin receptor
LHA	Lateral hypothalamic area
Log	Logarithmized
LOGIC	Long-term effects of lifestyle intervention in Obesity and Genetic Influence in Children
M	
M	Molar
MAF	Minor allele frequency
MAF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog
MALDI-TOF	Matrix assisted laser desorption / ionisation time of flight
MC4R	Melanocortin-4 receptor
MDD	Major depressive disorder
mg	milligram
µg	microgram
$MgCl_2$	Magnesium chloride
mg/dl	milligram/deciliter
min	Minute
ml	Milliliter
µl	Microliter
mM	Millimolar
µM	Micromolar
mmHg	Millimeters of mercury
µmol	Micromole
mmol	Millimole
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
MRC	Medical Research Council
mRNA	Messenger ribonucleic acid

MSH	Melanocyte sitmulating hormone
MSRA	Methionine sulfoxide reductase A
MTCH2	Mitochondrial carrier homolog 2
MTNR1B	Melatonin receptor 1B
mU/l	Milliunits/liter
m/z	Mass-to-charge ratio

N

N	Number
NaCl	Sodium choloride
Na_2EDTA	Dinatrium-ethylendiamin-tetraacetat
NCBI	National Center for Biotechnology Information
NCR3	Natural cytotoxicity triggering receptor 3 precursor
NEGR1	Neuronal growth regulator 1
Ng	Nanogram
NH_4Cl	Ammonium chloride
nl	nanoliter
nm	Nanometer
NPC	Niemann-Pick type C
NPC1	Niemann-Pick disease type C1
NPY	Neuropeptide Y
NTS	Nucleus of the solitary tract
NUGENOB	Nutrient-gene interaction in human obesity: implication for dietary guidelines

O

OD	Optical density
OH	Hydroxyl group
OR	Odds ratio

P

p	P-value
PA	Physical activity
PAPSS2	3`-phosphoadenosine 5`-phosphosulfate synthase 2
PC1, PC2	Proconvertase 1, 2
PCR	Polymerase Chain Reaction
PCSK1	Proprotein convertase subtilisin/kexin type 1
PFKP	Platelet-type phosphofructokinase
PI3K	Phosphoinositol 3-kinase
PLIN	Perilipin
POMC	Proopiomelanocortin
PPARG	Peroxisome proliferator-activated receptor gamma
PRL	Prolactin
PTER	Phosphotriesterase-related
PVN	Paraventricular nuclei
PYY	Peptide tyrosine-tyrosine

Q

Q	Questionnaire

R

r	Correlation
RASAL2	RAS protein activator like 2
RBC	Red blood cell
RBP-4	Retinol binding protein 4
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute

S

S1, S2, S3, S4	Survey 1, 2, 3, 4
SAP	Shrimp alkaline phosphatase
SAS	Statistical Analysis System
s.d.	Standard deviation
SDCCAG8	Serologically defined colon cancer antigen 8
SDS	Standard deviation score
SDS	Sodium dodecyl sulfate
SE	Standard error
SE buffer	Sodium chloride EDTA buffer
sec	Second
SEC16B	SEC16 homolog B
SFRS10	Splicing factor, arginine/serine-rich 10
SGA	Small for gestational age
SH2B1	Src-homology-2 (SH2) domain containing the putative adaptor protein 1
SH2	Src-homology-2
SHIP	Study of Health in Pomerania
SIM1	Single-minded homolog 1
SNP	Single nucleotide polymorphism
SOS	Swedish obese subjects

T

T2D	Type 2 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBE	Tris borat EDTA
TE	Tris EDTA
TFAP2B	Transcription factor AP-2 beta
TFEQ	Three-Factor Eating Questionnaire
TMEM18	Transmembrane protein 18
TNFalpha	Tumor necrosis factor α
TNKS	Tankyrase
TRAILS	Tracking Adolescents` Individual Lives Survey
TRHR	Thyrotropin-releasing hormone receptor
Tris	Tris(hydroxylmethyl)aminomethane
TRKB	Tyrosine kinase receptor
TSH	Thyreotropin
TULIP	Tübingen Lifestyle Intervention Program

U

U	Unit
UCP	Uncoupling protein
US	United States
UV	Ultraviolet

V

VID	Virtuelle Diabetes Institute
VMH	Ventromedial hypothalamus

W

WHO	World Health Organization
WW	Weight Watchers

Other

℃	Centigrade
%	Percent

1 Introduction

The worldwide increasing prevalence of obesity shows that the maintenance of body weight is difficult in our “obesogenic” environment. In Germany the obesity prevalence in adults aged 18 to 80 years is about 20 percent. A proportion of 51 percent women and of 66 percent men is overweight or obese (Body Mass Index (BMI) ≥ 25 kilogram/meter² (kg/m²)). Within the age range from 14 to 80 years 11.6 percent were sticking to a diet at the time of assessment (Max Rubner Institut BfEuL 2008). Experiences show that a short-term weight loss is often possible, whereas the following increase of body weight is difficult to avoid. Reasons of the difficulty of weight loss are of social, environmental and genetic nature. From former times the body is programmed to eat if food is available and to store energy in form of fat deposits.

1.1 Lifestyle intervention programmes and weight loss

1.1.1 Body weight regulation

Body weight regulation is based on multiple factors and complex mechanisms which interact to each other in order to reach energy homeostasis (energy intake = energy expenditure). Body weight regulation is programmed on the environmental conditions from the time period of hunters and gatherers. In that time, the accumulation of fat during periods of feast was necessary to survive during periods of famine. What was of vital importance in further times has become a liability in the nowadays “obesogenic” environment.

About 50 years ago, *James V Neel* proposed the so-called “thrifty genotype” which is less of an asset now than in the former time of feast and famine (Bouchard C 2007; Neel JV 1962). Selective forces led to a selection of genotypes which provide survival advantage due to efficient fat storage during famine periods. That might be an explanation why obesity became so prevalent in our western lifestyle where these thrifty genotypes are disadvantageous.

The environment changed from a “traditional” one with scarce food and high energy expenditure to a “westernized” one, where energy-rich foods are always and everywhere available and high physical activity is needless (**Figure 1-1**). This “obesogenic” environment favours the adoption of an “obesogenic” behaviour including consumption of large portion size meals, high fat and sugar intake, and sedentary lifestyle with low physical activity. Compared to the “traditional environment” with “leptogenic” behaviour, the “obesogenic” behaviour leads to a higher mean BMI confirmed by the obesity prevalence as well to a broader range of BMI levels confirmed by the fact, that even in places where obesity is common, many people are lean. In both environmental conditions the variability of BMI depends on the genetic propensity.

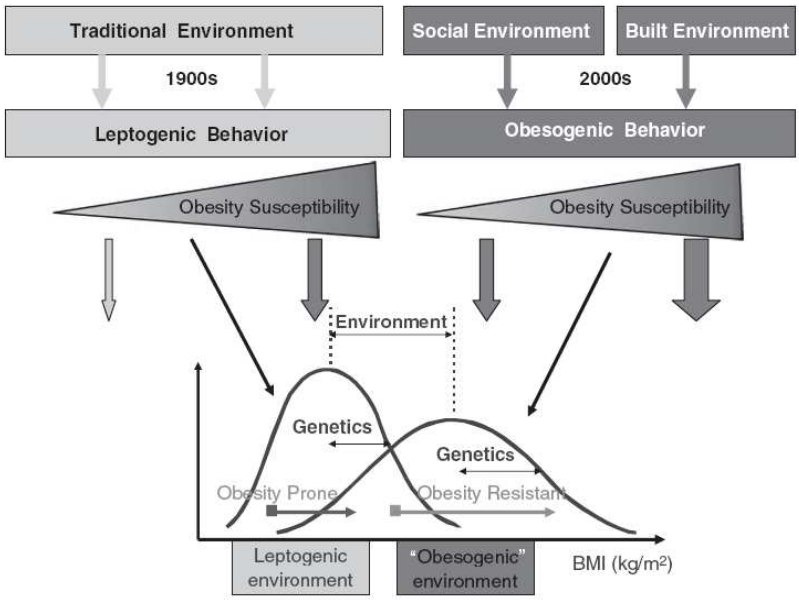
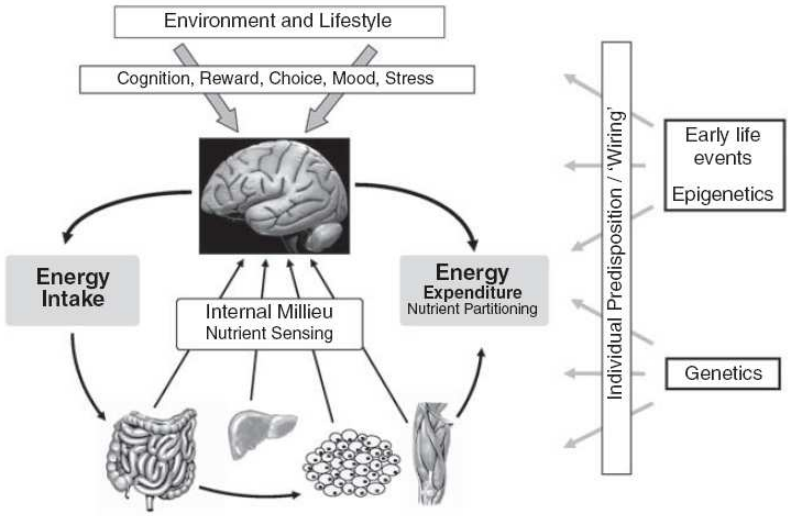


Figure 1-1: Genetic and environmental factors in the context of BMI. Left: The "traditional" environment with scarce food and high energy expenditure is presented. This environment leads to "leptogenic" behaviour in which the variability of BMI depends on the genetic propensity. Right: The "obesogenic" environment is presented. This environment leads to "obesogenic" behaviour with high caloric food and low physical activity. Also in this environment the BMI depends on the genetic propensity (Galgani J and Ravussin E 2008).

Although the biological predisposition appears to be largely genetic, it is also suggested that programming in fetal and early life as well as epigenetic mechanisms are involved. Furthermore, social, psychological, behavioural, and physiological factors play an important role for the regulation of body weight. Very complex interaction systems and feedback mechanisms keep energy intake and expenditure balanced and the central nervous system (CNS) is the pivot to modulate signals from adipose tissue, liver, muscle, and gastrointestinal tract in brain areas that process information about hunger and satiety (**Figure 1-2**).

Figure 1-2: Factors involved in energy homeostasis (Galgani J and Ravussin E 2008)

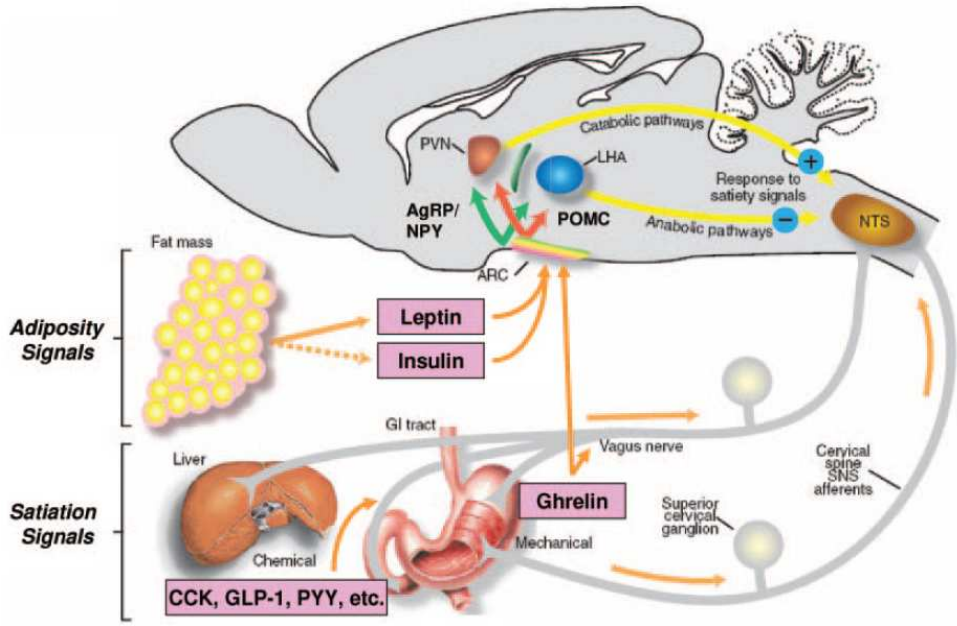


Food intake is regulated by hormones and nutrients which circulate depending on the nutritional state and the energy stores within the blood and interact with each other (**Figure 1-3**). Short-term or satiety signals like ghrelin are secreted after food intake in the

gastrointestinal tract and act as acute signals of hunger and satiety. Long-term or adiposity signals like leptin are secreted by the adipose tissue and reflect the long-term nutritional stage (Woods SC and D'Alessio DA 2008).

During meals, signals like cholecystokinin (CCK) or ghrelin as well as distension of the stomach are transmitted through the vagus nerve and sympathetic fibres to the nucleus of the solitary tract (NTS). Ghrelin also stimulates neurons in the arcuate nucleus (ARC). Signals like leptin and insulin circulate in the blood to the brain (ARC) and interact with neurons that synthesize proopiomelanocortin (POMC) or neuropeptide Y (NPY) as well as agouti-related peptide (AgRP). ARC neurons project to the paraventricular nuclei (PVN) to stimulate the catabolic pathway and to the lateral hypothalamic area (LHA) to inhibit the anabolic pathway. The metabolic parameters CCK, glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY), ghrelin, leptin and insulin highlighted in rosy (**Figure 1-3**) are described in a more detailed way in **appendix A**.

Figure 1-3: Signals regulating energy homeostasis



CCK=cholecystokinin; GLP-1= glucagon-like peptide-1; PYY= peptide tyrosine-tyrosine; GI=gastrointestinal; ARC= arcuate nucleus; NPY= neuropeptide Y; AgRP= agouti-related peptide, PVN= paraventricular nuclei; LHA= lateral hypothalamic area; NTS= nucleus of the solitary tract; (Woods SC and D'Alessio DA 2008)

There are several other metabolic parameters like oxyntomodulin which play a role in body weight regulation. They are not described in the framework of this thesis, but often reviewed (Chaudhri OB et al. 2008; Crowley VE 2008; Drazen DL and Woods SC 2003; Morton GJ et al. 2006; Schwartz MW et al. 2000; Stanley S et al. 2005; Woods SC and D'Alessio DA 2008; Wynne K et al. 2005).

1.1.2 Lifestyle components of weight loss intervention programmes (Holzapfel C and Hauner H 2008)

Dietary restraint and physical activity are the major lifestyle components regulating the weight status of a person (**Figure 1-4**). The combination of both leads to maintenance of body weight or a relatively low risk of developing obesity.

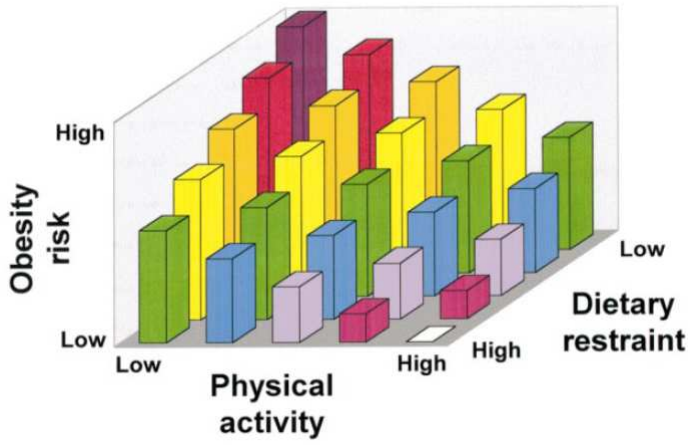


Figure 1-4: Hypothetical obesity risk dependent on physical activity and dietary restraint (Hill JO and Peters JC 1998)

The most effective therapy of obesity is a combination of nutrition, physical activity, and behavioural therapy which is also recommended in the German guideline for the prevention and therapy of obesity (Deutsche Adipositas-Gesellschaft et al. 2007). The offered strategies and programmes like self-monitoring or commercial weight loss programmes (Holzapfel C and Hauner H 2008) aim the change of lifestyle factors in order to reach a negative energy balance which is followed by a balanced one. Therefore, a long-term therapeutic concept is necessary in which individual preferences are considered, old behaviours are borne down, and a long-term behavioural change is established. Continued care after weight loss promises a stable maintenance of body weight. The most important aim – the negative energy balance – is reachable by reducing caloric intake and increasing physical activity.

Caloric intake

The caloric content seems to be more important than the composition of the diet; low-carbohydrate diets or low-fat diets are not more effective at producing weight loss than high-carbohydrate or high-fat diets, as long as the total caloric content is equivalent. A systematic review assessed the efficacy of low-fat diets including six studies (Pirozzo S et al. 2003). There was no significant difference in weight loss between low-fat diets and other diets. Another systematic review including 107 studies assessed the efficacy of carbohydrate diets (Bravata DM et al. 2003). The mean weight loss was not statistically different between low-carbohydrate diets (3.6 kg) and high-carbohydrate diets (2.1 kg). In a recent study four diets with different percentages of energy derived from fat, protein, and carbohydrates were

compared. The average weight loss after six months was 6 kg and after two years 4 kg (Sacks FM et al. 2009). Another study comparing four different weight loss programmes (Atkins, Slim Fast, WW, Rosemary Conley`s) also resulted in a weight loss effect of about 6 kg after six months (Truby H et al. 2006). Evaluating the efficacy of meal replacement therapies for weight loss, six studies were reviewed (Heymsfield SB et al. 2003). Participants who received meal replacement lost seven to eight percent body weight, compared to three to seven percent body weight by those who received conventional reduced-caloric diet.

Physical activity

Concerning body weight reduction and maintenance it seems that at least 60 minutes of moderate physical activity per day are necessary. Although this is difficult to combine with the western lifestyle, physical activity helps to reduce and maintain body weight by increasing energy expenditure (Jakicic JM et al. 2010; Jakicic JM and Otto AD 2005). In comparison to the dietary intervention, an intervention based on physical activity is less successful (Hagan RD et al. 1986). Nevertheless, the diet- or exercise-induced weight loss effects could be the same, given the fact that the same caloric deficit is reached in both intervention arms (Ross R et al. 2000). The observation of 42 twin pairs from Finland discordant for both intensity and volume of leisure physical activity showed after 30 years that the weight gain in the active group was 5.4 kg less than in the inactive group (p=0.003) (Waller K et al. 2008). Besides the structured aerobic exercise also daily life activity affects weight loss success (Andersen RE et al. 1999).

1.1.3 Effects of lifestyle intervention programmes on weight loss (Holzapfel C and Hauner H, in press)

A limited number of studies has investigated lifestyle interventions of at least one year (**Table 1-1**). In a two-year US study 423 overweight and obese adults were randomized to either attend weekly Weight Watchers (WW) meetings or a self-help programme (Heshka S et al. 2003). At twelve months, weight loss was 4.3±6.1 kg in the WW group and 1.3±6.1 kg in the self-help group. By 24 months weight was 2.9±6.5 kg less than at baseline in the WW group versus 0.2±6.5 kg in the self-help group. The „Dietary Approaches to Stop Hypertension“ (DASH) diet together with caloric restriction and physical activity reached a weight loss of 5.8±4.4 kg after six months (Hollis JF et al. 2008). A carbohydrate-restricted diet (30 grams (g) per day or less) led to a weight loss of 5.8±8.6 kg after six months (Samaha FF et al. 2003). A meta-analysis of 46 weight loss studies of at least six weeks and a nutritional component in the intervention showed a maximal effect of 1.9 BMI units after twelve months (Dansinger ML et al. 2007).

Table 1-1: Overview about weight loss studies of at least one year

Subjects	Inclusion-BMI (kg/m²)	Weight loss programme	Weight loss	Reference
Duration one year				
63 adults (20 men)	34 (mean)	a) Atkins (20 g carbohydrates in the first two weeks, then gradual increase) b) LEARN	a) 4.4 ± 6.7% b) 2.5 ± 6.3% BCF analysis	(Foster GD et al. 2003)
160 adults	27 to 42	a) Atkins b) Zone c) WW d) Ornish	a) 2.1 ± 4.8 kg b) 3.2 ± 6.0 kg c) 3.0 ± 4.9 kg d) 3.3 ± 7.3 kg BCF analysis	(Dansinger ML et al. 2005)
311 pre-menopausal women	27 to 40	a) Atkins b) Zone c) LEARN c) Ornish	a) 4.7 (CI: 6.3 - 3.1) kg b) 1.6 (CI: 2.8 - 0.4) kg c) 2.6 (CI: 3.8 - 1.3) kg d) 2.2 (CI: 3.6 - 0.8) kg BCF analysis	(Gardner CD et al. 2007)
181 women	31 (mean)	a) Atkins b) Zone c) Ornish	a) 5.3 ± 7.2 kg b) 2.2 ± 6.3 kg c) 3.0 ± 6.8 kg completer analysis	(Alhassan S et al. 2008)
454 adults (25% men)	36 (mean)	M.O.B.I.L.I.S.	6.4 ± 7.5 kg completer analysis	(Berg A et al. 2008)
97 women	30 to 40	a) Reduction of fat intake b) Reduction of fat intake and increase of water-containing foods (e.g. fruits)	a) 6.4 ± 0.9 kg b) 7.9 ± 0.9 kg completer analysis	(Ello-Martin JA et al. 2007)
118 adults (36% men)	33 (mean)	a) 4% carbohydrate, 35% protein, 61% fat, energy-reduced b) 46% carbohydrate, 24% protein, 30% fat, isocaloric	a) 14.5 ± 1.7 kg b) 11.5 ± 1.2 kg completer analysis	(Brinkworth GD et al. 2009)
5,145 adults (40% men)	Women: 36 Men: 35 (mean)	a) Lifestyle intervention (nutrition/physical activity) with group meeting b) Standard programme	a) 8.6 ± 6.9% b) 0.7 ± 4.8% completer analysis	(Pi-Sunyer X et al. 2007)
522 adults (33% men)	31 (mean)	a) Control group (one meeting, material) b) Lifestyle intervention (nutrition/physical activity) – regular meetings	a) 1.0 ± 3.7 kg b) 4.5 ± 5.0 kg completer analysis	(Lindstrom J et al. 2003)
130 adults (14.9 or 7.9% men)	44 (mean)	a) Diet and physical activity intervention for 12 months b) Diet intervention for 12 months and physical activity delayed for 6 months	a) 12.1 (CI: 10.0 - 14.2) kg b) 9.9 (CI: 8.0 - 11.7) kg intention to treat analysis	(Goodpaster BH et al. 2010)
Duration two years				
423 adults (65 men)	27 to 40	a) WW b) Self-help programme	a) 2.9 ± 6.5 kg b) 0.2 ± 6.5 kg intention to treat analysis	(Heshka S et al. 2003)
322 adults (86% men)	31 (mean)	a) 1500/1800 kcal (women/men); 30% fat b) 1500/1800 kcal (women/men); ≤ 35% fat; main source: 30-45 g olive oil; handful nuts (Mediterranean diet) c) 20 g carbohydrates in the first two months, then gradual increase up to 120 g	a) 2.9 ± 4.2 kg b) 4.4 ± 6.0 kg c) 4.7 ± 6.5 kg intention to treat analysis	(Shai I et al. 2008)
811 adults (40% men)	25 to 40	a) 20% fat, 15% protein, 65% carbohydrate b) 20% fat, 25% protein, 55% carbohydrate c) 40% fat, 15% protein, 45% carbohydrate d) 40% fat, 25% protein, 35% carbohydrate	4 kg; no difference between groups completer analysis	(Sacks FM et al. 2009)
442 women	25 to 40	a) Center-based group b) Telephone-based group c) Usual care group all groups based on diet and physical activity	a) 7.4 (CI: 6.1 - 8.7) kg b) 6.2 (CI: 4.9 - 7.6) kg c) 2.0 (CI: 0.6 - 3.3) kg intention to treat analysis	(Rock CL et al. 2010)
307 adults (99 men)	36 (mean)	a) Atkins (20 g carbohydrates in the first three months, then gradual increase) b) Fat-reduced diet	~ 7 kg; no difference between groups completer analysis	(Foster GD et al. 2010)

BCF=baseline carried forward; CI=confidence interval; kcal=kilocalories; (Holzapfel C and Hauner H, in press)

The level of dietary adherence is associated with weight loss success. Regardless of the type of diet, the twelve-month weight change was greater in the most adherent compared to the least adherent tertiles (-8.3±5.6 kg vs 1.9±5.8 kg (p=0.006, Atkins); -3.7±6.3 kg vs -0.4±6.8kg (p=0.12, Zone); -6.5±6.8 kg vs -1.7±7.9 kg (p=0.06, Ornish)) (Alhassan S et al. 2008). Persons who were more compliant according to self-reported attendance lost more weight with the WW programme (Heshka S et al. 2003). In supporting successful weight loss these results suggest that adherence is more important than the specific macronutrient composition of the weight loss diet. In general regardless of weight loss programme, there is a moderate weight loss success of 2 to 4 kg after one to two years (**Table 1-1**).

Despite this moderate weight change, the range of weight loss strongly varies. In an US study the weight changes after a one-year therapy was between -28 and +12 kg (commercial weight loss programme) as well as between -26 and +15 kg (self-help programme) (Heshka S et al. 2003). The combination of an energy-reduced diet and increased physical activity leads to a weight reduction between 4 and 30 kg (Svetkey LP et al. 2008). Furthermore, the use of weight loss medications results in a similar weight loss variation (Rucker D et al. 2007). Beside the heterogeneity of short-term results of weight loss programmes, there is also a large inter-individual range of long-term results. Some persons could not maintain their “new” weight, whereas other persons reduced their weight also in the weight maintenance phase (Svetkey LP et al. 2008).

Due to the complex mechanisms of the body to defend body weight and the strong need of a lifelong lifestyle change, it is difficult to maintain body weight at a lower level after weight loss (**Appendix B**). Although only 20 percent of overweight persons are successful in long-term results (Wing RR and Phelan S 2005), in a German analysis 18 percent of previous overweight and 30 percent of obese persons could maintain at least a ten percent weight loss over one year (de Zwaan M et al. 2008). In a weight loss maintenance study, weight regain of 4.0 to 5.5 kg occurred and after 30 months 71 percent of study participants remained below the entry weight (Svetkey LP et al. 2008). Another study confirmed these results (Lowe MR et al. 2008a). A meta-analysis of 29 studies showed that after five years a weight loss of about 3 kg could be maintained (Anderson JW et al. 2001). A review of 80 weight loss studies having at least a one year follow-up period confirmed these results. After four years three to six percent of weight loss were maintained (Franz MJ et al. 2007). A survey conducted 14(±10) months after a behavioural weight loss programme showed that a proportion of 77 percent could maintain a weight loss of at least five percent of the initial weight (Befort CA et al. 2008).

1.2 Genetic susceptibility of weight loss (Holzapfel C and Hauner H 2009)

1.2.1 Evidence from adoption and twin studies

Individual differences in the predisposition to gain and lose weight exist and genetic variation has much to do with the risk of becoming obese or with weight loss success. Data to support this notion come from experimental studies conducted with pairs of identical twins. These studies demonstrated that the amount of gained or lost weight was strongly dependent on a predisposition that appeared to be largely inherited (Bouchard C et al. 1994; Bouchard C and Tremblay A 1997).

Bouchard C et al. undertook a very well-controlled study to determine whether there are differences in the responses to fasting among individuals and to assess the possibility that genotypes are involved in such differences.

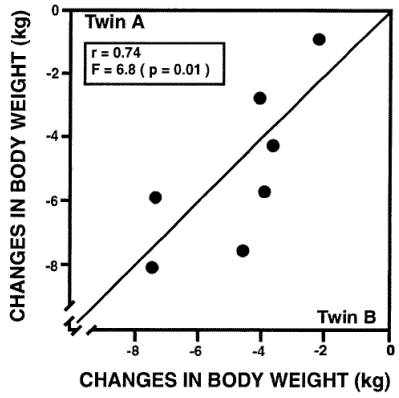


Figure 1-5: Changes of body weight in identical twins following negative energy balance (Bouchard C and Tremblay A 1997)

In male monozygotic twins, the response on negative energy balance induced by increased physical activity is influenced by genetic factors. Under identical conditions there are large inter-individual differences concerning weight loss, but only small differences within twin pairs (**Figure 1-5**) (Bouchard C et al. 1994; Bouchard C and Tremblay A 1997). Furthermore, also the resting metabolic rate is affected by genetic factors (Bouchard C et al. 1993; Fontaine E et al. 1985).

In 14 pairs of female identical twins – lost weight with a very low-calorie diet – huge variability among pairs in loss of weight (5.9 to 12.4 kg) after 28 days and a high intra-pair correlation (r=0.85) was shown (Hainer V et al. 2000; Hainer V et al. 2001). This gives evidence that not only environmental and behavioural factors are responsible for inter-individual variability of weight loss success or resistance, but also inherited factors.

1.2.2 Evidence from candidate gene studies

Also in studies of unrelated subjects, genetic contribution to weight loss success was identified. Results from candidate gene studies concerning weight loss were already reviewed (Hainer V et al. 2008; Holzapfel C and Hauner H 2009; Martinez JA et al. 2008; Moreno-Aliaga MJ et al. 2005). Most of the loci and their results concerning weight loss are listed and described in the following (**Table 1-2**).

Table 1-2: Overview of loci which were already reported in the context of weight loss

Gene	Abbreviation
Leptin	*LEP*
Leptin receptor	*LEPR*
Uncoupling protein 1, 2, 3	*UCP1, UCP2, UCP3*
Beta-3-adrenergic receptor	*ADRB3*
Beta-2-adrenergic receptor	*ADRB2*
Alpha2A-adrenergic receptor	*ADRA2A*
Guanine nucleotide-binding protein, beta-3 subunit	*GNB3*
Guanine nucleotide binding protein alpha stimulating activity polypeptide 1	*GNAS*
Peroxisome proliferator-activated receptor gamma 2	*PPARG2*
5-hydroxytryptamine (serotonin) receptor 2C	*HTR2C*
Insulin-induced gene 2	*INSIG2*
Perilipin	*PLIN*
Insulin receptor substrate 1	*IRS-1*
Interleukin 6	*IL-6*
Adiponectin	*ADIPOQ*
Proprotein convertase subtilisin/kexin type 1	*PCSK1*
Tumor necrosis factor	*TNFalpha*

Although physiological data on leptin suggest a significant role in development of obesity, few data on genetic variations in the **leptin (*LEP*)** and **leptin receptor (*LEPR*)** gene exist. Leptin deficiency caused by a frameshift mutation leads to monogenic obesity. This extreme obese phenotype can be successfully treated by administration of exogenous recombinant human leptin (Farooqi IS et al. 2002; Gibson WT et al. 2004). Leptin treatment of polygenic obesity is not successful (Fogteloo AJ et al. 2003; Heymsfield SB et al. 1999). Weight loss programmes showed that high baseline leptin concentrations are associated with decreased weight loss (Naslund E et al. 2000; Verdich C et al. 2001b). During a low-calorie diet patients homozygous for the C allele of the single nucleotide polymorphism (SNP) C-2549A in the promoter region of the *LEP* gene showed greater weight loss compared to carriers of the A allele (Mammes O et al. 1998). In addition, women carrying the C allele compared to non-carriers of the polymorphism Ser343Ser (T/C) within the *LEPR* gene had greater weight reduction (Mammes O et al. 2001). Furthermore, the Lys656Lys group of the Lys656Asn polymorphism lost less weight compared to the Asn-carriers (De Luis DA et al. 2010a). During antipsychotic treatment neither the *LEP* nor the *LEPR* gene was associated with weight gain (Perez-Iglesias R et al. 2010).

Uncoupling proteins represent a family of transmembrane proteins and are involved in heat formation and energy expenditure. Polymorphism A-3826G within the promoter region of the **uncoupling protein 1 (*UCP1*)** gene affects the magnitude of weight change during energy reduction (Fumeron F et al. 1996). In Korean females, a *UCP1* haplotype was associated with improved weight loss (Shin HD et al. 2005). Weight change during a low-calorie diet differed not between A-3826G genotypes (Hamada T et al. 2009). Weight loss was similar across genotypes within the **uncoupling protein 3 (*UCP3*)** (De Luis DA et al. 2008; De Luis DA et al. 2009a; De Luis DA et al. 2010b; Kim OY et al. 2004). A haplotype constructed from six *UCP3* SNPs was associated with increased reduction in body weight and BMI after a very

low-energy diet (Cha MH et al. 2006). The expression of *UCP3* messenger ribonucleic acid (mRNA) in skeletal muscle was higher in responders to a weight loss programme compared to non-responders, while no changes were found for the **uncoupling protein 2 (*UCP2*)** mRNA levels (Harper ME et al. 2002). In another study both *UCP3* and *UCP2* mRNA expression decreased after weight loss (Vettor R et al. 2003). By contrast SNP G-866A within the *UCP2* gene and one haplotype within the *UCP2-3* gene cluster were associated with the effects of a very low-caloric diet on body fat reduction (Yoon Y et al. 2007). After adiposity surgery the carriers of A-866A lost more weight compared to the other genotypes (Sesti G et al. 2005). Under sibutramine therapy there was no significant weight loss in individuals with the wild-type genotype GG (Hsiao TJ et al. 2010). Furthermore, body fat reduction after a caloric restriction differed according to the Ala55Val polymorphism (Cha MH et al. 2007). Also after gastric banding weight loss differed between genotypes (Chen HH et al. 2007).

The beta-adrenergic receptors are expressed in adipose tissue (Krief S et al. 1993). The beta-3-adrenergic receptor is a regulator of catecholamine-induced lipolysis and influences adipocyte metabolism. Polymorphism Trp64Arg within the **beta-3-adrenergic receptor (*ADRB3*)** gene is linked to lower lipolytic activity and to lipid accumulation in adipose tissue (Arner P 2001). Patients carrying the Arg64 allele had lower weight loss success than Trp64 carriers (Sakane N et al. 1997; Shiwaku K et al. 2003; Yoshida T et al. 1995). Japanese men carrying the Arg64 allele increased their body weight over four years, whereas non-carriers had no changes in body weight (Yamakita M et al. 2010). There are also studies which found no differences in weight loss between genotypes (Fumeron F et al. 1996; Kim OY et al. 2004; Kuriyama S et al. 2008; Rawson ES et al. 2002; Tchernof A et al. 2000). Although carriers and non-carriers of the risk allele significantly reduced weight the metabolic effects of mild weight loss differed between genotypes with greater effects in the non-risk group (De Luis DA et al. 2007; De Luis DA et al. 2009b). It was reported for polymorphism Gln27Glu within the **beta-2-adrenergic receptor (*ADRB2*)** gene that the Glu allele carriers had a greater reduction in body weight in 78 Spanish obese women following an energy-restricted diet (Ruiz JR et al. 2010b). The Gly allele of polymorphism Arg16Gly was more frequent in obese persons with further weight gain (Kawaguchi H et al. 2006). Patients who regain weight after weight reduction had a significantly higher frequency of the Gly16 allele compared to patients who have maintained their weight (Masuo K et al. 2005). There are studies investigating the combined effect of *UCP1* and *ADRB3* polymorphisms. Subjects with risk alleles of both genes lost less weight than either those with the *ADRB3* or the *UCP1* risk variant alone (Fogelholm M et al. 1998; Kogure A et al. 1998). The C-1291G polymorphism within the **alpha2A-adrenergic receptor (*ADRA2A*)** gene was reported to be associated with

abdominal body fat, whereas the results were inconsistent across gender and races (Garenc C et al. 2002). Sibutramin treatment resulted in greater weight loss in a combination of genotypes from *ADRA2A* and **guanine nucleotide-binding protein, beta-3 subunit (*GNB3*)** gene (Grudell AB et al. 2008). There was a marginal relationship between G1780A SNP (*ADRA2A*) and percent body fat in African Americans (Lima JJ et al. 2007).

G proteins mediate many pathways including the beta-adrenergic signalling pathway (Hamm HE 1998). They are known to be involved in the control of lipolysis. The C825T polymorphism within the *GNB3* gene might affect beta-adrenergic control of lipolysis (Hauner H et al. 2002). A fasting period over eight days was associated with better mood and less hunger in homozygous CC genotype carriers compared to TT carriers (Michalsen A et al. 2009). The CC genotype of SNP C825T showed greater weight loss during sibutramine treatment, but reduced weight loss under placebo (Hauner H et al. 2003). There was a significant gene by treatment (sibutramine) interaction reflecting different effects of treatment (Grudell AB et al. 2008). In Taiwanese patients sibutramine caused no significant additional weight loss in CC genotype carriers (Hsiao DJ et al. 2009). After gastric banding the C825T polymorphism did not predict long-term weight loss (Potoczna N et al. 2004).

The GG genotype carriers of polymorphism A-1211G within the **guanine nucleotide binding protein alpha stimulating activity polypeptide 1 (*GNAS*)** gene lost significantly more weight during a low-calorie diet, whereas sibutramine was effective only in A allele carriers (Frey UH et al. 2008a). During modified fasting weight loss significantly differed between *GNAS* genotypes (Frey UH et al. 2008b).

Peroxisome proliferator-activated receptor gamma (PPARG) is a member of the nuclear hormone receptor family of transcription factors. There are two isoforms, PPARG1 and PPARG2, whereas the latter is considered to be more specific for adipose tissue. The transcription factor stimulates the transcription of genes responsible for growth and differentiation of adipocytes. The Ala allele of polymorphism Pro12Ala within the ***PPARG2*** gene reduces transcription and adipogenesis (Masugi J et al. 2000). Homozygotes for the Ala allele were more successful in long-term weight loss than subjects with other genotypes (Lindi V et al. 2001; Lindi VI et al. 2002). Another study found no differences in weight loss between carriers and non-carriers of the Ala allele (Nicklas BJ et al. 2001). Furthermore, there are studies showing that the frequency of genotypes is different between persons with successful and non-successful weight loss maintenance (Nicklas BJ et al. 2001; Vogels N et al. 2005). Six *PPARG* polymorphisms significantly affect the response to a caloric restriction (Matsuo T et al. 2009).

Serotonin is a neurotransmitter and plays an important role in the CNS by inducing satiety. Serotonin receptor agonists cause a small reduction in body weight and appetite (Halford JC et al. 2007; Sargent PA et al. 1997). Subjects with the heterozygous genotype of the polymorphism C-759T of the **5-hydroxytryptamine (serotonin) receptor 2C (*HTR2C*)** gene lost less weight than homozygous carriers of the risk allele during psychological weight loss treatment (Pooley EC et al. 2004). Furthermore, the SNP Cys23Ser played a role in weight reduction in teenage girls (Westberg L et al. 2002).

Results concerning an association between the **insulin-induced gene 2 (*INSIG2*)** and obesity are inconsistent (Boes E et al. 2008; Bressler J et al. 2009; Herbert A et al. 2006; Hotta K et al. 2008; Lyon HN et al. 2007; Vimaleswaran KS et al. 2009a; Wiedmann S et al. 2009). A recent meta-analysis published by *Heid IM et al.* showed that there is no evidence that *INSIG2* is associated with obesity (Heid IM et al. 2009). The *INSIG2* gene encodes endoplasmatic reticulum proteins that regulate transcription factors. Children homozygous for the C allele of polymorphism rs7566605 near *INSIG2* had lower weight loss than children carrying the G allele (Reinehr T et al. 2008). Furthermore, the combination of risk alleles of the *INSIG2* and the **fat-mass- and obesity-associated (*FTO*)** gene was associated with the lowest overweight reduction in children (Reinehr T et al. 2009b). In the Diabetes Prevention Program (DPP) weight loss differed between *INSIG2* genotypes (Franks PW et al. 2008).

Perilipin plays a role in the regulation of lipid storage and is essential for triglyceride deposition and mobilization (Mottagui-Tabar S et al. 2003; Tansey JT et al. 2004). Lipolysis and perilipin content of adipocytes differed between genotypes of the rs891460 polymorphism of the **perilipin (*PLIN*)** gene (Tansey JT et al. 2004). In mice, perilipin overexpression protects against diet-induced adipocyte hypertrophy, obesity, and glucose intolerance (Miyoshi H et al. 2010). In GG subjects of the polymorphism G11482A there was a significant decrease of body weight after dietary treatment. There was resistance to a low-energy diet in carriers of the A allele (Corella D et al. 2005). Furthermore, haplotypes of *PLIN* genes differed in weight loss response (Deram S et al. 2008; Soenen S et al. 2009).

The insulin receptor substrate 1 (IRS-1) is one of the primary substrates of insulin signalling. The ***IRS-1*** gene did not modify the weight change response to a lifestyle intervention programme (Laukkanen O et al. 2004). Furthermore, after bariatric surgery weight loss did not differ between genotypes of the *IRS-1* gene (Sesti G et al. 2005). The combination of *ADRB3* and *IRS-1* risk alleles led to a significantly lower weight loss (Benecke H et al. 2000).

The immune-regulating cytokine interleukin 6 (IL-6) is released by adipose tissue and its serum concentrations are correlated with obesity (Eder K et al. 2009; Fried SK et al. 1998). The ***IL-6*** gene was not associated with anthropometric variables after dietary intervention. After one year the C allele of the -174G>C polymorphism was more frequently observed in subjects maintaining body weight (Goyenechea E et al. 2006). After adiposity surgery the carriers of the GG genotype had lost more weight than the carriers of the G allele (Sesti G et al. 2005). In a Dutch population followed-up for more than six years, the *IL-6* gene variant was associated with weight gain (Heidema AG et al. 2010).

Adiponectin is an adipose tissue-related hormone which is negatively correlated with visceral adiposity (Arita Y et al. 1999; Matsuzawa Y 2010). The **adiponectin (*ADIPOQ*)** gene was associated with weight regain after a low-calorie diet (Goyenechea E et al. 2009). Although the *ADIPOQ* gene was not associated with weight loss, gene-diet interaction effects on weight loss existed (Sorensen TI et al. 2006). In another study *ADIPOQ* genotypes tended to be associated with three year body weight gain (Razquin C et al. 2010b).

Sorensen TIA et al. investigated 42 SNPs in 26 candidate genes for their association with weight loss (Sorensen TI et al. 2006). None of the genetic loci was associated with weight loss, whereas the **proprotein convertase subtilisin/kexin type 1 (*PCSK1*)** and the **tumor necrosis factor alpha (*TNFalpha*)** gene were associated with weight loss assuming a specific genetic model (Sorensen TI et al. 2006).

Several obesity-related candidate genes might affect weight loss in response to weight reducing programmes. The listed genes are candidate genes for weight loss. In a candidate gene approach genes likely to be involved in obesity or weight regulation by their function or a role in an affected metabolic pathway are chosen to investigate the association between these genes and weight loss. For the listed genes the results concerning weight loss are inconsistent and often with weak evidence regarding statistical significance (p-value not very different from 0.05). Due to the often very small sample size (N ~ 100), the lack of homogeneity of study designs and the absence of replication studies, it is not sure whether the truth was observed. Replication in larger cohorts could bring more light into this field.

1.2.3 Evidence from genome-wide association studies

Genome-wide association studies investigate a large number of SNPs over the whole genome without prior hypothesis of plausibility for a specific disease. This approach resulted in the identification of many new genetic loci strongly associated with BMI in population-based studies.

In 2009 strong evidence for 17 genetic loci (**Table 1-3**) associated with obesity (Hofker M and Wijmenga C 2009) existed, whereas the list has already been extended - also to other obesity measurements (Heard-Costa NL et al. 2009; Heid IM et al. 2010; Scherag A et al. 2010; Speliotes EK et al. 2010). Most of the genes are expressed in the brain and thus might exert their effect on body weight via central mechanisms (Frayling TM et al. 2007; Loos RJ et al. 2008; Meyre D et al. 2009; Thorleifsson G et al. 2009; Willer CJ et al. 2009). In the following the loci (**Table 1-3**) are described.

Table 1-3: Overview and properties of 17 loci associated with BMI

Genes and chromosomal location	Proposed molecular or cellular function	Additional phenotypes	Expression[a]
NEGR1 (1p31)	Neuronal outgrowth	—	A
SEC16B, *RASAL2* (1q25)	—	—	L
TMEM18 (2p25; closest gene)	Neural development	Associated with T2D[c]	None
ETV5 (3q27; locus with three genes, strongest association in *ETV5*)	—	—	None
Gene desert; *GNPDA2* is one of three genes nearby (4p13)	—	Associated with T2D[c]	A
PRL (6p22.2–p21.3)	—	—	Pituitary only
Locus containing *NCR3*, *AIF1* and *BAT2* (6p21)	—	Associated with weight, not BMI	*NCR3*: A, H *AIF1*: H *BAT2*: H
PTER (10p12)	—	—	H, L
BDNF (11p14; locus with four genes, strongest association near *BDNF*)	BDNF expression is regulated by nutritional state and MC4R signaling	Associated with T2D[c]. Individuals with WAGR syndrome with *BDNF* deletion have BMI > 95th percentile. *Bdnf* knockdown in mouse hypothalamus causes hyperphagia and obesity	H
MTCH2 (11p11.2; locus with 14 genes)	Cellular apoptosis	—	**A**, H, **L**
FAIM2 (12q13; locus also contains *BCDIN3D*)	Adipocyte apoptosis	—	A, **H**
SH2B1 (16p11.2; locus with 19–25 genes)	Neuronal role in energy homeostasis	*Sh2b1*-null mice are obese and diabetic	A, H
MAF (16q22–q23)	Transcription factor involved in adipogenesis and insulin-glucagon regulation	—	No data
FTO (16q22.2)	Neuronal function associated with control of appetite	Associated with T2D[c]	A, **H**
NPC1 (18q11.2)	Intracellular lipid transport	*Npc1*-null mice show late-onset weight loss and poor food intake. NPC1 interferes with function of raft-associated insulin receptor signaling	A, **H**
MC4R (18q22)	Hypothalamic signaling	Haploinsufficiency in humans is associated with morbid obesity. MC4R-deficient mice show hyperphagia and obesity	A, H, L
KCTD15 (19q13.11)	—	—	A, H

WAGR=Wilms` tumor, aniridia, genitourinary anomalies and mental retardation; A=adipocyte; H=hypothalamus; L=liver; T2D=type 2 diabetes mellitus; bold=high expression; (Hofker M and Wijmenga C 2009)

The ***FTO*** gene has been reported to code for an oxygenase involved in deoxyribonucleic acid (DNA) methylation (Gerken T et al. 2007). The *FTO* gene is the strongest genetic risk factor of polygenic obesity identified as yet (Dina C et al. 2007; Frayling TM et al. 2007; Scuteri A et al. 2007). Some negative results concerning an association with weight loss exist (Haupt A et al. 2008; Lappalainen TJ et al. 2009; Mueller TD et al. 2008; Reinehr T et al. 2009b). There was comparable weight loss induced by moderate exercise across *FTO* genotypes, whereas subjects homozygous for the minor A allele of rs8050136 had a greater weight loss when exercise was at or above the physical activity recommendations (Mitchell JA et al. 2010). Regardless of nutritional intervention, subjects carrying the BMI risk allele had the lowest body weight gain after three years (Razquin C et al. 2010a).

The melanocortin-4 receptor (MC4R) is a G protein coupled receptor which is expressed in the hypothalamus and plays, as part of the melanocortinergic pathway, a crucial role in energy homeostasis. Rare mutations within the ***MC4R*** gene are the most common cause for monogenic obesity and account for up to six percent of severe, early-onset obesity (Farooqi IS et al. 2003; Hinney A et al. 2006). Genome-wide association studies found a strong association between variants near the *MC4R* gene and increased obesity risk and waist circumference (Chambers JC et al. 2008; Loos RJ et al. 2008). Other studies provided evidence that the *MC4R* 103I variant is associated with a lenient phenotype (Geller F et al. 2004; Heid IM et al. 2005; Young EH et al. 2007). There are also studies which have investigated *MC4R* polymorphisms as well as mutations and weight reduction during lifestyle intervention (Haupt A et al. 2009a; Reinehr T et al. 2009a).

The **transmembrane protein 18 (*TMEM18*)** gene is widely expressed in human tissues and seems to modulate cell migration (Jurvansuu J et al. 2008). The *TMEM18* mRNA expression was detected in all major brain regions, but it was more abundant in neurons than in other cell types (Almen MS et al. 2010). The TMEM18 protein has three or four membrane spanning domains and contains a nuclear localization signal sequence at the C-terminus.

The **neuronal growth regulator 1 (*NEGR1*)** gene is highly expressed in the brain and hypothalamus (Willer CJ et al. 2009) and the NEGR1 protein is a member of the IgLON family of cell adhesion molecules and plays a role in the development of the CNS. Also in rats *Negr1* is primarily expressed in the brain (Funatsu N et al. 1999).

Tumour phenotypes (e.g. metastases) are influenced by the **mitochondrial carrier homolog 2 (*MTCH2*)** gene (Yu K et al. 2008). The MTCH2 protein on the surface of mitochondria might play a role in mitochondrial apoptosis (Grinberg M et al. 2005).

Variants within the **Src-homology-2 (SH2) domain containing the putative adaptor protein 1 (*SH2B1*)** gene are associated with serum leptin, total fat, waist circumference, and body weight in female twins (Jamshidi Y et al. 2007). In mice SH2B is a key regulator of leptin sensitivity, energy balance, and body weight (Ren D et al. 2005), and knockout mice develop hyperinsulinemia, hyperglycemia, and glucose intolerance (Duan C et al. 2004b). Through binding to Janus kinase 2 (JAK2), SH2B activates the phosphoinositol 3-kinase (PI3K) pathway (Duan C et al. 2004a).

Mutations within the **Niemann-Pick disease type C1 (*NPC1*)** gene cause autosomal recessive inherited Niemann-Pick type C (NPC) disease which is characterized by disordered cholesterol homeostasis (Pentchev PG 2004). The NPC1 protein is involved in controlling cholesterol levels (Amigo L et al. 2002). Npc1 null mice show a cellular defect in cholesterol transport (Xie C et al. 1999). The NPC1 heterozygous mouse model has significantly increased weight gain when fed a high-fat diet compared to homozygous normal mice (Jelinek D et al. 2010).

The **ets variant gene 5 (*ETV5*)** mRNA is widely expressed across organs (Kobberup S et al. 2007; Monte D et al. 1994; Monte D et al. 1996). Knockout mice demonstrated that males have reduced body weight and are infertile (Schlesser HN et al. 2008). The *ETV5* gene is located near the polymorphism rs7647305 showing an association with BMI and obesity (Thorleifsson G et al. 2009). Also the **diacylglycerol kinase gamma (*DGKG*)** gene encoding an enzyme which regulates diacylglycerol by phosphorylating it to form phosphatidic acid and the **splicing factor, arginine/serine-rich 10 (*SFRS10*)** gene are located near this polymorphism.

A locus containing **allograft inflammatory factor-1 (*AIF1*)**, **natural cytotoxicity triggering receptor 3 precursor (*NCR3*),** and **HLA-B associated transcript-2 (BAT2)** gene was associated with weight (Thorleifsson G et al. 2009). The AIF1 protein is a calcium-binding protein involved in immune response (Deininger MH et al. 2002). There is a relationship between the *AIF1* gene and systemic sclerosis (Alkassab F et al. 2007; Otieno FG et al. 2007). The NCR3 is a natural killer cell activating receptor (Sato M et al. 2001). A ***NCR3*** sequence variant was associated with increased risk of mild malaria (Delahaye NF et al. 2007). The **BAT2** protein is a proline-rich protein with similarities to proteins with large proline-rich domains such as some nuclear proteins, collagens, elastin, and synapsin (Banerji J et al. 1990). A relationship between *BAT2* microsatellite marker and age-at-onset of insulin-dependent diabetes was suggested (Hashimoto M et al. 1999).

The **brain derived neurotrophic factor (*BDNF*)** gene is expressed in the brain (Jones KR and Reichardt LF 1990) and its high expression in the ventromedial hypothalamus (VMH) is regulated by nutritional state and *MC4R* signalling (Xu B et al. 2003). Mice with lower expression of the *BDNF* receptor were hyperphagic and gained excessive weight on high-fat diets (Xu B et al. 2003). A sequence variant showed a risk effect on eating disorders (Gratacos M et al. 2007). Homozygotes for the minor allele had a lower BMI compared to other genotypes (Gunstad J et al. 2006). Haplotypes within the *BDNF* gene were associated with bulimia and anorexia nervosa (Mercader JM et al. 2007).

The **Fas apoptotic inhibitory molecule 2 (*FAIM2*)** gene protects against Fas mediated apoptosis and is widely expressed (Schweitzer B et al. 1998; Somia NV et al. 1999).

Prolactin is an essential regulator of mammary development, acting with other hormones during puberty and pregnancy. In obese women there was a negative correlation between graded prolactin response to hypoglycaemia and increasing waist to hip ratio (Weaver JU et al. 1990). The **prolactin (*PRL*)** gene is well characterized (Berwaer M et al. 1994; DiMattia GE et al. 1990; Truong AT et al. 1984). An association between *PRL* polymorphisms and breast cancer risk is not clear (Lee SA et al. 2007; Vaclavicek A et al. 2006). The *PRL* gene is associated with systemic lupus erythematosus (Stevens A et al. 2001a; Stevens A et al. 2001b).

Knowledge about the **phosphotriesterase-related (*PTER*)** gene, the **V-maf musculoaponeurotic fibrosarcoma oncogene homolog (*MAF*)** gene, the **potassium channel tetramerisation domain containing 15 (*KCTD15*)** gene, the **SEC16 homolog B (*SEC16B*)** gene, the **RAS protein activator like 2 (*RASAL2*)** gene, and the **glucosamine-6-phosphate deaminase 2 (*GNPDA2*)** gene and their proteins is very limited, but an association with obesity has been described in recent genome-wide association studies (Meyre D et al. 2009; Thorleifsson G et al. 2009; Willer CJ et al. 2009).

Loci described in the following are not listed in **table 1-3**, but were also identified as obesity-related loci in genome-wide association studies.

The **methionine sulfoxide reductase A (*MSRA*)** gene encodes the methionine sulfoxide reductase A – a repair enzyme for oxidative damage (Lindgren CM et al. 2009). Oxidation of proteins by reactive oxygen species (ROS) is associated with oxidative stress. Also obesity is associated with oxidative stress (de Ferranti S and Mozaffarian D 2008).

The **tankyrase (*TNKS*)** gene encodes a polymerase (tankyrase) which interacts with insulin-responsive aminopeptidase (IRAP) in glucose transporter type 4 (GLUT4) vesicles (Chi NW and Lodish HF 2000; Yeh TY et al. 2007). Tankyrase-deficient mice show increased energy expenditure, fatty-acid oxidation, and insulin-stimulated glucose utilization (Yeh TY et al. 2009). Polymorphisms in this genomic region were associated with waist circumference in adults (Lindgren CM et al. 2009) and obesity in children (Scherag A et al. 2010).

The enzyme phosphofructokinase is the rate-limiting enzyme in glycolysis converting D-fructose-6-phosphate to fructose-1,6-biphosphate. The **platelet-type phosphofructokinase (*PFKP*)** gene could alter the balance between glycolysis and glycogen production (Hannemann A et al. 2005; Scuteri A et al. 2007). In an obese mouse model a locus that includes *Pfkp* was associated with liver weight, insulin, and reproductive fat pad weight (Ehrich TH et al. 2005). In humans the *PFKP* gene was associated with obesity, but replication failed (Andreasen CH et al. 2008a; Scuteri A et al. 2007).

The **thyrotropin-releasing hormone receptor (*TRHR*)** gene encodes the TRHR. Thyrotropic-releasing hormone is secreted by the hypothalamus (Liu XG et al. 2009). Mutations in the *TRHR* gene may decrease affinity of TRHR for thyrotropic-releasing hormone and result in central hypothyroidism (Collu R et al. 1997). *TRHR* polymorphisms are associated with lean body mass (Liu XG et al. 2009).

The **melatonin receptor 1B (*MTNR1B*)** gene was predicted to encode the melatonin receptor 1B which was reported to be expressed in human retina and brain (Reppert SM et al. 1995). It has been shown that the gene is also transcribed in human pancreatic islet cells (Lyssenko V et al. 2009; Ramracheya RD et al. 2008). Melatonin has an inhibitory effect on insulin secretion in clonal beta cells (Lyssenko V et al. 2009; Peschke E et al. 2002; Peschke

E et al. 2006; Ramracheya RD et al. 2008). *MTNR1B* polymorphisms are associated with fasting glucose (Bouatia-Naji N et al. 2009; Lyssenko V et al. 2009; Prokopenko I et al. 2009). Also an association with BMI was observed (Andersson EA et al. 2010).

The ***PCSK1*** gene encodes an enzyme expressed in neuroendocrine cells that converts prohormones into functional key hormones regulating central and/or peripheral energy metabolism. *PCSK1* mutations lead to human congenital PC1/3 deficiency characterized by obesity and small intestinal dysfunction (Farooqi IS et al. 2007; Jackson RS et al. 1997; Jackson RS et al. 2003). *PCSK1* polymorphisms are associated with obesity in humans (Benzinou M et al. 2008; Chang YC et al. 2010; Kilpelainen TO et al. 2009).

Knowledge about the **serologically defined colon cancer antigen 8 (*SDCCAG8*)** gene and its protein is very limited, but an association with obesity has been described (Scherag A et al. 2010).

The described obesity-related genes identified by genome-wide association studies (except *MTNR1B* and *PCSK1*) might be potential candidate genes for weight loss. As described above *FTO* and *MC4R* were already investigated in the context of weight loss. For the other genetic loci, no data concerning weight reduction are published so far.

1.3 Genetic susceptibility of lifestyle factors (dietary intake / physical activity)

Individual differences in dietary intake and physical activity exist and there is evidence that the genetic background affects food preferences as well as being active or inactive.

1.3.1 Evidence from family, twin, linkage and association studies

Dietary intake

The correlation and association between genetic differences in energy and nutrient intake as well as in eating behaviour phenotypes shown in many studies was reviewed by Rankinen and Bouchard (Rankinen T and Bouchard C 2006). Results from family studies gave evidence for familial aggregation of individual differences observed in caloric and macronutrient intake (Billon S et al. 2002; Cai G et al. 2004; Garn SM et al. 1979; Mitchell BD et al. 2003; Perusse L et al. 1988; Sellers TA et al. 1991; Vauthier JM et al. 1996). Heritability estimates of the three eating behaviour traits (restraint, disinhibition, hunger) from the Three-Factor Eating Questionnaire (TFEQ) were 28, 40, and 23 percent in the Amish Family Diabetes Study and 28, 18, and six percent in the Quebec Family Study (Provencher V et al. 2005; Steinle NI et al. 2002).

Furthermore, twin studies show evidence. Heritable factors account for about one third of the variance in whether a person consumes a specific food item (van den Bree MB et al. 1999). A study of mono- and dizygotic twin pairs reared apart provides evidence that the heritability of dietary characteristics is about one third of the phenotypic variance (Hur YM et al. 1998). The genetic variance for total caloric intake ranged from 24 to 33 percent in twins eating without presence of the co-twin (Faith MS et al. 1999). *De Castro JM et al.* published a lot of work concerning food and nutrient intake in mono- and dizygotic twins (de Castro JM 1993a; de Castro JM 1993b; de Castro JM 1999a; de Castro JM 1999b; de Castro JM 1999c; de Castro JM 2002; de Castro JM 2004b). He summarized that heredity accounts for 42 percent of the variance in average overall dietary intake, for 28 percent of the variance in the meal size and for 34 percent of the variance in the meal frequencies of twins (de Castro JM 1993a; de Castro JM 1993b; de Castro JM 2004a). The analyses of 600 twin pairs demonstrated genetic effects on dietary intake (e.g. energy, fiber) with heritability estimates ranging from 0.25 to 0.49 (Hasselbalch AL et al. 2008). An investigation of mono- and dizygotic twins from the Swedish Twin Registry showed that genetic factors affect food preferences and the frequency of intake of some foods (Heitmann BL et al. 1999).

In **table 1-4** the correlation patterns concerning food intake phenotypes are shown for various types of relatives. The higher correlations in dizygotic twins compared to siblings and the highest correlations in monozygotic twins are the result of both genetic and environmental factors (Perusse L et al. 1988; Rankinen T and Bouchard C 2006).

Table 1-4: Correlations for various pairs of relatives for energy and macronutrient intake

Variable	Siblings by adoption	Foster parent-adopted child	Spouses	Parent-offspring	Siblings	Di-zygotic twins	Mono-zygotic twins
Number of pairs	115	314	339	1,212	361	59	59
Energy intake (kcal/kg/day)	0.21	0.29	0.31	0.26	0.30	0.58	0.69
Carbohydrate (% energy)	0.21	0.08	0.50	0.29	0.37	0.49	0.70
Fat (% energy)	0.04	0.18	0.45	0.31	0.36	0.59	0.61
Protein (% energy)	0.22	0.22	0.28	0.27	0.38	0.55	0.71

Kcal=kilocalories; (Perusse L et al. 1988; Rankinen T and Bouchard C 2006)

Furthermore, there are genome-wide linkage scans as well as some candidate gene studies for food intake phenotypes which were already reviewed (Rankinen T and Bouchard C 2006). For example, in the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study the performed linkage scan found linkage for dietary energy and nutrient intakes (Collaku A et al. 2004).

Physical activity

Twin and family studies suggest that genetic factors contribute to the propensity of being sedentary or physically active (Bouchard C et al. 1986; Simonen R et al. 2004). If one of the parents or co-twins is active in sports, it is more likely that the child or co-twin is also active (Beunen G and Thomis M 1999). The analysis of 37,051 twin pairs resulted in heritability estimates of exercise participation from 48 to 71 percent (Stubbe JH et al. 2006). In another twin study the monozygotic twin correlations were higher than dizygotic suggesting that genes are involved in the magnitude of physical activity (Lauderdale DS et al. 1997). In a Portuguese twin study the genetic effects explained a considerable amount of variation in sports participation as well as in leisure-time physical activity (Maia JA et al. 2002).

In Dutch and American adults the first genome-wide association study of exercise behaviour was conducted (de Moor MH et al. 2009). The strongest association was observed for the 3`-phosphoadenosine 5`-phosphosulfate synthase 2 (*PAPSS2*) gene locus. Furthermore, an association with DNA polymerase-transactivated protein 6 (*DNAPTP6*) and chromosome 18 open reading frame 2 (*C18orf2*) polymorphisms was found.

Until 2009 seven versions of the human gene map for fitness- and performance-related traits were published (Bray MS et al. 2009; Perusse L et al. 2003; Rankinen T et al. 2001; Rankinen T et al. 2002; Rankinen T et al. 2004; Rankinen T et al. 2006; Wolfarth B et al. 2005). In 2010 a review called “advances in exercise, fitness, and performance genomics” summarized high quality publications on this topic (Rankinen T et al. 2010b).

1.3.2 Lifestyle factors and obesity-related genes (*FTO*, *MC4R*)

Obesity-related genes identified by genome-wide association studies might be potential candidate genes for lifestyle factors like dietary intake and physical activity. A recent Dutch study in 1,700 females showed a borderline significant association for two loci (*SH2B1* and *KCTD15*) with fat and carbohydrate intake, but not for *FTO* and *MC4R* (Bauer F et al. 2009). The best investigated obesity-related loci *FTO* and *MC4R* are reviewed in the following concerning the association with lifestyle factors.

FTO

Some studies report no association between *FTO* polymorphisms and caloric intake (Hakanen M et al. 2009; Hasselbalch AL et al. 2010; Johnson L et al. 2009; Liu G et al. 2010), whereas in other studies associations with increased energy intake have been shown (Cecil JE et al. 2008; Haupt A et al. 2009b; Speakman JR et al. 2008). Children homozygous for the risk allele had a significantly reduced satiety responsiveness score (Wardle J et al. 2008). Non-risk allele carriers ate less than children carrying the risk allele (Wardle J et al. 2009). In an experimental setting with 103 adults, individuals with a low postprandial

decrease in hunger were overrepresented in risk allele carriers (den Hoed M et al. 2009). The percent of children reporting “loss of control eating” differed between genotypes and children with the risk allele consumed more energy from fat (Tanofsky-Kraff M et al. 2009). In another study risk allele carriers consumed more fat and had a higher total energy intake than those not carrying the risk allele (Timpson NJ et al. 2008). In 711 Korean children an association between dietary fat intake and *FTO* genotype was seen (Lee HJ et al. 2010). Moreover, interactions between *FTO* locus and diet on BMI as well as on reduction in resting energy expenditure, beta cell function, and insulin resistance after a hypo-energetic diet were observed (Grau K et al. 2009; Sonestedt E et al. 2009).

Fto deficient mice show significantly reduced adipose tissue and lean body mass and an increased energy expenditure through increased sympathetic nervous system activity. *FTO* is assumed to have a function in energy homeostasis via control of energy expenditure (Church C et al. 2009; Fischer J et al. 2009). Up to now no study has reported an association between *FTO* and physical activity (Berentzen T et al. 2008; Hakanen M et al. 2009; Vimaleswaran KS et al. 2009b) or resting energy expenditure (Berentzen T et al. 2008; Do R et al. 2008; Haupt A et al. 2009b; Speakman JR et al. 2008). Results from several cohorts have suggested that physical activity may attenuate the effect of *FTO* (Andreasen CH et al. 2008b; Rampersaud E et al. 2008). Similar interaction results were observed in other studies (Cauchi S et al. 2009; Scott RA et al. 2010; Sonestedt E et al. 2009; Vimaleswaran KS et al. 2009b). Adolescents meeting the daily physical activity recommendations in the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA) may overcome the effect of the *FTO* gene on obesity (Ruiz JR et al. 2010a). In Finnish and Swedish adults the interaction between physical activity and *FTO* genotype was not significant (Jonsson A et al. 2009). In European and African-American youth neither a direct association nor an interaction was found between *FTO* and physical activity (Liu G et al. 2010). Furthermore, in the Tracking Adolescents` Individual Lives Survey (TRAILS) Study no modification by physical activity in the association between *FTO* and overweight was observed (Liem ET et al. 2010).

MC4R

Studies suggested that *MC4R* polymorphisms are associated with dietary intake (Heid IM et al. 2008; Pichler M et al. 2008; Qi L et al. 2008; Stutzmann F et al. 2009). There was a trend for an association between *MC4R* polymorphisms and intake of energy from whole grains (Hasselbalch AL et al. 2010). In obese Chilean children the *MC4R* gene may affect eating behaviour. A genetic variant might be associated with satiety responsiveness and enjoyment of food scores (Valladares M et al. 2010). Using data on dietary energy intake recorded from

food frequency questionnaire (FFQ), no association with *MC4R* polymorphisms was found in a Scottish population (Tenesa A et al. 2009). Furthermore, linkage findings mapped carbohydrate intake and physical activity to the region on chromosome 18 containing the *MC4R* gene (Cai G et al. 2006). In the Viva la Familia cohort *MC4R* genetic variants are likely to play a functional role in energy expenditure (Cole SA et al. 2010). This was not confirmed in obese young men (Kring SI et al. 2010). In the Quebec Family Study the *MC4R* C2745T variant showed significant associations with physical activity phenotypes (Loos RJ et al. 2005). In the first genome-wide association study concerning exercise behaviour the *MC4R* gene could not be replicated as a locus associated with exercise participation (de Moor MH et al. 2009). An interaction between *MC4R* variants and physical activity was not found (Cauchi S et al. 2009; Liem ET et al. 2010).

1.4 Aim of thesis

Environmental, lifestyle, and genetic factors modulate body weight and obesity risk. Meta-analyses of genome-wide association studies indicate that genetic factors are associated with BMI. However, knowledge about the association between genetic factors and lifestyle-induced weight loss is limited. It was hypothesized that the BMI associations might be evoked by a modulation of nutritional intake and energy expenditure.

The aim of this thesis was to investigate genetic loci for their association with changes of anthropometric traits after a lifestyle intervention programme as well as for their association with lifestyle factors.

1.4.1 Genetic association analysis for anthropometric changes

Little is known about the genetic loci which might be associated with weight loss success or resistance and no data exists – with the exception of *FTO* and *MC4R* - whether obesity-related loci identified by genome-wide scans are associated with changes of anthropometric traits during lifestyle intervention. This work addresses the genetic effect on changes of anthropometric traits in lifestyle intervention programmes (**Figure 1-6**). Therefore, a literature-based candidate gene approach including genes in the context of weight loss (e.g. *LEPR*, *ADRB2*, *PPARG2*) and of obesity (e.g. *FTO*, *MC4R*, *NEGR1*, *TMEM18*, *SH2B1*) was chosen. In particular the selected polymorphisms were analyzed in

(i) adults from a randomized controlled weight loss trial for their association with delta weight, fat mass, and waist circumference
(ii) children participating in an in-patient weight loss study for their association with delta weight and BMI standard deviation score (SDS).

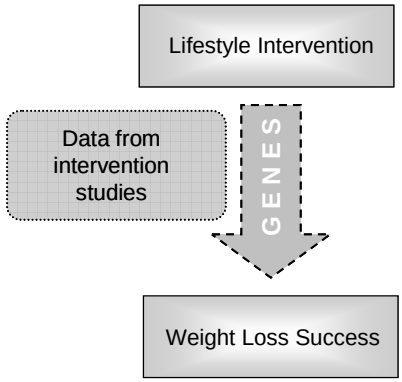


Figure 1-6: Schematic view of hypothesis tested. Investigation of an association between selected polymorphisms and weight loss success

This approach provides the chance to detect gene variants predicting the response to weight loss programmes.

1.4.2 Genetic association analysis for lifestyle factors

There is limited knowledge whether genetic factors associated with BMI interact with modifiable environmental factors. Investigations of associations between SNPs and dietary intake as well as SNPs and physical activity and the role of these lifestyle factors as mediators within the SNP-BMI association are scarce.

In this work seven obesity-related loci (*FTO*, *MC4R*, *NEGR1*, *TMEM18*, *SH2B1*, *MTCH2*, *KCTD15*) were selected in order to investigate in a large population-based study whether

(i) these loci are associated with lifestyle factors like dietary intake and physical activity

(ii) these factors are mediators within the SNP-BMI association (**Figure 1-7**).

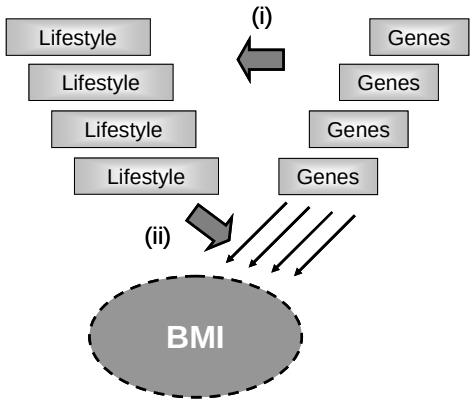


Figure 1-7: Schematic view of hypotheses tested: (i) investigation of an association between selected polymorphisms and lifestyle factors; (ii) investigation whether lifestyle factors are mediators within the gene-BMI association

Examining the direct association between obesity-related genes and lifestyle factors will contribute to the knowledge whether obesity-related genes are also genes affecting lifestyle factors. Furthermore, the examination of lifestyle factors as modifiers within the genotype-BMI associations can contribute to the understanding of the physiological pathways through which these genetic loci mediate their effect on obesity.

2 Study populations

2.1 Weight Watchers (WW) Global Efficacy Study

The WW Global Efficacy Study was an investigator-initiated intervention trial sponsored by WW (Weight Watchers International, Inc, New Work). The study title was “A randomized controlled trial to investigate the effectiveness of two commonly-used lifestyle-based weight-loss programmes across three countries” and for the German WW site “Investigation of the effectiveness of the WW method compared to a weight loss programme of general practitioner (GP) in overweight persons”. The co-ordinator was the Medical Research Council (MRC) Human Nutrition Research in Cambridge (United Kingdom). The primary purpose was to examine the effectiveness of the WW method for weight loss compared to current standard GP care, as per national guidelines, in three countries – United Kingdom (MRC Human Nutrition Research, Dr. S. Jebb), Australia (Boden Institute of Obesity, Nutrition & Exercise, Prof. I. Caterson) and Germany (Else Kroener-Fresenius-Centre for Nutritional Medicine (EKFZ), Prof. H. Hauner). Secondary objectives are listed in the following:

- To investigate numbers of participants losing five or ten percent of baseline weight
- To examine the cost of WW versus standard GP care for weight loss
- To investigate changes in a number of indicators of metabolic disease in both groups
- To explore effects of treatment on eating behaviour, physical activity, quality of life
- To qualitatively explore participants’ experiences in the two weight-loss programmes
- **To identify gene variants predicting weight loss or resistance to weight loss efforts**

The study protocol including all documents for participants has been approved by the ethics review committee of each site, in Germany by the ethical committee of the Faculty of Medicine of the Technische Universität München. The study has been performed in accordance with the ethical principles in the Declaration of Helsinki 2000 version and applicable regulatory requirements. Study data has been stored in a computer database maintaining confidentiality in accordance with national data legislation. Subjects in this database were identified by initials and subject number only.

The following information (**Chapters 2.1.1** to **2.1.4**) is for the German part of the WW study and is in general the same for all three countries, whereas there are some country-specific differences, for example in used instruments.

A total of 772 participants has been recruited across the three countries. In Germany, 268 participants were recruited from GP surgeries in Munich.

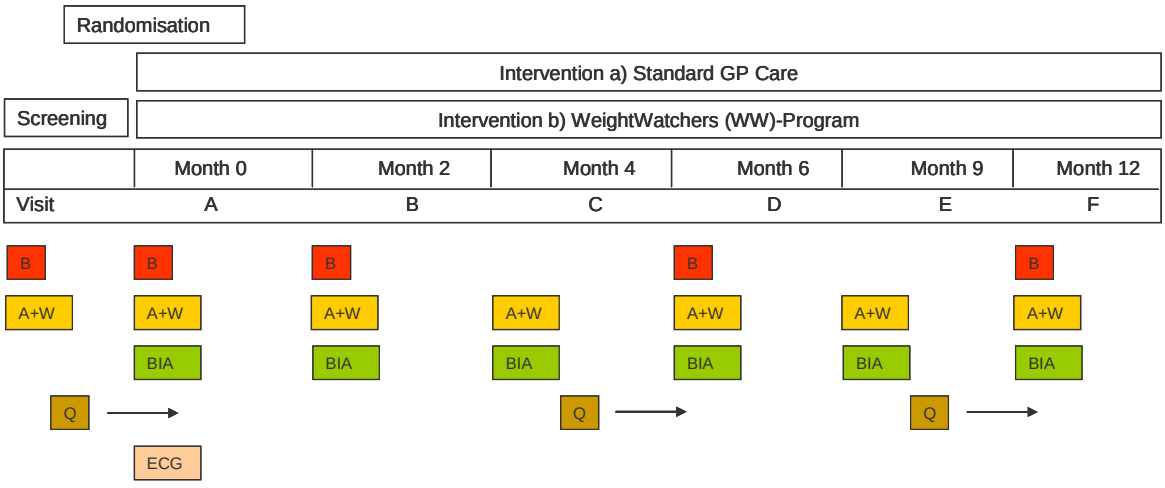
2.1.1 Intervention programme: Weight Watchers (WW) and "usual care" (GP)

Participants randomized to the **WW group** received the standard WW weight loss programme (**Appendix C**) offered through the local WW meetings. Participants got vouchers to attend WW on a weekly basis for twelve months and a user code for free access to WW "eSource" online resources. "eSource" is an internet accompaniment to the WW meetings which enables users to chart their weight loss, to access weight loss tips and recipes and to communicate with other online users. The participants were requested not to mention their study involvement to the group leader or other attendees at the meetings. They were allowed to visit their GP if required, but the GP did not offer any weight loss advice. Participants randomized to the **GP group** received individual weight loss advice provided by the GP (**Appendix D**) for twelve months. GPs and participants got a list of internet resources, a flyer with information for weight loss, and recommendations for a energy-restricted diet.

2.1.2 Study design

The study was a multi-centre randomized controlled trial. The participant was required to give written consent before any procedures were performed. Once a participant was deemed eligible (**Chapter 2.1.3**) for randomisation, he was allocated to a treatment group, with groups stratified for gender and type 2 diabetes mellitus (T2DM). Random group allocation was performed by the study database upon entry of the participant's details. Following on from Visit A, participants attended five visits (month 2, 4, 6, 9 and 12) at their GP`s office. The follow-up for further twelve months (month 18 and 24) to assess weight maintenance was optional. The study flow chart with summary of measurements made at each visit within the first study year is shown in **figure 2-1**. The schedule of study procedures lists the activities performed (**Appendix E**).

Figure 2-1: Study flow chart of outcome measurements at each visit within the first twelve months



B=blood sample; A+W=anamnesis and weight measurement; BIA=bio-impedance measurement; Q=questionnaires; ECG=electrocardiogram; GP=general practitioner

The two weight loss programmes were free of charge for participants and their health insurances. In Germany for each bio-impedance (BIA) measurement the participant got 15 euros to compensate for travel expenses and time loss. The quarterly practise fee has been refunded, if the patient had successfully finished the study. The same procedure was made for the follow-up time. GPs got 250 euros per participant fulfilling visit A and again 250 euros per participant completing visit F. During follow-up the GP got 25 euros per visit. The first year of intervention was finished in January 2010.

2.1.3 Inclusion and exclusion criteria

The participant's eligibility was assessed with the screening questionnaire. Inclusion criteria are shown in **table 2-1** and exclusion criteria in **table 2-2**.

Table 2-1: Inclusion criteria used in the WW study

Gender	male or female
Age	> 18 years
BMI	27 to 35 kg/m^2
Answer YES for at least ONE of the risk factors	family history of T2DM stable T2DM not treated with insulin previous gestational diabetes mellitus impaired glucose tolerance / impaired fasting glycaemia mild-moderate dyslipidaemia, or treatment for dyslipidaemia treatment for hypertension central adiposity (waist circumference > 88 cm women or > 102 cm men) polycystic ovary syndrome / infertility without other cause than weight osteoarthritis in lower limbs abdominal hernia

T2DM=type 2 diabetes mellitus

Table 2-2: Exclusion criteria used in the WW study

Participants have been excluded for ANY of the reasons	
Factors which may affect weight	weight loss of > 5 kg in the previous three months history of clinically-diagnosed eating disorder orthopaedic limitations preventing participation in regular physical activity untreated thyroid disease or more than one change in thyroid medication over previous six months taking any prescription medication with known effects on appetite or weight chronic / inflammatory gastrointestinal disorders (irritable bowel syndrome accepted) previous surgical procedure for weight loss major surgery within previous three months pregnancy or lactation
Co-existing disease	insulin-treated diabetes mellitus HbA1c > 9.0 percent diagnosis of T2DM within previous six months heart problems within previous three months (e.g. angina, myocardial infarction, stroke) or implanted cardiac defibrillator or pacemaker uncontrolled hypertension (> 160/95 mmHg) start of taking a new prescription medication within the previous three months change in dose of a prescription medication within the previous one month history or presence of cancer (resected basal or squamous cell carcinoma acceptable if treatment completed more than six months prior to enrolment)
Additional excluded medications	non-prescription weight-loss medications drugs for weight reduction including herbal preparations neuroleptics, prolonged use of laxatives, oral steroids gastrointestinal prokinetic drugs antidepressants / psychotropic medications with appetite effects

T2DM=type 2 diabetes mellitus; HbA1c=glycosylated hemoglobin

Participants have also been excluded if they have participated in another trial within 30 days prior to enrolment. Participants had to be willing to be involved in a weight loss programme and able to attend weekly WW meetings for a one-year period if allocated to the WW group.

2.1.4 Measured parameters

Questionnaires were used to obtain **demographic variables**. **Body height** (centimeter (cm)) was measured to the nearest 0.5 cm using a rigid stadiometer. **Body weight** (kg) was assessed to the nearest 0.1 kg using GP`s regular scale or, if not available, the provided scales (HD 327 S, Tanita Europe B.V., Hoofddorp, The Netherlands). The participant was dressed in light clothing, shoes removed and bladder emptied. **BMI** (kg/m²) was calculated as body weight in kg divided by squared body height in m). **Waist circumference** (cm) was measured using a non-stretch measuring tape to the nearest 0.5 cm. Waist circumference was measured midway between the top of the iliac crest and the most inferior part of the rib cage. The measurement was taken at the end of usual inspiration. **BIA analysis** was assessed using a BC-418 segmental Body Composition Analyzer (Tanita Europe B.V., Hoofddorp, The Netherlands). The participant was dressed in light clothing, shoes removed and bladder emptied. For clothes 1 kg was subtracted. **Blood pressure** (BP, millimeters of mercury (mmHg)) and **radial pulse rate** (beats per minute) were measured under standardized conditions. A twelve-lead **electrocardiogram** (ECG) was recorded using GP´s standard method. **Medications** as well as any changes or additions to concomitant medications were documented. **Dietary intake** was assessed using an open diet diary over four days. A **pedometer** (WW™, Weight Watchers GmbH, Düsseldorf, Germany) was provided to each participant in order to record the number of steps they walk for seven days. Participants were asked for the number of WW meetings as well as of visits with GP recorded in the **compliance** diary. The following three **questionnaires** were handed out for completion:

- TFEQ-R21
- International Physical Activity Questionnaire – short version (IPAQ-short)
- Impact of Weight on Quality of Life-Lite (IWQOL-Lite)

The German version of the IWQOL-Lite questionnaire was not validated yet. The IWQOL-Lite data from the WW study were used together with data from the Department of Psychosomatic Medicine and Psychotherapy of the University of Erlangen – Nuremberg (Germany) to evaluate the German IWQOL-Lite version (Mueller A et al. 2010).

Fasting blood samples were collected for analysis of biochemical parameters according to standardized analytical methods at “Medizinisches Versorgungszentrum Labor München Zentrum” (Munich, Germany).

Glucose was measured with the hexokinase method (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany) and **insulin** with an electrochemiluminescence immunoassay (ECLIA) method (Immulite 2000, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Using the turbidimetric immunoassay Tina-quant (Integra 800, Roche Diagnostics GmbH, Mannheim, Germany) **glycosylated hemoglobin** (HbA1c) was measured. **Total cholesterol** was determined by CHOD-PAP method (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany) and determination of **triglycerides** as well as **high and low density lipoprotein** (HDL, LDL) **cholesterol** was performed with an enzymatic colour assay (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany). Using Biuret method (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany) **total protein** and using turbidimetry (Integra 800, Roche Diagnostics GmbH, Mannheim, Germany) **high-sensitivity C reactive protein** (hsCRP) were measured. **Bilirubin** was determined using 2,5-dichlorophenyldiazonium (DPD) reagens (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany). Liver enzymes were measured by photometry: **alkaline phosphatase** (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany), **glutamic-oxaloacetic transaminase** (GOT, Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany), **glutamic-pyruvate transaminase** (GPT, Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany), **γ-glutamyltransferase** (GGT, Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany). **Thyreotropin** (TSH) was measured by ECLIA (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany) and **creatinine** with the Jaffé method (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany). Ethylenediaminetetraacetic acid (EDTA) and serum blood samples were collected for **DNA isolation** as well as the investigation of **adipokines** and **chemokines**.

2.2 LOGIC

The Long-term effects of lifestyle intervention in Obesity and Genetic Influence in Children (LOGIC) study is a clinical intervention trial initiated and coordinated by the Chair of the Preventive and Rehabilitative Sports Medicine (Technische Universität München, Prof. M. Halle). The study is performed at the Rehabilitation Hospital Schönsicht in Berchtesgaden (Dr. H. Langhof, Berchtesgaden, Germany). The determination of cardiovascular risk parameters is performed in cooperation with the University of Ulm (Internal Medicine, Prof. W. Koenig) and the genetic analysis is done together with the Helmholtz Zentrum München (HMGU, Institute of Epidemiology, PD Dr. T. Illig) and the EKFZ (Prof. H. Hauner). The study has been approved by the ethics review committee of the Technische Universität München.

The primary purpose of this study was to examine the association between genetic loci and short- and long-term (four weeks / one year) BMI-SDS change after a controlled lifestyle intervention in overweight and obese children and adolescents. Secondary objectives are the association of genetic loci and changes of adipokines and inflammation markers as well as the ten year effects of a four-week lifestyle intervention on nutrition, physical activity, and quality of life.

2.2.1 Intervention programme: lifestyle intervention

The Rehabilitation Hospital Schönsicht is certified as a centre for childhood obesity treatment by the German Society of Obesity (DAG) and by the consortium of Obesity in Childhood and Adolescence (Arbeitsgemeinschaft Adipositas im Kindes- und Jugendalter (AGA)). The multidisciplinary team consisting of medical specialists for children and adolescents, psychologists and psychotherapists, dieticians, pedagogues and educators, physiotherapists and nurses, works according to DAG and AGA guidelines. The weight loss programme is in-patient for regularly four to six weeks and is based on nutrition, physical activity, and behaviour therapy (**Appendix F**). The duration of the intervention is documented. There is a moderate energy reduction of about 500 kilocalories (kcal) per day. The physical activity part consists of eleven hours per week and due to spare time activities six hours per week are added. Behaviour therapy consists of psychological therapy within group sessions including training of eating behaviour. If there is need, an additional psychological single therapy up to 45 minutes three times per week is offered.

2.2.2 Study design

This mono-centre study is divided into phase I (pilot phase) and II. During the pilot phase the first recruiting step was done from January 2006 to June 2008 including 512 children and adolescents. In phase II the second recruitment step is done. Once a child arrives at the Rehabilitation Hospital Schönsicht, the study team ask for participating. Parent is asked to give written informed consent before any study procedures are performed. In total 1,500 children are planned to be included until December 2012. The follow-up for ten years to assess weight control will last until June 2018 (phase I) or October 2022 (phase II), respectively. Visit 1 and 2 are in-patient. Three follow-up visits (six months, one and two years) are performed by the GP/pediatrician at the residence of the child, whereas the last two visits (five, ten years) are again in-patient.

The study flow chart with all visits is shown in **appendix F**. Beside these visits weight data was collected every week during the initial stay. The schedule of study procedures lists the activities performed at each visit (**Appendix F**). Participating in the study is free of charge for children and their health insurances.

2.2.3 Study population

A proportion of 66 percent from the first recruitment step completed the follow-up visit after six months. The return rate after one and two years was 49 and 42 percent, respectively. Children without follow-up visits are systematically recorded and interviewed by phone. To keep drop out rate low, children are regularly called and motivated for further participation.

The participant's eligibility is assessed according to the following criteria:

- gender: male or female
- age: six to 18 years
- referral to Rehabilitation Hospital Schönsicht with indication of obesity therapy
- written informed consent of parent for participation as well as for follow-up

Secondary obesity or monogenic disorders influencing obesity development (e.g. Prader-Willi syndrome) as well as drop out during the in-patient phase of the study are considered as exclusion criteria.

2.2.4 Phenotypes and measured parameters

Questionnaires are used to obtain **demographic variables**. **Body height** (cm) is measured to the nearest 0.5 cm using a rigid stadiometer. **Body weight** (kg) is assessed in underwear to the nearest 0.1 kg (Tanita BC-420 P MA Profi, Tanita Europe B.V., Hoofddorp, The Netherlands). **BMI-SDS** is calculated using an established equation (Cole TJ 1990; Kromeyer-Hauschild K et al. 2001). **Waist circumference (cm)** is measured midway between the lowest rib margin and the superior border of the iliacal crest using a non-stretch measuring tape to the nearest 0.5 cm. The **BP** (mmHg) is measured under standardized conditions. **Pubertal status** is assessed according to Tanner staging. A **pedometer** (Omron, Walking Style Pro HJ-720IT) is provided to a subgroup of 200 children, in order to record the number of steps they walk for two weeks. A standardized **questionnaire for parents** is used in order to collect data on age, height, and weight of parents, ethnical background of parents and grandparents, education and job of parents, obesity-related illness within the family as well as information on the private situation of the child. Furthermore, the following three **questionnaires** are handed out for completion:

- Questionnaire for quality of life
- Questionnaire for physical activity
- Questionnaire for nutritional behaviour

Fasting blood samples are collected for analysis of biochemical parameters according to standard methods. **Glucose** is measured with the hexokinase method (INTEGRA® 800, Roche Diagnostics) and **insulin** by an enzyme-linked immuno sorbent assay (ELISA) (Mercodia, Uppsala, Sweden). **Homeostasis model assessment of insulin resistance (HOMA-IR)** as a measure of insulin sensitivity and **HOMA of beta cell function (HOMA-B)** as an index of beta cell function are calculated according to *Matthews DR et al.* (Matthews DR et al. 1985). These indexes have been validated in healthy children (Gungor N et al. 2004). Lipids (**total cholesterol**, **LDL and HDL cholesterol**, **triglycerides**), **pro-insulin**, **leptin**, **adiponectin multimer**, **resistin**, **IL-6**, **TNFalpha**, **hsCRP**, **retinol binding protein 4 (RBP-4)**, **TSHbasal**, and **uric acid** are measured according to standardized protocols.

2.3 MONICA/KORA

The “Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)” Augsburg project was part of the World Health Organization (WHO) MONICA project (Bothig S 1989; Keil U et al. 1998). The main aim of the WHO project was “to measure the trends and determinants in cardiovascular mortality and coronary heart disease and cerebrovascular disease morbidity and to assess the extent to which these trends related to changes in known risk factors, daily living habits, health care, or major socioeconomic features measured at the same time in defined communities in different countries”. Three independent cross-sectional surveys were conducted (S1 1984/85, S2 1989/90, S3 1994/95). One centre is in southern Germany, in the city of Augsburg and two adjacent counties (Augsburg and Aichach-Friedberg). Subjects were prospectively followed within the “Cooperative Health Research in the Region of Augsburg (KORA)”. The survey S4 (1999/2001) was conducted under the same conditions as the previous three surveys. The MONICA/KORA study has been approved by the ethics review committee of the Bavarian Medical Association and the Bavarian commissioner for data protection and privacy.

2.3.1 Study design

The MONICA/KORA study is a population-based cross-sectional study. Therefore, a random sample stratified by age and gender was drawn from the study region. Persons with German nationality, residency in the study region and aged between 25 and 64 for the first survey (S1) and between 25 and 74 years for the further surveys (S2, S3, S4) were included. The participants were required to give written consent before any procedures were performed. Following on from the first examination, participants were followed-up (Holle R et al. 2005). Details of the study design have been described previously (Holle R et al. 2005; Loewel H et al. 2005; Wichmann HE et al. 2005).

2.3.2 Study population

In total 18,079 participants (9,000 men, 9,079 women) were included in the MONICA/KORA Augsburg baseline surveys (Wichmann HE et al. 2005). 12,462 participants (6,271 men, 6,191 women) from the surveys S2, S3 and S4 were included into the present analysis. The potential of population stratification was reported to be small in KORA (Steffens M et al. 2006).

2.3.3 Phenotypes and measured parameters

Standardized interviews to obtain **demographic** and **lifestyle** variables and **medical examination** were conducted by trained medical staff. **BMI** (kg/m²) was calculated as body weight in kg measured in light clothing to the nearest 0.1 kg divided by squared body height in m measured to the nearest 0.5 cm. **Waist circumference** (cm) was measured at the level midway between the lower rib margin and the iliac crest with the participants breathing out gently. Venous **blood samples** were drawn and used for determination of biochemical parameters as well as for bio-banking. Details of measured blood parameters are published elsewhere (Keil U et al. 1998; Rathmann W et al. 2003).

A four-category seasonal **physical activity score** was assessed from questions on leisure time sports in summer and winter: 1 = regularly more than 2 hours, 2 = regularly about 1 hour, 3 = irregularly about 1 hour, 4 = no sports on a weekly basis during leisure time (Meisinger C et al. 2005). For **smoking** specific questions were asked. From self-reported alcohol intake for the previous workday and the previous weekend, **alcohol consumption** was calculated in g per day (g/day) (Doering A et al. 1993; Wellmann J et al. 2004). Scores of the frequency of **consuming fat or carbohydrate** containing foods were constructed based on a validated qualitative FFQ with 24 items. Subjects were asked for the frequency (almost daily, several times per week, about once a week, several times per month, once a month or less, and never) of the usual intake of food groups (Winkler G and Doering A 1998).

3 Materials

3.1 Equipment

Equipment used for DNA preparation and analysis at HMGU is listed in **appendix G**.

3.2 Software and databases

Software for genotyping processes as well as online databases for SNP selection and statistical software are listed in **appendix G**.

3.3 Buffer, solutions, reagents, and enzymes

Buffer and solutions for DNA extraction as well as materials for agarose gel electrophoresis, polymerase chain reaction (PCR), and SNP detection are listed in **appendix G**.

3.4 Expendable items

Expendable items like silicium-chip, dimple plates, falcon tubes, and micro-plates are listed in **appendix G**.

3.5 Oligonucleotides

For genotyping three different oligonucleotides are used (sequence-specific forward and reverse primers for iPLEX PCR and extension primers for iPLEX primer extension reaction). All oligonucleotides were designed by the MassARRAY software (Sequenom, Hamburg, Germany) to avoid overlapping peaks in mass spectra. The software also considers potential unwanted intra- and inter-primer interactions in order to avoid non-template extensions. The primers were produced by Metabion (Metabion, Martinsried, Germany). PCR primers were used in a concentration of 100 micromolar (µM), the extension primers of 300 µM. Extension primers were purified by high performance liquid chromatography (HPLC) and checked by matrix assisted laser desorption / ionisation time of flight (MALDI-TOF) mass spectrometry. Additionally to the primer sequence a ten-mer tag consisting of 5´-ACGTTGGATG-3` and non-complementary to the DNA sequence in the genome was attached. The thereby generated primer mass of 9000 dalton (Da) cannot be detected in the mass spectrum of extension primers and their elongation products. Therefore, unused PCR primers fall outside the mass range of analytical peaks. Used primers are listed in **appendix H**.

4 Methods

4.1 Single nucleotide polymorphism (SNP) selection

SNPs are substitutions of single base pairs (bp), mostly biallelic, with a minor allele frequency (MAF) greater than one percent in the population. There are about 23 millions SNPs in the human genome and approximately every 150 bp a SNP is found (www.ensemble.org). The 1,000 genomes project sequencing the genome of a large number of people will result in new numbers (www.1000genomes.org). SNP selection for genotyping was based on a literature search as well as on the public databases “National Center for Biotechnology Information” (NCBI; www.ncbi.nlm.nih.gov), Ensembl (www.ensembl.org) and HapMap (www.hapmap.org).

For the outcome BMI and obesity mainly genome-wide association studies were considered, whereas data for weight loss was only based on candidate gene studies. For every SNP the most published and best described SNP was selected for genotyping.

For an association between genetic variants and weight loss success in the WW and the LOGIC study BMI- and weight loss-related SNPs were selected (**Appendix H** and **Chapter 5.2.1**). For lifestyle factor analysis BMI-associated SNPs according to the published data by *Willer C et al.* (Willer CJ et al. 2009) were genotyped in the MONICA/KORA study (**Appendix H** and **Chapter 5.4.1**). All selected gene loci were described in the introduction part. There are only a few gene loci (*LEP*, *UCP3*, *ADRB3*, *GNAS*, *TNFalpha*, *AIF1 NCR3 BAT2*, *FAIM2*) which were mentioned in the introduction part, but not genotyped.

4.2 Deoxyribonucleic acid (DNA) extraction from blood

The DNA isolation from 2.7 and 9.0 milliliters (ml) frozen EDTA anti-coagulated blood samples was based on the salting-out method of *Miller SA et al.* (Miller SA et al. 1988) with slight modifications. Blood was solved with 30 ml lysis buffer (erylysis) and removed by centrifugation at 2,500 revolutions per minute (rpm) from cells with intact nuclei. Leukocytes were lysed by adding 5 ml sodium chloride EDTA buffer (SE buffer), 25 microliters (µl) proteinase K and 250 µl of 20 percent sodium dodecyl sulfate (SDS) (leukolysis) and by digesting overnight at 55 centigrades (°C). Remaini ng proteins were precipitated with 3 ml saturated sodium chloride (NaCl) solution and 5 ml SDS buffer (protein precipitation). After vigorous shaking and centrifugation at 3,500 rpm, supernatant was solved with 13 ml 100 percent isopropanol, whereby DNA precipitated. DNA pellet washed with 10 ml 70 percent ethyl alcohol and dried was dissolved in 1.2 ml 0.1x tris(hydroxylmethyl)aminomethane (Tris) EDTA buffer (TE buffer). DNA samples were stored at 4 °C. This method was used for the WW and MONICA/KORA samples. The LOGIC samples were extracted at the Preventive and Rehabilitative Sports Medicine where a Wizard® Genomic DNA Purification Kit (Promega, Mannheim, Germany) was used.

4.3 Deoxyribonucleic acid (DNA) quantification (concentration and quality)

4.3.1 Spectrophotometry

Spectrophotometry is the quantitative measurement of reflection or transmission properties of a material as a function of wavelength. DNA absorbs ultraviolet (UV) light very efficiently. The nitrogenous bases in nucleotides have an absorption maximum at 260 nanometers (nm). Using a 1 cm light path, the extinction coefficient for nucleotides at this wavelength is 20. Based on this extinction coefficient the absorbance at 260 nm in a 1 cm quartz cuvette of 50 micrograms (µg)/ml solution of double stranded DNA is equal to one (optical density, OD). The sample concentrations are automatically calculated as follows: *DNA concentration (µg/ml) = (OD 260) x (dilution factor) x (50 µg DNA/ml) / (1 OD 260 unit)*.

In contrast to nucleic acids, proteins have a UV absorption maximum of 280 nm, mostly due to the tryptophan residues. The absorbance of a DNA sample at 280 nm gives an estimate of the protein contamination of the sample based on the fact that the OD 260 is twice as high as that at 280 nm, if the solution contains pure DNA. The ratio of absorbance (260 nm / 280 nm) is a measure of the purity of a DNA sample. It should be between 1.70 and 2.00.

The NanoDrop® (Thermo Fisher Scientific Inc., Wilmington, USA) is a full-spectrum (220-750 nm) spectrophotometer that measures 1 µl samples with high accuracy and reproducibility. In addition, the ND-1000 has the capability to measure highly concentrated samples without dilution. The OD 260 / OD 280 ratios measured were in almost all cases 1.70 to 2.00, demonstrating good deproteinization during DNA extraction.

4.3.2 Agarose gel electrophoresis

The agarose gel electrophoresis is a molecular biological method separating DNA fragments in the electrical field according to size. The size of the fragments can be estimated by comparison with commercially available known fragments (DNA ladders). Nucleic acids are negatively charged because of the phosphodiester backbone. Thus, in the electrical field DNA migrate to the anode. The smaller the DNA fragments the farther the movement through the gel matrix. A 1.5 percent agarose gel is performed with agarose and Tris borat EDTA buffer (TBE buffer). After heating the agarose solution in the microwave ethidium bromide – a colour for nucleic acids flourescencing at 266 nm – is added. Afterwards the sample is put with 3 µl blue juice (loading dye) on the cooled gel. For size measurement a molecular weight marker is also put on the gel. The electrophoresis lasts 120 minutes at 90 volt. Results were visualized by an agarose gel documentation system. The amplicons of a successful PCR contain about 100 bp. The gel electrophoresis is used to check whether enough DNA was built by the PCR reaction. Negative controls detect contamination and positive controls give hints for a successful PCR. The gel electrophoresis is also used as quality control for genomic DNA after DNA preparation.

4.3.3 Amelogenin

The amelogenin gene is a pseudogene which is localized on the X chromosome and shows a length polymorphism. For gender determination a specific part (forward primer: CTGATGGTTGGCCTCAAGCCTGTG / reverse primer: TAAAGAGATTCATTAACTTGACTG) of this gene is checked which has 977 bp in women and 788 bp in men. The amelogenin test is made by PCR and gel electrophoresis according to standardized protocols and checks for mistakes of DNA extraction or DNA amplification as well as mix-up errors.

4.3.4 Polymerase Chain Reaction (PCR)

PCR (Saiki RK et al. 1988), a standard method for amplification of a specific DNA region, was used to amplify an approximately 80-120 bp sequence around the SNP of interest. Two primers (forward/reverse) complementary to the end sequences of the DNA region of interest are used. The forward primer is complementary to the anti-strand and the reverse one to the sense strand. The double stranded DNA is disentangled in the first PCR step (Phase I: denaturation). Denaturation performed at a temperature of 94 °C leads to a destruction of the hydrogen bonds between bases of single strands. For annealing the temperature is lowered to 56 °C. This allows the excess of primers to anne al to their complementary sequences on both DNA strands (Phase II: annealing). The primers are usually only 18 to 25 bp long and designed to bracket the DNA region to be amplified. Due to the catalytical effect of Taq polymerase and the four deoxyribonucleotides (dNTP; dATP, dGTP, dCTP, dTTP) new DNA strands are synthesized at a temperature of 72 °C (optimal temperature of Taq polymerase; Phase III: elongation). Polymerase reads from 3`-end to 5`-end and adds dNTPs from 5´-end to 3`-end. The 3` hydroxyl group (OH) group of the primers is used by polymerase to catalyze the DNA synthesis out of the provided dNTPs. A final elongation step is frequently used after the last cycle to ensure that any remaining single stranded DNA is completely copied. The amplification consists of 30 to 45 cycles of denaturation, annealing, and elongation. Both DNA strands are copied and thus lead to an exponential reaction for which new synthesized DNA segments act as matrices for the next cycles. DNA is copied according to the formula 2^n (n = number of cycles). 35 cycles produce 2^{36} DNA copies.

4.4 Polymorphism detection via MALDI-TOF mass spectrometry

4.4.1 Pipetting of 384-well plates

Four 96-well plates are combined to one 384-well plate with the pipetting robot TeMo (Tecan, Crailsheim, Germany), whereby 5 µl (1 nanogram (ng) DNA/µl) are pipetted per well. 384-well plates dried overnight at room temperature are stored afterwards at 4 °C. Allocation of the positions on the 384-well plate are administrated by excel and controlled by two independent persons.

4.4.2 iPLEX Gold Assay

A widely-used and well developed method for high-throughput SNP genotyping is the iPLEX Gold assay. Allele-specific extension products are generated by primer extension and can be distinguished by their molecular weight using mass spectrometry. This assay is a method with high plexing possibility to detect up to 40 SNPs in one approach. In extension all four mass-modified nucleotides are present. During iPLEX reaction the primer is extended by one of the nucleotides which terminates the extension of the primer.

The here analyzed SNPs were genotyped together with other SNPs not considered in this work. Thus, in the WW and LOGIC study the SNPs were genotyped in five assays – one of 23 SNPs (23-plex), one of 33 SNPs (33-plex), one of 35 SNPs (35-plex), and two of 37 SNPs each (37-plex) – and in the MONICA/KORA study in one assay (25-plex) (**Appendix H**).

4.4.3 Polymerase chain reaction (PCR) amplification for iPLEX assay

The PCR for the iPLEX assay was carried out according to a standard protocol. The master mix (**Table 4-1**) was pipetted with the Genesis RSP 150 workstation (Tecan, Crailsheim, Germany). PCR was performed in the DNA Engine Tetrad PCR block (MJ Research, now Bio-RAD, Munich, Germany) under standardized temperature conditions (**Table 4-2**).

Table 4-1: PCR master mix for iPLEX assay

Reagent	Concentration	Volume / well
PCR buffer (10x) with $MgCl_2$		0.625 µl
dNTP mix	25 mM	0.100 µl
$MgCl_2$	25 mM	0.325 µl
Primer mix (forward)	100 µM	0.005 µl per primer
Primer mix (reverse)	100 µM	0.005 µl per primer
HotStar-Taq (polymerase)*	5 U/µl	0.100 µl 0.200 µl
Nanopure water		fill up to 5 µl
+ genomic DNA		5-10 ng/µl

$MgCl_2$=magnesium chloride; mM=millimolar; µM=micromolar; U=unit; µl=microliter; dNTP=deoxynucleotide; ng=nanogram; *Taq addition ≤ 27-plex 0.100 µl, ≥ 28-plex 0.200 µl

Table 4-2: Temperature profile of PCR for iPLEX assay

PCR step	Temperature [°C]	Time	Cycle
	94	15 min	1
Denaturation	94	20 sec	45
Annealing	56	30 sec	45
Elongation	72	1 min	45
	72	3 min	1
	20	forever	1

Min=minute; sec=second

4.4.4 Shrimp alkaline phosphatase (SAP) reaction

Shrimp alkaline phosphatase (SAP) inactivates by dephosphorylation dNTPs remained from PCR. This avoids the incorporation of the dNTPs instead of a dideoxynucleotide (ddNTP) in the following iPLEX extension reaction which would lead to other products than the specified extension ones. SAP reaction is necessary for a correct SNP detection after the primer extension reaction and for a good interpretation of the peaks from the mass analysis. The SAP mix and pipetting scheme for SAP reaction is shown in **table 4-3**. The SAP mix was dispensed using a pipetting robot (Multimek^{TM} 96 automated 96 channel pipettor, Beckman Coulter, Krefeld, Germany). For activation and inactivation of phosphatase (temperature optimum of 37 ℃) the temperature profile shown in **table 4-4** was used. The reaction was performed on PCR DNA Engine Tetrad (MJ Research, now Bio-RAD, Munich, Germany).

Table 4-3: Shrimp alkaline phosphatase (SAP) mix for iPLEX assay

Reagent	Concentration	Volume / well
Nanopure water		1.53 µl
SAP buffer (10x)		0.17 µl
SAP enzyme	1 U/µl	0.30 µl
Total		2.00 µl

U=unit; µl=microliter

Table 4-4: Temperature profile of shrimp alkaline phosphatase (SAP) reaction

SAP step	Temperature [℃]	Time	Cycle
Activation	37	40 min	1
Inactivation	85	5 min	1
	20	forever	1

Min=minute

4.4.5 Primer extension reaction

The iPLEX reaction is based on the termination reaction according to *Sanger F*. The 3`-OH-group on the carbon atom C3 of desoxyribose of dNTPs binds to the phosphoric acid rest of the nearby dNTP. This leads to a 3´-5´-phosphodiester binding. In contrast to dNTPs the used ddNTPs in the primer extension reaction have no 3`-OH-group. This leads to chain termination after incorporation of a ddNTP. Analogue to PCR reaction the primer extension reaction is performed by annealing, binding of thermosequenase, and primer elongation.

The used extension primers are hybridized directly to the polymorphic site of interest. There is an inverse correlation between primer mass and peak intensity assessed by MALDI-TOF due to the more difficult ionisation of large heavy molecules compared to light molecules. Thus, as the extension primer with the highest mass has 25 percent less intensity than the average of the low mass primers, primers were adjusted by concentration dependent on their

mass. Three different methods could be used for adjustment: dividing primers into two or four groups or adjusting each primer separately. While lower plexes (up to 19-plex) were processed adjusting each primer separately, higher plexes were adjusted in four groups. Thus, added volumes of the primers were adapted according to the adjustment result from the plate editor tool included in the MassARRAY software (Sequenom, Hamburg, Germany). Compared to primers of low mass, primers with higher mass were added to the iPLEX mix in double amount.

Furthermore, ddNTPs are mass modified and each ddNTP shows a specific mass. Mass differences of elongation products were detected allele-specific via MALDI-TOF mass spectrometry. Therefore masses were chosen in order to have a distance of 30 kilo (k) Da to each other to get well analyzed spectra. Through specific software mass information from mass spectrometry is transferred into genotype information. The primer mix was prepared according to the primer adjustment protocol and mixed up with the other reagents. The reaction volume of 2 µl was pipetted by means of a pipetting robot (Multimek^{TM} 96 automated 96 channel pipettor, Beckman Coulter, Krefeld, Germany) (**Table 4-5**).

Table 4-5: Standard mix for primer extension reaction

Reagent	Volume / well
Nanopure water	0.755 µl
iPlex Gold buffer plus (10x)	0.200 µl
iPlex termination mix*	0.200 µl
Primer mix (7.0 µM, 9.3 µM, 11.6 µM, 14.0 µM)	0.804 µl
iPlex Enzyme*	0.041 µl
Total	2.000 µl

µM=micromolar; µl=microliter * ≥ 19-plex

The primer extension reaction was performed by thermal cycling (DNA Engine Tetrad, MJ Research, now Bio-RAD, Munich, Germany) following the conditions presented in **table 4-6**.

Table 4-6: Standard temperature profile for iPLEX extension reaction

Step	Temperature [°C]	Time	Cycles	
	94	30 sec	1	
Denaturation	94	5 sec	1	40
Annealing	52	5 sec	5	
Elongation	80	5 sec		
	72	3 min	1	
	20	forever	1	

Sec=second; min=minute

4.4.6 Clean Resin

After the extension reaction, samples were purified to remove extraneous salts that would interfere with MALDI-TOF mass spectrometry. Clean Resin (SpectroClean^{TM}, Sequenom, Hamburg, Germany) is an ion exchanger and removes cations like sodium, potassium, or magnesium from the extension products which would disturb mass spectrometry. Per well 6 milligrams (mg) Clean Resin were used. The dried ion exchanger is added to the DNA samples. Afterwards 20 µl water are pipetted per well by the robot (Multimek^{TM} 96 automated 96 channel pipettor, Beckman Coulter, Krefeld, Germany). After shaking by rotator for 20 minutes and centrifugation plates can be spotted or stored.

4.4.7 MALDI-TOF mass spectrometry

The introduction of the MALDI-TOF mass spectrometry (Karas M and Hillenkamp F 1988) has offered a solution for fast and accurate SNP genotyping in a high-throughput manner. During MALDI-TOF mass spectrometry the sample is staggered with a 100 to 1,000 fold excess of matrix, co-cristallized on a sample plate and irradiated with an intensive laser pulse for a few nanoseconds in the high vacuum chamber of the mass spectrometer (Karas M and Hillenkamp F 1988; Kirpekar F et al. 1998). Mass determination is performed via the mass-/charge-ratio of proteins and peptides. Therefore the matrix plays an important role for the absorption of applied laser energy as well as for the induction of ionization. Additionally the matrix should prevent the analyte against a photolytic damage and avoid interaction of analyte molecules with each other or with the sample carrier (Hillenkamp F et al. 1991). The DNA sample co-crystallizes with the chip matrix consisting of 3-hydroxypicolinic acid which is especially well applicable for DNA analysis (Gut IG 2001; Little DP et al. 1997a; Little DP et al. 1997b). The transfer of laser energy to sample molecules in the matrix generates mainly single charged molecule ions that trespass into the gas phase (Hillenkamp F et al. 1991). Under high vacuum conditions the matrix crystals were irradiated with nanosecond duration laser pulses leading to formation of a plume of volatilized matrix and analyte as well as charge transfer from matrix ions to analyte molecules. After electric field-induced acceleration in the mass spectrometer source region, the gas phase ions travel through a field-free region at a velocity inversely proportional to their mass-to-charge ratios (m/z) until they hit the detector (Buetow KH et al. 2001; Griffin TJ and Smith LM 2000).

The so-called “Time of Flight” is the flight time of ions from the ion source to the detector. Mass of ions is proportional to their “Time of Flight” because distance between ion source and detector is known and only ions with charge “one” are analyzed (Karas M and Hillenkamp F 1988; Kirpekar F et al. 1998). Ions with low m/z values are faster than ions with higher m/z values. The TOF-analyzer measures exactly the time until the ions hit the detector (Griffin TJ and Smith LM 2000).

With MALDI-TOF mass spectrometry DNA fragments from 1,000 to 9,000 Da (three to 30 bases) can be determined with an accuracy of 0.1 to 0.01 percent.
The resulting time-resolved spectrum is translated into a mass spectrum upon calibration. These mass spectra were further processed and analyzed by the software Spectro Typer RT (Sequenom, Hamburg, Germany) for baseline correction and peak identification.
Following the primer extension reaction, 1 to 2 nanoliters (nl) from the ion removed sample are transferred to a silicon chip. The silicon chip contains 384 matrix spots for the samples and ten matrix spots for the calibrant (a mix of three oligonucletides). After loading the chip was transferred to a metallic sample carrier and put in the vacuum lock of the mass spectrometer (Autoflex® Sequenom™ / Bruker Daltonics®, Hamburg / Bremen, Germany). Via MassARRAY software (Sequenom, Hamburg, Germany) SNP and sample data were transferred to the analyzing system. The mass analysis provides per sample a spectrum which is the average of a lot of spectra and which shows the masses of all SNP-specific extension primers and their single extension products.

4.4.8 Evaluation of spectra

The MassARRAY software (Sequenom, Hamburg, Germany) divides the different genotype qualities („calls“) according to their probability into six groups. If there is a probability of higher or equal 99 percent for the real occurrence of the specific genotype the call is termed as *conservative*. *Moderate* means a security of greater or equal 95 percent and *aggressive* a security of greater or equal 90 percent. In case of no allele determination by the software the call was termed *low probability*, *bad spectrum* or *no allele*, respectively. Genotypes can be manually evaluated as *user call* in the MassARRAY software (Sequenom, Hamburg, Germany).

4.4.9 Quality assurance during genotyping

To avoid sample mix-up and contamination errors eight asymmetric negative (0.1 TE) as well as eight positive (defined DNA) controls were included on each 384-well plate. A minimum of ten percent of routine duplicates were genotyped. Furthermore, sex determination was performed with a validated genotyping assay. This assay detected DNA variants on chromosome X and their homologous sequence on chromosome Y. Furthermore, the amelogenin check (**Chapter 4.3.3**), calculation of Hardy-Weinberg equilibrium (HWE, **Chapter 4.5.1**), gel electrophoresis (**Chapter 4.3.2**), and the check of laboratory steps by a second person are used for quality control. The “call” or “success” rates are a measure of the success of genotyping calculating the percentage of all genotyped samples for which a genotype is available. The success rate should be above 90 percent and is also considered as quality control.

4.5 Statistical methods

All analyses unless otherwise noted were performed using the Statistical Analysis System (SAS) Version 9.1 (SAS Institute Inc., Cary, NC, USA).

4.5.1 Hardy-Weinberg equilibrium (HWE)

The frequency of alleles and genotypes in a population remain constant from generation to generation if the population is stable and in genetic equilibrium (Hardy GH 1908; Weinberg W 1908). For a stable population a sufficient large population, panmixie, no new mutations, no selection and no migration are required. The HWE is calculated by $p^2+2pq+q^2 = (p+q)^2 = 1$ (p^2 or q^2 = probability of homozygous genotype for allele 1 or 2, respectively, 2pq = probability of heterozygous genotype for allele 1 and 2). Each SNP was tested for deviation from HWE by means of Chi-square (ChiSq) test and Fisher`s exact test (Fisher). SNPs with deviations from HWE ($p<0.05$) were handled with care or excluded from statistical analysis.

4.5.2 Linkage disequilibrium (LD)

The linkage disequilibrium (LD) analysis (Weir BS and Wilson SR 1986) reveals a possible co-segregation and the non-random association of alleles across two or more linked polymorphic loci due to lacking recombination events. As measures for pair wise LD between each pair of SNP D` and $r^2$ were estimated using Haploview (Barrett JC et al. 2005). D` is a measure of the Lewontin LD coefficient D and estimates the frequency of recombination. A D`-value of one ($D` = D/D_{max}$) means no recombination. The parameter r^2 ($r^2 = D^2/p_X p_x p_Y p_y$) is the correlation between two alleles. In this work r^2 was used for LD interpretation because r^2 was reported to be less dependent on sample size and genotype frequency (Ardlie KG et al. 2002; Weiss KM and Clark AG 2002). Strong LD was defined as r^2 above 0.8.

4.5.3 Power analysis

In the WW study power and sample size for weight loss have been calculated assuming a 50 percent drop out rate and a significance level of five percent. The remaining sample size would be sufficient to detect a difference in weight loss between treatments of 1.9 kg, with a power of 90 percent, allowing for a standard deviation (s.d.) of 8 kg. There would be a power of 90 percent to detect a centre difference in weight loss between treatments of 3.7 kg.

In the MONICA/KORA study the power analysis was done using the programme QUANTO version 1.2.4 (University of Southern California, Los Angeles, CA, USA; http://hydra.usc.edu/gxe). Given the effect sizes of the outcome parameter as well as MAFs reported in literature, the number of subjects and the s.d., the percentage of power to detect an association with a two- or one-sided alpha of 0.05 was calculated for an additive genetic model. According to the results reported by *Willer C et al.* (Willer CJ et al. 2009) (*NEGR1*,

rs2815752, 0.10 kg/m², MAF=0.38 / *TMEM18*, rs6548238, 0.26 kg/m², MAF=0.16 / *MTCH2*, rs10838738, 0.07 kg/m², MAF=0.34 / *FTO*, rs9939609, 0.33 kg/m², MAF=0.41 / *MC4R*, rs17782313, 0.20 kg/m², MAF=0.21 / *SH2B1*, rs7498665, 0.15 kg/m², MAF=0.41 / *KCTD15*, rs11084753, 0.06 kg/m², MAF=0.33), the power to detect these associations in the analyzed MONICA/KORA study population was 99 percent for *FTO*, 92 percent for *TMEM18*, 82 percent for *MC4R*, 74 percent for *SH2B1*, 40 percent for *NEGR1*, 21 percent for *MTCH2*, and 17 percent for *KCTD15*.

4.5.4 Normal distribution

Quantitative variables were regarded as normally distributed, if the median/mean ratio was between 0.9 and 1.1 and the three fold s.d. below the mean. In case of violation of one of these criteria the variable was log-transformed (natural logarithm) and tested under the same conditions. Traits which do not fulfil the criteria of normal distribution were checked with quantile-quantile-plots and a distribution curve. Variables following closely a normal distribution were considered as normally distributed.

4.5.5 Genetic predisposition score (GPS)

A genetic predisposition score (GPS) for the cumulative effect of combined SNPs was calculated according to *Li S et al.* (Li S et al. 2010). The GPS was calculated for each individual by adding the number of risk alleles (0, 1, or 2 per SNP). Genetic variants in nine out of twelve loci included in the GPS by *Li S et al.* are available in the WW and the LOGIC study. On literature basis the risk alleles were determined (**Table 4-7**).

Table 4-7: SNPs included in the genetic predisposition score (GPS)

Locus	SNP	Risk allele	WW	LOGIC
NEGR1	rs2815752	major allele A	T	T
SEC16B, *RASAL2*	rs10913469	minor allele C	C	C
TMEM18	rs7561317	major allele G	G	G
SFRS10, ETV5, DGKG	rs7647305	major allele C	C	C
BDNF	rs16917237	major allele G of rs6265	G	G
MTCH2	rs10838738	minor allele G	G	G
FTO	rs9939609	minor allele A	A	A (major allele)
MC4R	rs17782313	minor allele C	C	C
KCTD15	rs29941	major allele C	C	C

Genotyped *BDNF* SNP rs16917237 is in LD with rs6265 (r^2=0.8); for rs6265 the major allele is the risk allele; we considered also for rs16917237 the major allele as risk allele; in the LOGIC study the A allele of rs9939609 (*FTO*) is the major allele

Subjects with missing genotypes for all SNPs used to generate the GPS were excluded (N=2 in the WW study and N=0 in the LOGIC study). For individuals with missing genotypes for individual SNPs the specific “average” genotype which varies between zero and two was given. This leads to a larger set of individuals with a score. It will likely not change the results, but gives a bit more “stability”.

4.5.6 Datasets Weight Watchers (WW) and LOGIC study

In the completer analysis of the WW study, only persons with data available for the specific time point were included in the analysis (completer). In the "baseline carried forward" (BCF) analysis the missing values were replaced by the baseline values assuming that these persons had no changes in a certain trait from baseline to the specific time point. For statistical analysis especially the time points two, six, and twelve months were of interest because in the first two months the motivation was highest, the six months time point was the middle of the intervention period, and the twelve months time point was the end of the study.
In the LOGIC study weight and BMI-SDS data are available for every week, but the values after four and six weeks were of special interest because these time points indicated also the child´s duration of stay. The four weeks measurement is the last measurement (visit 2) of children who left the hospital at this time point and an interim control visit for children who stayed until the 6^{th} week. For statistical analysis – in order to get more power – the data after four and six weeks were separately analyzed as well as in combination (four and six weeks together). The latest one was with adjustment for duration of stay.

4.5.7 Descriptive statistics

Mean and s.d. for normally distributed variables and proportion (percent; %) for categorized variables were computed. For not normally distributed variables the median and inter quartile range (IQR) are additionally reported. As a descriptive test the non-parametric Kruskal-Wallis test was used to get a first overview about differences in a specific trait across genotypes. Therefore, in the three genotype groups the values of each parameter were ranked between the groups. For each group a sum of squares was estimated and calculated. The difference between calculated and estimated sum of squares indicates changes in values due to specific genotypes.

4.5.8 Association analysis

The additive genetic model which calculates beta estimates per minor allele (gene-dosis effect) was assumed. It is the most common model in genetic analysis.

4.5.8.1 Weight Watchers (WW) study

The primary endpoint "weight at twelve months" and the difference in weight loss between the two intervention groups (WW, GP) were analyzed at the MRC in Cambridge (**Appendix I**). For the association between SNPs and parameters which were available at different time points (weight, fat mass, and waist circumference) the delta of a parameter was built by subtraction of the "new" value from the baseline value (e.g. delta weight after 12 months = 12 months weight – baseline weight). The more negative the value the higher the weight loss.

To test the association between SNPs and delta weight, fat mass, and waist circumference, logistic, linear, and linear mixed effect models were calculated. In the logistic regression model the probability (odds ratio (OR)) to be in a specific group (1 or 2) of the dichotomized variable was calculated and in the linear regression model the amount of mean increase or decrease (beta estimate) of the outcome variable was estimated. In the linear mixed effect model the outcome variable is considered at different time points. For logistic and linear regression two adjustment approaches were performed: (i) age and sex, (ii) age, sex, country, intervention, height, and baseline value of the analyzed trait. In the mixed effect model adjustment for (i) age and sex or (ii) age, sex, country, intervention, and height was done. For the GPS analysis (only performed for delta weight) three adjustment approaches were chosen: (i) age and sex, (ii) age, sex, country, and intervention, (iii) age, sex, country, intervention, height, baseline weight. As indicated in the results section, some analyses were performed in the completer as well as in the BCF dataset. For logistic regression the variables were dichotomized by their time point-specific median. For delta weight a sensitivity analysis was performed by dichotomization according to percent weight loss (five, ten percent) adjusted for (i) age and sex as well as (ii) age, sex, country, and intervention.
For the changes of anthropometric traits during intervention 40 SNPs were tested. After calculating LD there were 31 independent SNPs. Adjustment for multiple testing leads to a significance level of ≤ 0.002 calculated as 0.05 (alpha) / 31 (number of SNPs).

4.5.8.2 LOGIC study

For the association between SNPs and weight and BMI-SDS, which were available at different time points, the delta of a parameter was built by subtraction of the “new” value from the baseline value (e.g. delta weight after 4 weeks = 4 weeks weight – baseline weight). The more negative the value the higher the weight loss.
To test for an association between SNPs and delta weight and BMI-SDS logistic, linear and linear mixed effect models were calculated. For logistic and linear regression two adjustment approaches were performed: (i) age, sex, and if required (four and six weeks together) duration of stay, (ii) age, sex, height, baseline value of the analyzed trait, and if required duration of stay. In the mixed effect model adjustment for (i) age, sex, and if required duration of stay or (ii) age, sex, height and if required duration of stay was done. For the GPS analysis (only performed for delta weight) two adjustment approaches were chosen: (i) age, sex and if required duration of stay, and (ii) age, sex, height, baseline weight, and if required duration of stay. For logistic regression the variables were dichotomized by their time point-specific median and for the linear regression delta weight was changed to a positive variable (weight loss), then log-transformed, and at the end the estimates were changed concerning the direction in order to get the delta weight for interpretation.

For the changes of anthropometric traits during intervention 44 SNPs were tested. After calculating LD there were 35 independent SNPs. Adjustment for multiple testing led to a significance level of ≤ 0.001 calculated as 0.05 (alpha) / 35 (number of SNPs).

4.5.8.3 MONICA/KORA study (Holzapfel C et al. 2010b)

Lifestyle variables were evaluated for their potential as mediators within genotype-BMI association according to the guidelines for surrogacy analyses (Prentice RL 1989) and as applied previously for genetic data (Heid IM et al. 2008). Briefly, this involves the following criteria (**Figure 4-1**): a) the genotype is associated with the outcome BMI (model 1); b) the mediator (lifestyle variable) is associated with the outcome (model 2); c) the genotype is associated with the mediator (model 3); d) including the mediator as an additional covariate into model 1, the genotype-outcome association is abolished (model 4). Regarding model 2, we applied two approaches: modelling each lifestyle factor separately ("single lifestyle factor model") as well as modelling all lifestyle factors together ("multiple lifestyle factor model").

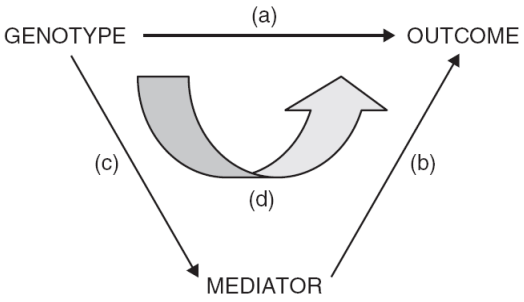


Figure 4-1: The four models to test whether a variable is a mediator within the genotype-outcome association

Linear regression models were used to analyze associations of the SNPs with BMI and logistic regression models for the association with dichotomized lifestyle factors. Dichotomization was done according to the sex-specific median in the case of carbohydrate and fat score. Smoking was dichotomized according to "ever" and "never", alcohol consumption according to the alcohol intake per day (≥ 40 g for men, ≥ 20 g for women), and physical activity according to the scores (high activity = score 1 and 2 / low activity = score 3 and 4). All analyses were adjusted for sex, age, and survey, and also conducted by gender. The significance level was set to 0.7 percent to account for the tested seven polymorphisms.
Gene-environment or gene-gene interactions were calculated including an interaction term (i) of each genotype and each lifestyle factor (SNP*lifestyle factor) or (ii) of the *TMEM18* genotype and each other genotype (*TMEM18**SNP) or of the *FTO* genotype and each other genotype (*FTO**SNP) or (iii) of *TMEM18* genotype and *FTO* genotype and each lifestyle factor (*TMEM18***FTO**lifestyle factor) in the model. *TMEM18* and *FTO* SNP were selected because of their strong association with BMI.

5 Results

5.1 Characteristics of study samples

5.1.1 Weight Watchers (WW) study

Characteristics of the WW study cohort are shown in **table 5-1** to **5-4**. The study cohort was not only characterized by anthropometric parameters but also by selected biochemical traits. Characteristics were not shown sex-specific because there were only 13 percent men (N=87) in the study. 88 percent of the participants were Caucasians. Study characteristics restricted to Caucasian persons (N=577) are assembled in **appendix J**.

In **table 5-1** means (s.d.) are shown for age, BP, heart rate, anthropometric and biochemical parameters in the whole study population (completer) over twelve months (0 months and after 2, 4, 6, 9, 12 months). Analogue to **table 5-1**, BCF data are shown in **table 5-2**.

Table 5-1: Characteristics of the whole study population (completer)

	Visit A (0 months)		Visit B (2 months)		Visit C (4 months)		Visit D (6 months)		Visit E (9 months)		Visit F (12 months)	
Parameter	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**
Age [years]	653	48.19 (12.54)	-	-	-	-	-	-	-	-	-	-
Height [m]	653	1.66 (0.08)	-	-	-	-	-	-	-	-	-	-
Systolic blood pressure [mmHg]	653	124.83 (16.12)	637	121.18 (15.51)	563	122.17 (15.86)	519	121.71 (15.59)	456	122.41 (15.60)	431	122.79 (15.82)
Diastolic blood pressure [mmHg]	653	78.68 (9.19)	637	77.09 (9.36)	563	77.20 (9.72)	519	77.08 (9.52)	455	76.85 (9.55)	431	76.87 (9.66)
Heart rate [mmHg]	635	71.67 (10.03)	624	71.02 (9.60)	554	72.31 (10.29)	502	70.94 (9.53)	443	71.94 (10.55)	409	70.38 (10.45)
BMI [kg/m²]	653	31.40 (2.57)	637	30.51 (2.65)	563	29.99 (2.88)	519	29.73 (3.03)	457	29.62 (3.16)	434	29.58 (3.24)
Weight [kg]	653	86.59 (11.53)	637	84.11 (11.55)	563	82.70 (11.94)	519	82.02 (12.50)	457	81.89 (12.59)	434	81.65 (12.48)
Waist circumference [cm]	646	99.78 (9.24)	632	96.76 (9.47)	561	95.41 (9.70)	512	94.53 (9.97)	449	94.45 (10.63)	424	94.17 (10.55)
Fat mass [kg]	592	33.07 (7.17)	584	31.09 (7.31)	510	29.65 (7.78)	474	29.18 (7.90)	412	28.99 (7.99)	397	29.01 (8.02)
Plasma glucose [mmol/l]	648	5.01 (0.81)	624	4.96 (0.78)	-	-	502	4.96 (0.72)	-	-	420	5.01 (0.80)
HbA1c [%]	646	5.65 (0.53)	-	-	-	-	302	5.57 (0.44)	-	-	240	5.52 (0.46)
Triglycerides [mmol/l]	649	1.44 (0.81)	624	1.38 (0.84)	-	-	502	1.37 (0.88)	-	-	419	1.36 (0.77)
Total cholesterol [mmol/l]	649	5.34 (1.00)	624	5.13 (0.98)	-	-	502	5.28 (1.00)	-	-	421	5.43 (1.02)
HDL cholesterol [mmol/l]	626	1.45 (0.36)	618	1.39 (0.33)	-	-	501	1.49 (0.43)	-	-	419	1.55 (0.38)
LDL cholesterol [mmol/l]	625	3.25 (0.87)	616	3.14 (0.84)	-	-	499	3.22 (0.89)	-	-	418	3.31 (0.87)

Means (s.d.) of anthropometric and biochemical parameters are shown at different time points (visit A to visit F); the time point-specific (visit A, B, D, F) median (IQR) for the not normally distributed triglycerides (mmol/l) is 1.28 (0.88), 1.20 (0.77), 1.17 (0.72), and 1.19 (0.71), respectively

Table 5-2: Characteristics of the study population

	Visit A (0 months)		Visit B (2 months)		Visit C (4 months)		Visit D (6 months)		Visit E (9 months)		Visit F (12 months)	
Parameter	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**
Systolic blood pressure [mmHg]	653	124.83 (16.12)	653	121.25 (15.38)	653	122.31 (15.87)	653	121.88 (15.45)	653	122.58 (15.38)	653	123.09 (16.04)
Diastolic blood pressure [mmHg]	653	78.68 (9.19)	653	77.17 (9.33)	653	77.39 (9.62)	653	77.27 (9.36)	653	77.30 (9.28)	653	77.45 (9.54)
Heart rate [mmHg]	635	71.67 (10.03)	647	70.98 (9.66)	647	72.48 (10.17)	647	71.21 (9.63)	646	72.36 (10.43)	645	71.24 (10.38)
Weight [kg]	653	86.59 (11.53)	653	84.18 (11.59)	653	83.24 (11.89)	653	82.82 (12.12)	653	83.13 (12.21)	653	83.28 (12.29)
Waist circumference [cm]	646	99.78 (9.24)	652	96.94 (9.47)	651	96.22 (9.86)	652	95.62 (9.93)	651	96.08 (10.35)	651	96.21 (10.48)
Fat mass [kg]	592	33.07 (7.17)	596	31.13 (7.28)	589	30.16 (7.74)	590	29.99 (7.88)	585	30.23 (8.00)	594	30.44 (7.98)
Plasma glucose [mmol/l]	648	5.01 (0.81)	651	4.96 (0.77)	-	-	649	4.95 (0.71)	-	-	648	4.97 (0.76)
HbA1c [%]	646	5.65 (0.53)	-	-	-	-	647	5.60 (0.52)	-	-	646	5.58 (0.52)
Triglycerides [mmol/l]	649	1.44 (0.81)	651	1.38 (0.84)	-	-	650	1.38 (0.86)	-	-	649	1.38 (0.82)
Total cholesterol [mmol/l]	649	5.34 (1.00)	651	5.13 (0.98)	-	-	650	5.29 (1.01)	-	-	649	5.39 (1.01)
HDL cholesterol [mmol/l]	626	1.45 (0.36)	642	1.39 (0.34)	-	-	643	1.48 (0.41)	-	-	638	1.51 (0.38)
LDL cholesterol [mmol/l]	625	3.25 (0.87)	641	3.15 (0.85)	-	-	642	3.24 (0.89)	-	-	637	3.29 (0.87)

Missing values are replaced by baseline values (BCF). Means (s.d.) of anthropometric and biochemical parameters are shown at different time points (visit A to visit F); the time point-specific (visit A, B, D, F) median (IQR) for the not normally distributed triglycerides (mmol/l) is 1.28 (0.88), 1.20 (0.79), 1.20 (0.80), and 1.20 (0.80), respectively

In **table 5-3** changes of anthropometric parameters (after 2, 6 and 12 months) are shown for the BCF dataset and completers as well as separately for the WW and the GP group.

Table 5-3: Changes of anthropometric parameters

	BCF		Completers		WW BCF		WW completers		GP BCF		GP completers	
Parameter	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**
After 2 months (visit B)												
Delta weight [kg]	653	-2.41 (2.82) -2.10 (3.60)	637	-2.47 (2.83) -2.20 (3.50)	330	-3.03 (3.03) -2.90 (3.60)	319	-3.14 (3.03) -3.00 (3.50)	323	-1.77 (2.44) -1.50 (2.90)	318	-1.80 (2.45) -1.50 (2.80)
Delta waist circumference [cm]	646	-2.85 (4.72) -2.00 (5.00)	626	-2.94 (4.77) -2.00 (5.00)	328	-3.23 (4.91) -2.00 (6.00)	315	-3.37 (4.97) -2.00 (6.00)	318	-2.46 (4.50) -2.00 (4.50)	311	-2.51 (4.53) -2.00 (5.00)
Delta fat mass [kg]	581	-2.00 (2.98) -1.70 (3.60)	569	-2.07 (3.01) -1.80 (3.60)	297	-2.51 (3.28) -2.40 (3.80)	288	-2.62 (3.32) -2.60 (3.60)	284	-1.47 (2.53) -1.20 (3.20)	281	-1.50 (2.54) -1.30 (3.20)
After 6 months (visit D)												
Delta weight [kg]	653	-3.77 (4.74) -2.80 (6.50)	519	-4.74 (4.86) -4.20 (5.80)	330	-5.00 (5.23) -4.50 (7.40)	273	-6.05 (5.17) -5.70 (5.70)	323	-2.50 (3.78) -1.30 (4.70)	246	-3.29 (4.02) -2.65 (5.00)
Delta waist circumference [cm]	646	-4.17 (5.63) -3.00 (7.50)	506	-5.32 (5.86) -5.00 (7.50)	328	-5.22 (6.08) -4.00 (9.00)	268	-6.39 (6.15) -5.55 (8.00)	318	-3.08 (4.89) -1.40 (6.00)	238	-4.11 (5.27) -3.00 (6.70)
Delta fat mass [kg]	578	-3.18 (4.36) -2.15 (5.60)	462	-4.01 (4.55) -3.70 (5.80)	297	-4.34 (4.93) -3.70 (7.30)	248	-5.27 (4.94) -4.75 (6.25)	281	-1.95 (3.26) -0.70 (4.10)	214	-2.55 (3.54) -2.35 (4.80)
After 12 months (visit F)												
Delta weight [kg]	653	-3.31 (5.41) -0.90 (5.60)	434	-4.98 (5.98) -3.90 (6.90)	330	-4.48 (6.19) -2.60 (7.50)	224	-6.60 (6.52) -5.40 (7.60)	323	-2.12 (4.16) -0.10 (3.90)	210	-3.26 (4.78) -2.30 (5.50)
Delta waist circumference [cm]	646	-3.59 (6.22) 0.00 (6.00)	419	-5.54 (7.00) -5.00 (9.00)	328	-4.37 (6.80) -1.60 (8.00)	215	-6.66 (7.44) -5.50 (9.00)	318	-2.79 (5.46) 0.00 (5.50)	204	-4.35 (6.30) -4.00 (8.00)
Delta fat mass [kg]	584	-2.62 (4.56) -0.50 (4.60)	387	-4.03 (5.13) -3.10 (6.20)	302	-3.56 (5.19) -1.35 (6.50)	207	-5.35 (5.53) -4.60 (7.30)	282	-1.60 (3.50) 0.00 (2.90)	180	-2.52 (4.15) -2.00 (4.70)

Means (s.d.) as well as medians (IQR) are shown for the BCF dataset as well as for completers. Furthermore, results are shown for both intervention groups (WW and GP). Delta = value visit B or D or F – value visit A

The range of delta weight after twelve months was -27.5 kg to +11.4 kg in completers (N=434). Regarding the range separately for both intervention groups, the range was -27.5 kg to +9.0 kg in the WW group (N=224) and -21.0 kg to +11.4 kg in the GP group (N=210).

In **table 5-4** the number of persons reached the cut off points of five or ten percent weight loss is given.

Parameter	**Visit B** (2 months)	**Visit D** (6 months)	**Visit F** (12 months)
		N	
Delta weight ≥ 5% initial weight	130	262	201
Delta weight < 5% initial weight	507	257	233
Delta weight ≥ 10% initial weight	21	83	98
Delta weight < 10% initial weight	616	436	336

Table 5-4: Number of persons with weight loss ≥/< 5 or 10 percent of initial weight is shown at different time points (two, six, and twelve months)

The mean (s.d.) percent weight loss in completers was -2.85 (3.24) kg (N=637), -5.49 (5.53) kg (N=519), and -5.74 (6.74) kg (N=434) after two, six, and twelve months, respectively. The corresponding median (IQR) of percent weight loss was -2.52 (3.94) kg, -5.06 (6.96) kg, and -4.45 (8.28) kg after two, six, and twelve months, respectively.

The results in the whole study population (**Table 5-1** to **5-4**) are not very different from the results in the Caucasian study population (**Appendix J**).

5.1.2 LOGIC study

Characteristics of the LOGIC study cohort are shown in **table 5-5** to **5-6**. The study cohort was not only characterized by the further analyzed anthropometric parameters but also by selected biochemical traits. Due to the small sample size (N=358) characteristics were not shown sex-specific. In the following tables 87 percent are Caucasians. Study characteristics restricted to Caucasian children (N=312) is assembled in **appendix K**.

In **table 5-5** means (s.d.) are shown for age, anthropometric and biochemical parameters at study entry (visit 1) and after four and six weeks of intervention (visit 2). Weight and BMI-SDS are available for all children after four weeks, whereas biochemical parameters are available after four or six weeks dependent on child`s duration of stay (visit 2).

Table 5-5: Characteristics of the study population

	Visit 1 (0 weeks)		Visit 2 (4 weeks)		Visit 2 (6 weeks)	
Parameter	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**
Age [years]	358	13.85 (2.26)	-	-	-	-
Height [m]	358	162.90 (10.97)	-	-	-	-
Weight [kg]	358	90.20 (23.07)	344	82.20 (20.93)	217	82.55 (19.90)
BMI-SDS	358	2.74 (0.55)	344	2.40 (0.61)	217	2.37 (0.62)
Plasma glucose [mmol/l]	355	3.95 (0.44)	119	4.02 (0.43)	204	4.03 (0.47)
Plasma insulin [mU/l]	354	11.36 (6.10)	123	10.81 (4.84)	209	10.75 (5.90)
Triglycerides [mg/dl]	355	63.66 (24.69)	123	73.12 (34.19)	208	69.11 (26.87)
Total cholesterol [mg/dl]	354	156.86 (30.97)	123	138.60 (25.69)	209	134.40 (26.77)
HDL cholesterol [mg/dl]	351	50.73 (12.65)	122	51.63 (11.78)	208	49.65 (13.12)
LDL cholesterol [mg/dl]	354	103.20 (32.93)	117	82.60 (23.56)	209	78.81 (24.64)

Means (s.d.) of anthropometric and biochemical parameters are shown at different time points (visit 1 and 2); the time point-specific median (IQR) for the not normally distributed plasma insulin (mU/l) is 10.10 (6.11), 9.51 (5.47), 9.72 (6.81) and for triglycerides (mg/dl) is 60.00 (29.00), 66.00 (35.00), 68.00 (35.00)

Changes of anthropometric parameters (after four or six weeks) are shown in **table 5-6**. The range of delta weight (N=344) after four weeks was -18.20 kg to -2.70 kg and after six weeks (N=217) was -23.60 kg to -4.40 kg.

Parameter	**N**	**mean (s.d.) median (IQR)**
After 4 weeks		
Delta weight [kg]	344	-8.19 (2.84) -7.80 (3.50)
Delta BMI-SDS	344	-0.36 (0.10) -0.35 (0.13)
After 6 weeks		
Delta weight [kg]	217	-10.88 (3.66) -10.40 (4.70)
Delta BMI-SDS	217	-0.47 (0.14) -0.45 (0.18)

Table 5-6: Changes of anthropometric parameters. Means (s.d.) as well as medians (IQR) are shown after four and six weeks for weight and BMI-SDS. Delta = value visit 2 – value visit 1 (visit 2 is after four or six weeks depending on child´s duration of stay)

The results in the whole study cohort were not different from the results in the Caucasian study cohort (**Appendix K**).

5.1.3 MONICA/KORA study

Baseline characteristics concerning age, education, anthropometric factors, and lifestyle factors of the analyzed study population are given in **table 5-7**.

Table 5-7: Characteristics of the study population

	Overall		Men		Women	
	N	Mean (s.d.) or %	N	Mean (s.d.) or %	N	Mean (s.d.) or %
General factors						
Age [years]	12,462	49.38 (13.97)	6,271	49.82 (14.10)	6,191	48.94 (13.82)
Education (< 12 years)	12,462	68.73 %	6,271	60.87 %	6,191	76.69 %
Anthropometric factors						
BMI [kg/m²]	12,357	26.97 (4.49)	6,231	27.32 (3.81)	6,126	26.61 (5.07)
Height [cm]	12,421	167.92 (9.32)	6,249	174.25 (7.06)	6,172	161.51 (6.52)
Waist circumference [cm]	12,383	89.82 (13.12)	6,252	96.13 (10.63)	6,131	83.38 (12.26)
Percentage body fat [%]	7,802	32.42 (7.63)	3,901	28.53 (6.29)	3,901	36.31 (6.84)
Lifestyle factors						
High carbohydrate score (≥ median)	12,426	54.30 %	6,250	55.55 %	6,176	52.80 %
High fat score (≥ median)	12,423	58.75 %	6,248	59.57 %	6,175	57.93 %
Smoking (ever smokers)	12,458	55.47 %	6,268	69.10 %	6,190	41.68 %
High alcohol ≥ 40g/d (men) / ≥ 20g/d (women)	12,438	22.09 %	6,271	26.76 %	6,191	17.28 %
High physical activity (scores 1 and 2)	12,441	43.47 %	6,257	45.02 %	6,184	41.90 %

Means (s.d.) or percentages (%) are shown for overall population and separately for men and women

5.2 Genotyping: Weight Watchers (WW) and LOGIC study

5.2.1 Genotyping results

In **table 5-8** genotyping results compared to the HapMap data are summarized for all SNPs and subjects in the WW and the LOGIC study. Data restricted to Caucasian persons is assembled in **appendix L**.

Genotyping success rates were above 90 percent for all successfully genotyped SNPs. Compared to the WW study genotyping success rates were always higher in the LOGIC study, except for one SNP (rs7832552, *TRHR*) with genotyping success rates of 99.39 percent (WW) and 98.61 percent (LOGIC).

Four SNPs (rs16917237, *BDNF*; rs1424233, *MAF*; rs17700144 and rs17782313, *MC4R*) violated HWE ($p<0.05$) in the LOGIC study, howerver the violation was not very strong (**Table 5-8**). In the WW study the polymorphism rs12145833 (*SDCCAG8*) marginally fulfilled HWE with p-values of 0.054 (ChiSq test) and 0.074 (Fisher test) and in the LOGIC study rs11084753 (*KCTD15*) with p-values of 0.050 (ChiSq test) and 0.056 (Fisher test). Because of no strong HWE violation SNPs were further investigated, but association results were considered with caution.

Two loci – the *GNPDA2* and the *IRS1* – failed genotyping at all in both studies (data not shown) and four loci (*IL6*, *MTNR1B*, *UCP2*, *SH2B1*) failed genotyping in the WW study.

Table 5-8: Genotype information of SNPs

Locus	SNP	Chr.	Minor allele	MAF	Minor allele	N (all)	HWE (ChiSq)	HWE (Fisher)	Genotyping Success Rate	MAF	Minor allele	N (all)	HWE (ChiSq)	HWE (Fisher)	Genotyping Success Rate	MAF
	HapMap				WW Study						LOGIC Study					
LEPR	rs1805134	1	-	-	C	649	0.299	0.291	99.39	21.34	C	357	0.145	0.203	99.44	20.87
NEGR1	rs2568958	1	G	36	G	649	0.163	0.170	99.39	35.52	G	358	0.825	0.907	99.72	34.64
	rs2815752		G	36	**C**	649	0.163	0.169	99.39	35.52	**C**	359	0.784	0.812	100	34.68
SDCCAG8	rs10926984	1	G	11	G	640	0.119	0.153	98.01	12.73	G	357	0.999	1.000	99.44	14.01
	rs12145833		G	13	G	637	0.054	0.074	97.55	12.79	G	356	0.992	1.000	99.16	14.04
	rs2783963		T	12	T	647	0.184	0.244	99.08	13.60	T	356	0.761	0.817	99.16	13.34
SEC16B, RASAL2	rs10913469	1	C	25	C	641	0.481	0.465	98.16	19.81	C	354	0.899	1.000	98.61	17.09
INSIG2	rs11684454	2	A	28	A	636	1.000	1.000	97.4	33.18	A	354	0.399	0.388	98.61	30.37
TMEM18	rs7561317	2	A	15	A	619	0.517	0.546	94.79	15.51	A	358	0.715	0.696	99.72	15.92
ADIPOQ	rs17300539	3	A	7	A	641	0.923	1.000	98.16	7.02	A	357	0.505	0.507	99.44	9.10
PPARG	rs1801282	3	G	10	G	638	0.809	1.000	97.7	12.30	G	359	0.602	0.801	100	11.84
SFRS10, ETV5, DGKG	rs7647305	3	T	20	T	630	0.961	1.000	96.48	21.75	T	357	0.211	0.245	99.44	19.61
UCP1	rs45539933	4	-	-	T	633	0.264	0.504	96.94	6.56	T	357	0.193	0.378	99.44	6.44
ADRB2	rs12654778	5	A	34	A	633	0.197	0.223	96.94	36.10	A	357	0.762	0.750	99.46	41.88
PCSK1	rs12186664	5	T	28	T	637	0.730	0.776	97.55	29.43	T	356	0.426	0.463	99.16	31.46
PRL	rs4145443	6	C	42	C	631	0.214	0.225	96.63	45.09	C	355	0.351	0.383	98.89	43.66
IL6	rs1554606	7	G	46	Genotyping failure						**T**	356	0.642	0.668	99.16	45.65
TNKS-MSRA	rs13278851	8	A	11	A	635	0.285	0.329	97.24	11.42	A	357	0.540	0.751	99.46	9.10
	rs17150703		A	11	A	643	0.359	0.320	98.47	10.96	A	357	0.573	1.000	99.44	8.96
	rs516175		A	11	**T**	640	0.112	0.120	98.01	12.81	**T**	358	0.060	0.071	99.72	11.17
TRHR	rs7832552	8	T	33	T	649	0.188	0.202	99.39	31.59	T	354	0.406	0.509	98.61	27.68
ADRA2A	rs1800544	10	-	-	G	640	0.832	0.846	98.01	28.75	G	358	0.883	1.000	99.72	27.23
PFKP	rs17132175	10	C	13	C	639	0.325	0.329	97.86	8.84	C	359	0.546	0.750	100	9.05
PTER	rs10508503	10	T	9	T	634	0.060	0.063	97.09	6.94	T	350	0.188	0.378	97.49	6.57
BDNF	rs16917237	11	T	22	T	628	0.319	0.413	96.17	21.18	T	357	**0.026**	**0.035**	99.44	20.59
MTCH2	rs10838738	11	G	36	G	639	0.592	0.592	97.86	33.10	G	358	0.460	0.471	99.72	33.38
MTNR1B	rs10830963	11	G	30	Genotyping failure						G	357	0.479	0.509	99.44	28.15
UCP2	rs659366	11	T	37	Genotyping failure						T	358	0.376	0.368	99.72	38.13
GNB3	rs5443	12	T	39	T	633	0.269	0.286	96.94	34.36	T	357	0.390	0.399	99.44	34.03
PLIN	rs894160	15	T	32	**A**	639	0.895	0.931	97.86	31.22	**A**	358	0.977	1.000	99.72	30.31
FTO	rs6499640	16	G	36	G	638	0.164	0.182	97.7	38.32	G	357	0.352	0.354	99.44	34.31
	rs7206010		A	36	A	641	0.500	0.513	98.16	38.69	A	357	0.352	0.342	99.44	34.31
	rs9935401		A	45	A	628	0.838	0.872	96.17	40.84	**G**	356	0.168	0.171	99.16	49.86
	rs9939609		A	46	A	638	0.892	0.932	97.7	41.30	**T**	357	0.153	0.158	99.44	49.72
MAF	rs1424233	16	C	44	**G**	635	0.978	1.000	97.24	48.90	**G**	357	**0.034**	**0.034**	99.56	47.34
SH2B1	rs7498665	16	G	38	Genotyping failure						G	359	0.301	0.328	100	42.62
MC4R	rs1673482	18	G	39	G	629	0.312	0.323	96.32	34.82	G	353	0.208	0.218	98.33	38.53
	rs17700144		A	25	A	641	0.457	0.473	98.16	21.22	A	357	**0.046**	**0.046**	99.44	27.73
	rs17782313		C	26	C	641	0.305	0.334	98.16	24.41	C	357	**0.046**	**0.046**	99.44	29.97
	rs502933		A	34	A	603	0.810	0.794	92.34	36.57	A	356	0.202	0.216	99.16	39.04
NPC1	rs1805081	18	C	47	**G**	634	0.534	0.567	97.09	40.06	**G**	357	0.054	0.061	99.44	36.13
KCTD15	rs11084753	19	A	31	A	631	0.079	0.085	96.63	33.12	A	356	0.050	0.056	99.16	32.72
	rs29941		A	32	**T**	648	0.407	0.413	99.23	32.72	**T**	358	0.387	0.469	99.72	32.40
HTR2C	rs6318*	X	C	17	C	551	0.346	0.398	97.35	14.61	C	212	0.817	1.000	99.07	16.04

Complementary minor alleles to the reference (HapMap) are bold/grey; in LOGIC for rs9935401 and rs9939609 (*FTO*) and for rs1554606 (*IL6*) the "other" allele is the minor allele also highlighted in bold/grey; violated p-values of HWE (<0.05) are bold/grey; *HWE only measured in women because SNP is on the X-chromosome; ChiSq=Chi-square test; Fisher=Fisher´s exact test; MAF=minor allele frequency in percent; HWE=Hardy-Weinberg equilibrium

The analysis has been repeated in Caucasians resulting in similar findings (**Appendix L**). Due to the fact that study characteristics and genotyping results are similar in the whole study population compared to Caucasian persons, all further analyses were performed in the whole study cohort and were not restricted to Caucasians.

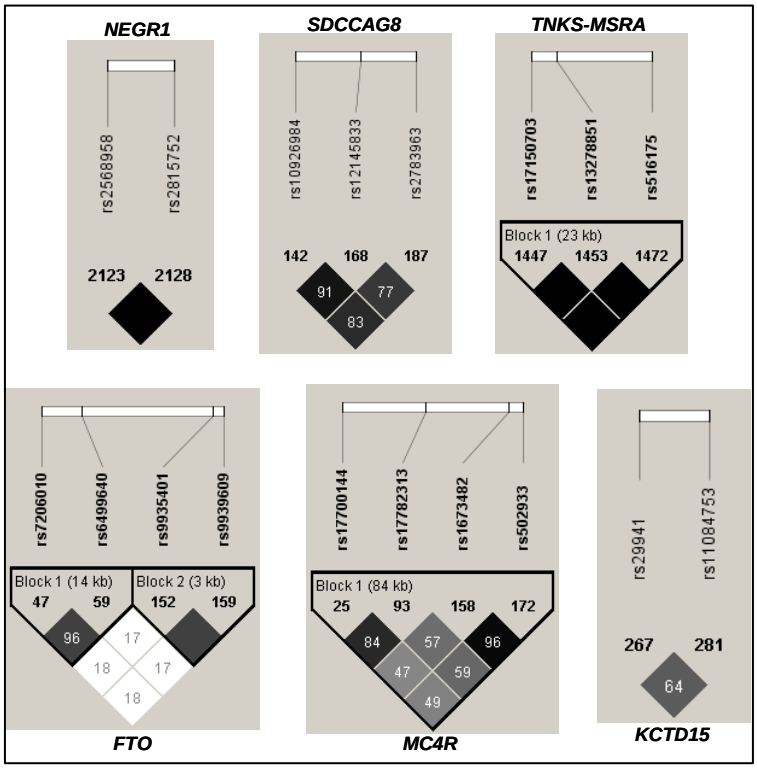
More SNP details like position on the chromosome, region, amino acid exchange, and proxy SNPs in the literature are shown in **appendix M**.

For a few SNPs with MAF below ten percent (**Table 5-8**) the sample size of persons homozygous for the minor allele was very small which has to be considered in the interpretation of results (**Appendix N**). For instance, one person (WW study) and no child (LOGIC study) was homozygous for the minor allele of *UCP1* SNP rs45539933. There was no subject in both studies homozygous for the minor allele of *PTER* SNP rs10508503.

5.2.2 Linkage disequilibrium (LD) results

For six loci (*NEGR1*, *SDCCAG8*, *TNKS-MSRA*, *FTO*, *MC4R*, *KCTD15*) more than one SNP was successfully genotyped. According to HapMap data LD was calculated for the genotyped SNPs in these specific loci. The correlation coefficients r^2 are shown in **figure 5-1**.

Figure 5-1: LD (r^2) results for *NEGR1*, *SDCCAG8*, *TNKS-MSRA*, *FTO*, *MC4R*, and *KCTD15*



Black quadrates mean $r^2 = 1.0$

For the *NEGR1* locus both SNPs were in high LD (r^2=1.0). Polymorphism rs10926984 (*SDCCAG8*) was in LD with both rs12145833 (r^2=0.91) and rs2783963 (r^2=0.83). The three *TNKS-MSRA* SNPs were completely in LD (r^2=1.0). There was high LD between the *FTO* SNPs rs7206010 and rs6499640 (r^2=0.96) as well as between rs9935401 and rs9939609 (r^2=1.0). The *MC4R* SNPs rs17700144 and rs17782313 (r^2=0.84) as well as rs1673482 and rs502933 (r^2=0.96) were in high LD. There was no LD block for *KCTD15* (**Figure 5-1**).

5.3 Association analyses: anthropometric traits

5.3.1 Weight Watchers (WW) study

5.3.1.1 Results for delta weight in the two intervention groups (Jebb S et al., in preparation)

The results for the primary outcome delta weight and its difference between the two intervention groups (WW, GP) are shown in the enclosed abstract (**Appendix I**).

5.3.1.2 Results from genetic analyses – delta weight

To test whether the genotyped polymorphisms are associated with delta weight at various time points, Kruskal-Wallis test, logistic and linear regression, mixed effect model as well as different adjustment models were calculated. Due to the multiple analysis approaches and the number of SNPs (N=40) especially results from the fully adjusted model with p-value ≤ 0.05 are mentioned.

In **appendix O** results from the Kruskal-Wallis test (p-values) are shown for delta weight after six and twelve months in both datasets (completers and BCF). Concerning delta weight significant p-values (≤0.05) were found for *NEGR1*, *SDCCAG8*, *SFRS10 ETV5 DGKG*, *PTER*, *BDNF* and *MC4R*. Often the p-values are not very different from 0.05. After adjustment for multiple testing only the p-value for *MC4R* SNP rs1673482 and delta weight after twelve months (BCF) stayed marginally statistically significant (p=0.002).

For logistic regression analysis delta weight was dichotomized by the time point-specific median. In **appendix P** the age- and sex-adjusted results are shown for delta weight after two (only completer), six and twelve months (completer and BCF) and are similar to the results from the fully adjusted model (age, sex, height, country, intervention, and baseline weight) as shown in **table 5-9**.

The *ADRB2* SNP rs12654778 showed an OR of 1.509 (95 percent confidence interval (CI): 1.165, 1.954; p=0.002) and the *PTER* SNP rs10508503 an OR of 2.054 (CI: 1.247, 3.383; p=0.005) for lower weight loss after two months. Three loci (*NEGR1*, *SFRS10 ETV5 DGKG*, *MC4R*) showed a significant result for both delta weight after six and twelve months. There was an OR of 0.769 (CI: 0.607, 0.975; p=0.030) or 0.714 (CI: 0.528, 0.966; p=0.029) for lower weight loss after six months (BCF) or twelve months (completer), respectively, for both *NEGR1* SNPs. SNP rs7647305 (*SFRS10 ETV5 DGKG)* showed an OR of 1.399 (CI: 1.015, 1.929; p=0.040) or of 1.470 (CI: 1.105, 1.956; p=0.008) for lower weight loss after six months (completers or BCF, respectively) and an OR of 1.518 (CI: 1.055, 2.185; p=0.025) for lower weight loss after twelve months (completers). For all four *MC4R* SNPs an OR below 1.0 with a p-value between 0.002 and 0.035 was observed for lower weight loss after six or twelve months (BCF). After adjustment for multiple testing the results for *ADRB2* and *MC4R* remained borderline significant (p=0.002) (**Table 5-9**).

There was no polymorphism which is associated with high/low weight loss over all three different time points as well as over all two datasets (completer and BCF). The highest consistency was seen for the *MC4R* locus which showed an association for all four analyzed SNPs which are in two LD blocks.

Table 5-9: Results from logistic regression concerning delta weight after two, six and twelve months

Locus	SNP	Delta weight (2 months)		Delta weight (6 months)		Delta weight BCF (6 months)		Delta weight (12 months)		Delta weight BCF (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	0.832	0.194	1.000	0.999	1.111	0.454	0.895	0.532	1.038	0.789
NEGR1	rs2568958	0.861	0.217	1.030	0.825	0.769	**0.030**	0.714	**0.029**	0.872	0.248
	rs2815752	0.861	0.217	1.030	0.825	0.769	**0.030**	0.714	**0.029**	0.872	0.248
SDCCAG8	rs10926984	1.040	0.833	1.003	0.987	0.975	0.891	1.458	0.097	1.292	0.158
	rs12145833	0.972	0.878	0.993	0.975	0.952	0.791	1.406	0.139	1.200	0.318
	rs2783963	1.182	0.346	1.254	0.254	1.121	0.513	1.551	**0.051**	1.333	0.099
SEC16B, RASAL2	rs10913469	0.930	0.623	1.059	0.722	0.937	0.657	0.889	0.514	0.839	0.230
INSIG2	rs11684454	1.086	0.516	0.834	0.203	1.041	0.751	0.920	0.585	0.900	0.399
TMEM18	rs7561317	1.371	0.059	0.927	0.675	0.886	0.462	0.714	0.091	0.838	0.277
ADIPOQ	rs17300539	0.727	0.173	1.113	0.689	1.071	0.768	1.016	0.957	1.030	0.897
PPARG	rs1801282	0.739	0.104	0.936	0.747	0.952	0.785	0.902	0.638	0.702	**0.052**
SFRS10, ETV5, DGKG	rs7647305	1.148	0.345	1.399	**0.040**	1.470	**0.008**	1.518	**0.025**	1.297	0.069
UCP1	rs45539933	0.871	0.582	0.870	0.596	0.749	0.242	0.662	0.163	1.032	0.896
ADRB2	rs12654778	1.509	**0.002**	1.174	0.257	1.145	0.289	1.252	0.142	1.033	0.798
PCSK1	rs12186664	0.919	0.519	0.815	0.154	0.839	0.173	1.059	0.711	1.075	0.570
PRL	rs4145443	0.933	0.557	1.015	0.909	1.010	0.931	0.917	0.549	1.187	0.137
TNKS-MSRA	rs13278851	0.949	0.776	0.892	0.578	1.019	0.917	0.998	0.994	1.119	0.537
	rs17150703	1.059	0.759	0.916	0.675	1.046	0.806	0.990	0.965	1.090	0.638
	rs516175	1.071	0.690	0.957	0.819	1.106	0.557	1.055	0.799	1.082	0.644
TRHR	rs7832552	0.974	0.831	0.934	0.610	0.769	**0.033**	1.020	0.897	0.867	0.241
ADRA2A	rs1800544	1.115	0.410	0.871	0.347	0.967	0.798	0.794	0.167	1.018	0.889
PFKP	rs17132175	0.943	0.776	1.091	0.697	1.041	0.844	0.839	0.491	1.022	0.913
PTER	rs10508503	2.054	**0.005**	1.952	**0.015**	1.448	0.130	1.711	0.072	1.117	0.647
BDNF	rs16917237	0.873	0.365	1.060	0.724	0.837	0.229	1.112	0.558	0.838	0.227
MTCH2	rs10838738	1.002	0.989	0.985	0.915	1.100	0.443	1.243	0.151	1.042	0.739
GNB3	rs5443	0.842	0.166	0.994	0.968	0.970	0.806	0.786	0.127	0.985	0.900
PLIN	rs894160	1.069	0.604	0.975	0.855	0.868	0.266	0.889	0.451	1.032	0.803
FTO	rs6499640	0.825	0.111	0.877	0.320	1.144	0.257	0.872	0.355	0.968	0.783
	rs7206010	0.822	0.106	0.867	0.282	1.095	0.450	0.884	0.412	0.932	0.555
	rs9935401	1.102	0.432	0.912	0.504	0.844	0.166	1.016	0.916	0.835	0.133
	rs9939609	1.115	0.375	0.938	0.636	0.857	0.205	1.071	0.646	0.873	0.259
MAF	rs1424233	1.038	0.756	0.946	0.677	1.012	0.923	0.875	0.358	1.018	0.876
MC4R	rs1673482	0.953	0.697	0.770	0.058	0.710	**0.006**	0.900	0.484	0.683	**0.002**
	rs17700144	0.843	0.235	0.740	0.055	0.686	**0.009**	0.819	0.234	0.659	**0.004**
	rs17782313	0.981	0.885	0.812	0.160	0.749	**0.035**	0.910	0.558	0.660	**0.002**
	rs502933	0.995	0.969	0.777	0.077	0.729	**0.013**	0.934	0.658	0.729	**0.012**
NPC1	rs1805081	1.075	0.562	0.934	0.621	0.925	0.528	0.763	0.073	0.962	0.748
KCTD15	rs11084753	0.878	0.293	0.853	0.239	0.898	0.377	0.787	0.109	0.859	0.205
	rs29941	0.803	0.080	0.916	0.521	1.002	0.989	1.032	0.834	0.944	0.636
HTR2C	rs6318*	1.102	0.610	1.571	**0.032**	1.115	0.556	0.998	0.992	0.902	0.570

Both datasets (completers and BCF) were analyzed for delta weight after six and twelve months; odds ratios (ORs) and p-values for lower weight loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age, sex, height, country, intervention and baseline weight was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Furthermore, dichotomization according to ≤ and > 5 or 10 percent weight loss was done for the six and twelve months time point. The number of individuals after two months was too small (**Table 5-4**). Age- and sex-adjusted results are shown in **appendix Q** and are similar to the fully adjusted (age, sex, country, and intervention) results shown in **table 5-10**.

Table 5-10: Results from logistic regression concerning percent weight loss after six and twelve months

Locus	SNP	5% delta weight (6 months)		5% delta weight (12 months)		10% delta weight (6 months)		10% delta weight (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	0.999	0.994	0.902	0.559	0.999	0.995	1.161	0.484
NEGR1	rs2568958	0.923	0.553	0.670	**0.009**	0.761	0.125	0.639	**0.011**
	rs2815752	0.923	0.553	0.670	**0.009**	0.761	0.125	0.639	**0.011**
SDCCAG8	rs10926984	1.108	0.617	1.362	0.177	1.416	0.242	1.605	0.106
	rs12145833	1.052	0.807	1.314	0.238	1.330	0.333	1.393	0.247
	rs2783963	1.381	0.108	1.448	0.101	1.614	0.105	1.558	0.117
SEC16B, RASAL2	rs10913469	1.009	0.957	0.964	0.840	1.101	0.661	0.915	0.665
INSIG2	rs11684454	0.837	0.216	0.927	0.615	0.822	0.297	1.086	0.646
TMEM18	rs7561317	0.887	0.509	0.740	0.125	0.709	0.127	0.669	0.063
ADIPOQ	rs17300539	1.055	0.842	0.842	0.552	0.893	0.750	0.851	0.625
PPARG	rs1801282	1.013	0.949	1.141	0.544	1.011	0.969	0.787	0.322
SFRS10, ETV5, DGKG	rs7647305	1.517	**0.012**	1.641	**0.008**	1.346	0.185	1.707	**0.021**
UCP1	rs45539933	0.905	0.709	0.570	0.059	0.700	0.261	0.873	0.672
ADRB2	rs12654778	1.122	0.418	1.246	0.150	0.967	0.859	1.064	0.726
PCSK1	rs12186664	0.913	0.529	1.130	0.434	1.076	0.706	1.012	0.948
PRL	rs4145443	1.024	0.854	1.026	0.860	1.059	0.743	0.998	0.991
TNKS-MSRA	rs13278851	0.879	0.536	1.048	0.831	1.005	0.986	1.061	0.819
	rs17150703	0.919	0.686	0.970	0.893	0.994	0.983	0.967	0.897
	rs516175	0.987	0.947	1.098	0.659	0.917	0.726	1.114	0.668
TRHR	rs7832552	0.977	0.865	1.084	0.592	1.082	0.669	1.258	0.204
ADRA2A	rs1800544	0.998	0.991	0.898	0.516	0.879	0.505	0.869	0.461
PFKP	rs17132175	1.280	0.278	0.865	0.565	1.874	0.084	1.588	0.164
PTER	rs10508503	1.859	**0.025**	1.537	0.152	1.457	0.334	1.236	0.553
BDNF	rs16917237	1.152	0.398	1.023	0.902	0.805	0.321	0.765	0.199
MTCH2	rs10838738	1.012	0.931	1.200	0.227	1.384	0.087	1.354	0.092
GNB3	rs5443	0.881	0.375	0.849	0.294	0.918	0.639	1.001	0.997
PLIN	rs894160	0.996	0.979	0.929	0.634	0.792	0.196	0.918	0.630
FTO	rs6499640	0.859	0.251	0.778	0.090	0.827	0.270	0.919	0.619
	rs7206010	0.885	0.363	0.796	0.127	0.864	0.405	0.977	0.892
	rs9935401	0.925	0.574	1.061	0.059	0.923	0.655	0.897	0.528
	rs9939609	0.950	0.707	1.100	0.522	0.925	0.663	0.929	0.669
MAF	rs1424233	0.966	0.799	0.877	0.363	0.995	0.978	0.979	0.901
MC4R	rs1673482	0.765	**0.054**	0.847	0.267	0.611	**0.006**	0.714	**0.049**
	rs17700144	0.689	**0.020**	0.741	0.075	0.666	**0.036**	0.824	0.308
	rs17782313	0.790	0.117	0.816	0.208	0.756	0.142	0.865	0.433
	rs502933	0.778	0.080	0.882	0.413	0.671	**0.027**	0.744	0.093
NPC1	rs1805081	0.865	0.298	0.820	0.186	0.815	0.260	0.835	0.301
KCTD15	rs11084753	0.822	0.154	0.740	**0.044**	0.942	0.732	0.939	0.710
	rs29941	0.890	0.401	0.906	0.510	0.944	0.751	1.070	0.703
HTR2C	rs6318*	1.351	0.147	1.030	0.898	1.106	0.717	0.956	0.867

Only completer dataset was analyzed; odds ratios (ORs) and p-values for lower percent weight loss are shown; variables were dichotomized according to their ≤ and > 5 or 10 percent weight loss; an additive genetic model was assumed; adjustment for age, sex, country, and intervention was done; p-values ≤ 0.05 are bold/grey;*) only analyzed in women

Three loci (*NEGR1*, *SFRS10 ETV5 DGKG*, *MC4R*) showed a significant result for both five and ten percent weight loss. Both *NEGR1* SNPs showed an OR of 0.670 (CI: 0.497, 0.904; p=0.009) or 0.639 (CI: 0.453, 0.903; p=0.011) for ≤ 5 or ≤ 10 percent weight loss after twelve months, respectively. SNP rs7647305 (*SFRS10 ETV5 DGKG)* showed an OR of 1.641 (CI: 1.136, 2.371; p=0.008) or of 1.707 (CI: 1.084, 2.689; p=0.021) ≤ 5 or ≤ 10 percent weight

loss after twelve months, respectively. For all four *MC4R* SNPs an OR below 1.0 with a p-value between 0.020 and 0.117 or between 0.006 and 0.142 was observed for ≤ 5 or ≤ 10 percent weight loss after six months. None of the results remained statistically significant after adjustment for multiple testing (p≤0.002) (**Table 5-10**).

Comparing the results between the two dichotomization strategies (median of delta weight or percent weight loss) revealed some consistency. The *NEGR1*, the *SFRS10 ETV5 DGKG*, and the *MC4R* locus showed a significant association with both delta weight and percent weight loss. Minor alleles of the SNPs near *NEGR1* and *MC4R* gene were associated with lower probability to be in the “low losers” group and the minor allele of the SNP near the *SFRS10 ETV5 DGKG* locus was associated with higher probability to be in the “low losers” group.

In the linear regression-based model delta weight was analyzed only in completers. In the BCF dataset delta weight neither as original nor as log-transformed variable was normally distributed. In **appendix R** the age- and sex-adjusted results from the linear regression analysis are shown for delta weight after two, six and twelve months and are similar to the results from the fully adjusted model (age, sex, height, country, intervention, and baseline weight) as shown in **table 5-11**.

The *NEGR1*, *SDCCAG8*, *PTER*, and *MC4R* locus showed an association with delta weight. Both *NEGR1* SNPs showed a significant association with greater delta weight after twelve months (beta=-1.086 kg, CI:-1.884, -0.287, p=0.008). For two out of three genotyped *SDCCAG8* polymorphisms a significant association was observed for lower delta weight after twelve months (rs10926984: beta=1.198 kg, CI: 0.011, 2.385, p=0.048; rs2783963: beta=1.336 kg, CI: 0.176, 2.496, p=0.024). The SNP near *PTER* was not associated with delta weight after twelve months, but with delta weight after two (beta=0.635 kg, CI: 0.009, 1.262, p=0.047) and six months (beta=1.328 kg, CI: 0.179, 2.477, p=0.024). Furthermore, the *MC4R* locus showed significant results for delta weight after six (rs1673482: beta=-0.828 kg, CI:-1.415, -0.241, p=0.006; rs17700144: beta=-0.902 kg, CI:-1.569, -0.235, p=0.008; rs502933: beta=-0.745 kg, CI:-1.362, -0.129, p=0.018) and twelve months (rs1673482: beta=-0.868 kg, CI: -1.654, -0.082, p=0.031). None of the results remained statistically significant after adjustment for multiple testing (p≤0.002) (**Table 5-11**).

Table 5-11: Results from linear regression concerning delta weight after two, six and twelve months

Locus	SNP	Delta weight (2 months)		Delta weight (6 months)		Delta weight (12 months)	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	-0.161	0.380	-0.008	0.981	-0.116	0.806
NEGR1	rs2568958	-0.244	0.121	-0.435	0.144	-1.086	**0.008**
	rs2815752	-0.244	0.121	-0.435	0.144	-1.086	**0.008**
SDCCAG8	rs10926984	0.145	0.546	0.596	0.184	1.198	**0.048**
	rs12145833	0.024	0.920	0.486	0.284	0.895	0.142
	rs2783963	0.343	0.136	0.869	**0.045**	1.336	**0.024**
SEC16B, RASAL2	rs10913469	-0.047	0.807	-0.104	0.769	-0.378	0.437
INSIG2	rs11684454	0.033	0.841	-0.016	0.959	-0.187	0.648
TMEM18	rs7561317	0.059	0.785	-0.369	0.356	-0.515	0.334
ADIPOQ	rs17300539	-0.490	0.106	-0.495	0.402	-0.571	0.462
PPARG	rs1801282	-0.104	0.661	-0.143	0.753	-0.626	0.286
SFRS10, ETV5, DGKG	rs7647305	0.279	0.140	0.537	0.134	0.899	0.066
UCP1	rs45539933	-0.132	0.687	-0.226	0.696	-0.087	0.911
ADRB2	rs12654778	0.202	0.227	0.071	0.820	0.213	0.599
PCSK1	rs12186664	-0.147	0.383	-0.138	0.661	0.092	0.825
PRL	rs4145443	0.028	0.857	-0.097	0.735	-0.059	0.878
TNKS-MSRA	rs13278851	-0.185	0.438	-0.132	0.772	0.126	0.833
	rs17150703	-0.122	0.616	-0.088	0.849	-0.009	0.989
	rs516175	-0.005	0.981	-0.048	0.911	-0.022	0.970
TRHR	rs7832552	0.073	0.652	-0.236	0.427	0.118	0.770
ADRA2A	rs1800544	0.010	0.953	-0.053	0.870	-0.414	0.354
PFKP	rs17132175	0.072	0.788	0.482	0.333	0.362	0.599
PTER	rs10508503	0.635	**0.047**	1.328	**0.024**	1.063	0.177
BDNF	rs16917237	-0.297	0.132	0.037	0.920	-0.564	0.250
MTCH2	rs10838738	0.073	0.655	0.291	0.337	0.791	**0.050**
GNB3	rs5443	-0.149	0.356	-0.174	0.573	-0.274	0.511
PLIN	rs894160	0.145	0.386	-0.130	0.675	-0.098	0.816
FTO	rs6499640	-0.099	0.527	-0.258	0.375	-0.381	0.339
	rs7206010	-0.083	0.596	-0.236	0.420	-0.337	0.401
	rs9935401	-0.027	0.863	-0.335	0.267	-0.343	0.397
	rs9939609	0.002	0.989	-0.268	0.371	-0.254	0.530
MAF	rs1424233	-0.141	0.362	-0.396	0.175	-0.438	0.260
MC4R	rs1673482	-0.230	0.152	-0.828	**0.006**	-0.868	**0.031**
	rs17700144	-0.244	0.190	-0.902	**0.008**	-0.732	0.104
	rs17782313	-0.096	0.587	-0.612	0.060	-0.584	0.179
	rs502933	-0.175	0.295	-0.745	**0.018**	-0.754	0.070
NPC1	rs1805081	-0.088	0.587	-0.300	0.325	-0.639	0.116
KCTD15	rs11084753	0.048	0.764	-0.219	0.463	-0.502	0.206
	rs29941	0.024	0.883	0.105	0.729	0.077	0.847
HTR2C	rs6318*	0.037	0.874	0.165	0.714	-0.257	0.682

Beta estimates (kg) and p-values are shown; an additive genetic model was assumed; adjustment for age, sex, height, country, intervention and baseline weight was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

In addition a linear mixed effect model was calculated taking into account delta weight loss at five different time points (2, 4, 6, 9, and 12 months). Results from three different adjustment models (age, sex / age, sex, country, intervention / age, sex, height, country, intervention) are shown in **table 5-12**.

Table 5-12: Results from mixed effect model concerning delta weight at five time points (2, 4, 6, 9, 12 months)

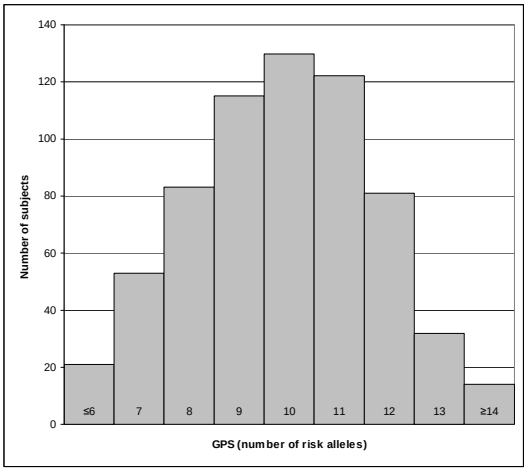
Locus	SNP	Delta weight (five time points) *adjusted for age and sex*		Delta weight (five time points) *adjusted for age, sex, country, intervention*		Delta weight (five time points) *adjusted for age, sex, height, country, intervention*	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	0.019	0.944	-0.126	0.635	-0.145	0.583
NEGR1	rs2568958	-0.604	**0.010**	-0.602	**0.008**	-0.547	**0.015**
	rs2815752	-0.604	**0.010**	-0.602	**0.008**	-0.547	**0.015**
SDCCAG8	rs10926984	0.546	0.131	0.413	0.232	0.408	0.235
	rs12145833	0.395	0.280	0.266	0.446	0.255	0.461
	rs2783963	0.688	**0.047**	0.671	**0.043**	0.654	**0.047**
SEC16B, RASAL2	rs10913469	-0.149	0.605	-0.197	0.475	-0.173	0.529
INSIG2	rs11684454	0.104	0.678	0.050	0.832	0.048	0.839
TMEM18	rs7561317	-0.456	0.161	-0.268	0.391	-0.328	0.290
ADIPOQ	rs17300539	-0.245	0.593	-0.505	0.250	-0.493	0.258
PPARG	rs1801282	-0.225	0.530	-0.370	0.280	-0.311	0.362
SFRS10, ETV5, DGKG	rs7647305	0.466	0.102	0.533	**0.051**	0.494	0.069
UCP1	rs45539933	-0.316	0.514	-0.140	0.762	-0.191	0.678
ADRB2	rs12654778	-0.051	0.840	0.073	0.762	0.100	0.676
PCSK1	rs12186664	-0.312	0.225	-0.292	0.232	-0.269	0.269
PRL	rs4145443	0.213	0.357	0.196	0.374	0.178	0.416
TNKS-MSRA	rs13278851	-0.383	0.292	-0.137	0.693	-0.109	0.751
	rs17150703	-0.375	0.308	-0.156	0.657	-0.115	0.742
	rs516175	-0.293	0.389	-0.030	0.928	-0.040	0.903
TRHR	rs7832552	-0.124	0.608	-0.121	0.601	-0.148	0.518
ADRA2A	rs1800544	-0.012	0.962	0.028	0.911	0.012	0.960
PFKP	rs17132175	0.306	0.450	0.320	0.409	0.311	0.419
PTER	rs10508503	0.814	0.089	0.731	0.109	0.820	0.071
BDNF	rs16917237	-0.447	0.132	-0.459	0.104	-0.443	0.115
MTCH2	rs10838738	0.176	0.473	0.149	0.525	0.190	0.416
GNB3	rs5443	-0.112	0.647	-0.081	0.730	-0.097	0.677
PLIN	rs894160	-0.064	0.799	0.087	0.719	0.056	0.814
FTO	rs6499640	-0.290	0.220	-0.204	0.366	-0.182	0.418
	rs7206010	-0.273	0.249	-0.197	0.384	-0.176	0.435
	rs9935401	-0.197	0.415	-0.258	0.261	-0.255	0.264
	rs9939609	-0.129	0.589	-0.199	0.385	-0.202	0.376
MAF	rs1424233	-0.293	0.212	-0.214	0.339	-0.214	0.335
MC4R	rs1673482	-0.596	**0.014**	-0.586	**0.011**	-0.607	**0.008**
	rs17700144	-0.626	**0.025**	-0.670	**0.012**	-0.676	**0.011**
	rs17782313	-0.321	0.228	-0.440	0.084	-0.470	0.063
	rs502933	-0.564	**0.025**	-0.517	**0.031**	-0.527	**0.028**
NPC1	rs1805081	-0.200	0.411	-0.269	0.249	-0.217	0.352
KCTD15	rs11084753	-0.153	0.527	-0.135	0.560	-0.141	0.540
	rs29941	0.123	0.612	0.094	0.686	0.087	0.707
HTR2C	rs6318*	0.229	0.529	-0.001	0.998	0.005	0.989

Beta estimates (kg) and p-values are shown; an additive genetic model was assumed; three different adjustment models were calculated (age, sex / age, sex, country, intervention / age, sex, height, country, intervention); p-values ≤ 0.05 are bold/grey; *) only analyzed in women

The fully adjusted model (age, sex, height, country, intervention) showed significant results for three loci: *NEGR1*, *SDCCAG8*, and *MC4R*. Both *NEGR1* SNPs were associated with higher delta weight (beta=-0.547 kg, standard error (SE): 0.225, p=0.015). The rs2783963 polymorphism (*SDCCAG8*) was associated with lower delta weight (beta=0.654 kg, SE: 0.329, p=0.047). A significant association was observed for rs1673482, rs17700144, and rs502933 (*MC4R*) with beta=-0.607 kg (SE: 0.230, p=0.008), beta=-0.676 kg (SE: 0.265, p=0.011), and beta=-0.527 kg (SE: 239, p=0.028), respectively. Different adjustment led to similar results concerning the direction and size of beta estimates as well as p-values. Despite the fact that p-values changed between the different models, after adjustment for multiple testing none of the results remained statistically significant (p≤0.002).

Comparing the results between the linear regression and the linear mixed effect model revealed some consistency. The *NEGR1*, the *SDCCAG8*, and the *MC4R* locus showed a significant association with delta weight in both approaches. Minor alleles of the SNPs near *NEGR1* and *MC4R* gene were associated with greater delta weight and the minor allele of the SNP within the *SDCCAG8* gene was associated with lower delta weight.

In addition to the single SNP analysis, a cumulative one was performed. Therefore – as described in the methods part (**Chapter 4.5.5**) – nine BMI-related SNPs from genome-wide association studies were analyzed for their cumulative effect by including them into the GPS.



The number of subjects for a specific number of risk alleles (≤6 to ≥14) is shown in **figure 5-2**. The mean/median (s.d./IQR) of delta weight after two, six, and twelve months in the different GPS categories is given in **table 5-13**. A graphical illustration is shown in **figure 5-3**. These descriptive results give no hint for a linear association or trend.

Figure 5-2: Number of subjects in the different GPS categories is shown

Table 5-13: Means (s.d.) and medians (IQR) of delta weight (kg) at different time points are shown for the specific GPS categories

Parameter	GPS (number of risk alleles)								
	≤6	7	8	9	10	11	12	13	≥14
	mean (s.d.) median (IQR)								
Delta weight (2 months)	-2.93 (3.04)	-2.96 (3.47)	-2.10 (2.75)	-2.67 (3.13)	-2.46 (2.75)	-2.15 (2.69)	-2.62 (2.32)	-2.67 (2.88)	-2.56 (1.87)
	-2.60 (2.80)	-2.80 (4.10)	-2.00 (3.10)	-2.40 (4.40)	-2.30 (3.70)	-1.70 (3.10)	-2.55 (2.55)	-2.70 (2.90)	-2.20 (2.80)
Delta weight (6 months)	-6.08 (6.27)	-5.65 (6.36)	-3.58 (4.75)	-5.19 (4.85)	-4.45 (4.86)	-4.36 (4.01)	-4.55 (3.98)	-5.76 (4.59)	-6.82 (6.31)
	-4.35 (7.80)	-4.50 (5.90)	-3.30 (6.00)	-4.65 (6.05)	-3.85 (6.10)	-3.90 (5.10)	-4.70 (4.90)	-4.85 (4.70)	-6.30 (7.00)
Delta weight (12 months)	-5.84 (5.64)	-6.56 (8.00)	-4.27 (5.96)	-5.80 (6.16)	-4.75 (6.04)	-4.46 (5.02)	-3.87 (4.51)	-5.85 (6.70)	-7.28 (8.13)
	-5.75 (5.90)	-5.00 (8.50)	-3.10 (5.20)	-4.30 (8.60)	-4.10 (7.30)	-3.60 (7.65)	-3.20 (5.10)	-5.30 (7.60)	-5.10 (11.20)

The lowest and the highest mean weight at the specific time point is grey

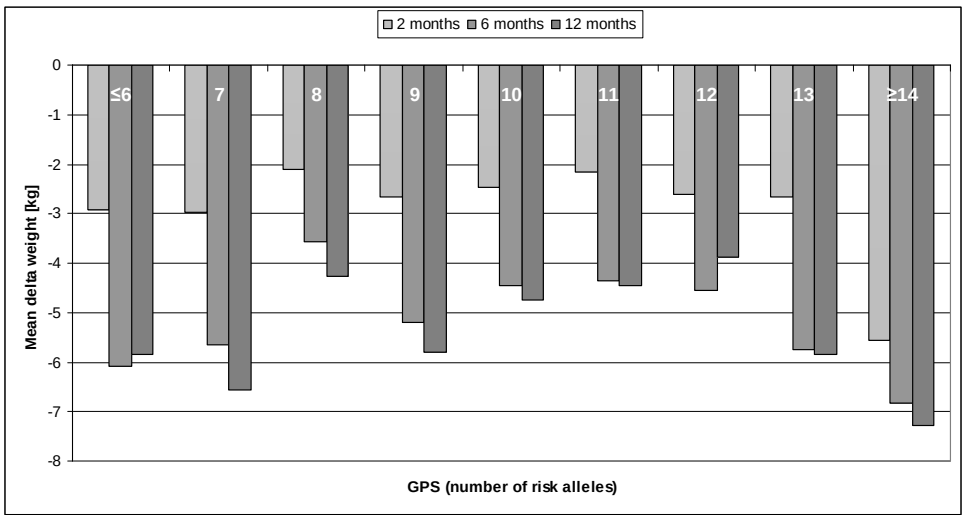


Figure 5-3: Graphical illustration of mean delta weight after two, six and twelve months in the different GPS categories

To test whether there is a statistically significant association between the number of risk alleles (GPS) and delta weight a linear regression with different adjustment approaches was performed. There were no significant results (**Table 5-14**). This is not unexpected given the results in **figure 5-3**. Subjects with a smaller number of risk alleles as well as subjects with a higher GPS had a greater mean of delta weight than subjects with the average GPS.

Table 5-14: Results from linear regression concerning delta weight after two, six, and twelve months

Parameter	*adjusted for age and sex*		*adjusted for age, sex, country, intervention*		*adjusted for age, sex, height, country, intervention, baseline weight*	
	beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
Delta weight (2 months)	0.037	0.535	0.012	0.835	0.018	0.756
Delta weight (6 months)	0.004	0.974	-0.061	0.567	-0.058	0.587
Delta weight (12 months)	0.148	0.327	0.103	0.475	0.096	0.501

Beta estimates (kg) and p-values are shown; an additive genetic model was assumed

5.3.1.3 Results from genetic analyses – delta fat mass

To test whether the genotyped polymorphisms are associated with delta fat mass at various time points, Kruskal-Wallis test, logistic and linear regression as well as different adjustment models were calculated. Analogue to the outcome delta weight a selection of results from the fully adjusted model was reported in the main text.

In **appendix O** results from the Kruskal-Wallis test (p-values) are shown for delta fat mass after six and twelve months in both datasets (completers and BCF). Concerning delta fat mass there were significant p-values for six loci (*SDCCAG8*, *PRL*, *TNKS-MSRA*, *FTO*, *MAF*, *MC4R*), whereas often the p-values were not very different from 0.05. After adjustment for multiple testing only the association between *TNKS-MSRA* SNP rs516175 and delta fat mass after six months (completers: p=0.0003; BCF: p=0.0004) remained statistically significant.

For logistic regression analysis delta fat mass was dichotomized by the time point-specific median. In **appendix S** the age- and sex-adjusted results are shown for delta fat mass after two (completer), six and twelve months (completer and BCF analysis) and are similar to the results from the fully adjusted model (age, sex, height, country, intervention, and baseline fat mass (**table 5-15**)).

Table 5-15: Results from logistic regression concerning delta fat mass after two, six and twelve months

Locus	SNP	Delta fat mass (2 months)		Delta fat mass (6 months)		Delta fat mass BCF (6 months)		Delta fat mass (12 months)		Delta fat mass BCF (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	0.976	0.870	1.003	0.987	1.059	0.701	0.967	0.862	0.948	0.714
NEGR1	rs2568958	0.743	**0.023**	0.845	0.246	0.847	0.193	0.698	**0.032**	0.887	0.343
	rs2815752	0.743	**0.023**	0.845	0.246	0.847	0.193	0.698	**0.032**	0.887	0.343
SDCCAG8	rs10926984	1.188	0.383	1.269	0.287	1.308	0.171	0.888	0.628	1.148	0.475
	rs12145833	1.145	0.491	1.259	0.304	1.338	0.137	0.856	0.526	1.116	0.566
	rs2783963	1.198	0.336	1.469	0.077	1.440	**0.052**	0.917	0.721	1.170	0.394
SEC16B, RASAL2	rs10913469	1.100	0.540	1.173	0.353	1.207	0.223	1.063	0.751	0.945	0.708
INSIG2	rs11684454	1.187	0.200	1.124	0.447	1.035	0.795	1.116	0.509	0.999	0.993
TMEM18	rs7561317	1.315	0.120	1.271	0.217	0.854	0.368	0.933	0.747	0.803	0.207
ADIPOQ	rs17300539	0.876	0.594	1.294	0.389	1.432	0.156	1.082	0.806	1.041	0.870
PPARG	rs1801282	0.979	0.914	0.960	0.854	0.906	0.606	0.924	0.741	0.675	**0.043**
SFRS10, ETV5, DGKG	rs7647305	1.013	0.933	1.392	0.055	1.254	0.133	1.273	0.221	1.169	0.295
UCP1	rs45539933	0.773	0.332	0.726	0.254	0.482	**0.007**	0.385	**0.005**	0.881	0.621
ADRB2	rs12654778	1.172	0.243	1.200	0.235	1.136	0.347	1.647	**0.003**	1.131	0.355
PCSK1	rs12186664	0.886	0.383	1.029	0.851	0.957	0.749	1.098	0.580	0.946	0.684
PRL	rs4145443	1.006	0.961	0.945	0.688	1.061	0.630	1.003	0.982	1.353	**0.014**
TNKS-MSRA	rs13278851	0.854	0.415	0.760	0.210	0.851	0.408	1.221	0.397	1.031	0.876
	rs17150703	0.859	0.443	0.746	0.186	0.828	0.338	1.187	0.474	1.017	0.932
	rs516175	0.907	0.590	0.768	0.201	0.958	0.813	1.107	0.650	1.045	0.805
TRHR	rs7832552	1.263	0.076	0.995	0.975	0.699	**0.007**	1.043	0.798	0.856	0.234
ADRA2A	rs1800544	0.826	0.169	1.147	0.388	1.049	0.726	0.943	0.744	1.112	0.436
PFKP	rs17132175	1.300	0.228	1.148	0.568	1.073	0.743	1.049	0.859	1.004	0.984
PTER	rs10508503	0.967	0.898	1.152	0.619	0.826	0.455	1.056	0.863	0.956	0.860
BDNF	rs16917237	1.015	0.926	1.121	0.522	0.786	0.130	1.210	0.331	0.727	**0.043**
MTCH2	rs10838738	0.993	0.957	0.979	0.887	1.029	0.825	1.071	0.675	1.071	0.599
GNB3	rs5443	1.074	0.583	1.179	0.272	1.321	**0.033**	0.842	0.301	1.095	0.477
PLIN	rs894160	1.284	0.064	0.943	0.693	0.994	0.961	0.830	0.267	1.100	0.468
FTO	rs6499640	0.942	0.639	0.942	0.674	1.162	0.236	0.910	0.555	1.073	0.568
	rs7206010	0.931	0.579	0.949	0.717	1.138	0.312	0.935	0.679	1.065	0.612
	rs9935401	0.811	0.107	0.801	0.129	0.743	**0.021**	0.942	0.709	0.775	**0.045**
	rs9939609	0.883	0.336	0.884	0.392	0.814	0.107	0.994	0.971	0.810	0.094
MAF	rs1424233	0.892	0.364	0.876	0.356	0.854	0.207	0.755	0.077	0.800	0.073
MC4R	rs1673482	0.949	0.683	0.919	0.560	0.872	0.286	0.821	0.218	0.678	**0.003**
	rs17700144	0.860	0.317	0.683	**0.024**	0.648	**0.005**	0.868	0.428	0.665	**0.007**
	rs17782313	0.992	0.954	0.813	0.193	0.730	**0.030**	0.981	0.910	0.667	**0.005**
	rs502933	1.027	0.842	0.957	0.768	0.901	0.434	0.862	0.366	0.717	**0.011**
NPC1	rs1805081	1.038	0.777	0.924	0.592	1.057	0.673	0.814	0.208	1.045	0.737
KCTD15	rs11084753	0.954	0.718	0.844	0.237	0.842	0.177	0.886	0.444	0.934	0.587
	rs29941	0.896	0.406	0.942	0.680	0.951	0.697	1.008	0.959	1.024	0.851
HTR2C	rs6318*	1.189	0.375	1.222	0.367	1.070	0.724	0.702	0.171	0.818	0.295

Both datasets (completers and BCF) were analyzed for delta fat mass after six and twelve months; odds ratios (ORs) and p-values for lower fat mass loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age, sex, height, country, intervention and baseline fat mass was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

The *NEGR1*, *UCP1*, *FTO* and *MC4R* locus showed significant results for delta fat mass at two different time points. *NEGR1* polymorphisms showed an OR of 0.743 (CI: 0.576, 0.959, p=0.023) for lower fat mass loss after two months and of 0.698 (CI: 0.502, 0.970, p=0.032, completer) after twelve months. The *UCP1* locus resulted in an OR of 0.482 (CI: 0.283, 0.822, p=0.007, BCF) and 0.385 (CI: 0.198, 0.750, p=0.005, completer) for lower fat mass loss after six or twelve months, respectively. For the *FTO* SNP rs9935401 an OR of 0.743 (CI: 0.577, 0.957, p=0.021) and 0.775 (CI: 0.604, 0.994, p=0.045) was observed for lower fat mass loss after six and twelve months (BCF). Two *MC4R* polymorphisms were associated with delta fat mass after six months (BCF), whereas all four *MC4R* polymorphisms were associated with delta fat mass after twelve months (BCF) with an OR below 1.0 and p-values between 0.003 and 0.011.
The SNP rs12654778 (*ADRB2*) showed a significant association with delta fat mass after twelve months (OR=1.647, CI: 1.180, 2.300, p=0.003, completer) and for polymorphism rs7832552 (*TRHR*) an association with delta fat mass after six months was observed (OR=0.699, CI: 0.539, 0.906, p=0.007, BCF) (**Table 5-15**). After adjustment for multiple testing all results lost statistical significance.

Analogue to the outcome delta weight, in the linear regression-based model delta fat mass was analyzed only in completers. In **appendix R** the age- and sex-adjusted results from the linear regression analysis are shown for delta fat mass after two, six and twelve months and are similar to the results from the fully adjusted model (age, sex, height, country, intervention, and baseline weight) as shown in **table 5-16**.

Polymorphism rs7832552 (*TRHR*) showed a marginally significant association with lower delta fat mass after two months (beta=0.357 kg, CI: 0.005, 0.709, p=0.047). The *MAF* locus (rs1424233) showed a marginally significant association with higher delta fat mass after twelve months (beta=-0.786 kg, CI: -1.458, -0.113, p=0.022). All other analyzed SNPs showed no association with delta fat mass (**Table 5-16**).

Table 5-16: Results from linear regression concerning delta fat mass after two, six and twelve months

Locus	SNP	Delta fat mass (2 months)		Delta fat mass (6 months)		Delta fat mass (12 months)	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	-0.110	0.593	0.034	0.921	0.054	0.899
NEGR1	rs2568958	-0.300	0.090	-0.171	0.554	-0.595	0.101
	rs2815752	-0.300	0.090	-0.171	0.554	-0.595	0.101
SDCCAG8	rs10926984	0.150	0.581	0.477	0.277	0.477	0.380
	rs12145833	0.094	0.728	0.412	0.349	0.414	0.443
	rs2783963	0.301	0.243	0.711	0.094	0.565	0.288
SEC16B, RASAL2	rs10913469	0.052	0.810	0.361	0.283	-0.158	0.710
INSIG2	rs11684454	0.147	0.423	0.257	0.399	0.150	0.683
TMEM18	rs7561317	-0.234	0.338	-0.186	0.632	-0.237	0.616
ADIPOQ	rs17300539	-0.308	0.374	0.087	0.882	-0.477	0.503
PPARG	rs1801282	0.087	0.743	-0.083	0.851	-0.575	0.270
SFRS10, ETV5, DGKG	rs7647305	-0.046	0.827	0.257	0.450	0.495	0.246
UCP1	rs45539933	-0.479	0.187	-0.618	0.264	-0.863	0.210
ADRB2	rs12654778	0.106	0.572	0.001	0.998	0.606	0.091
PCSK1	rs12186664	-0.059	0.758	-0.002	0.994	0.246	0.510
PRL	rs4145443	0.292	0.088	0.106	0.704	0.243	0.475
TNKS-MSRA	rs13278851	-0.241	0.370	-0.460	0.288	-0.149	0.777
	rs17150703	-0.218	0.426	-0.452	0.302	-0.244	0.647
	rs516175	-0.192	0.447	-0.506	0.216	-0.361	0.470
TRHR	rs7832552	0.357	**0.047**	-0.082	0.776	0.277	0.446
ADRA2A	rs1800544	-0.146	0.448	-0.068	0.829	-0.035	0.930
PFKP	rs17132175	0.032	0.914	0.189	0.696	0.451	0.452
PTER	rs10508503	-0.397	0.271	0.290	0.605	0.487	0.483
BDNF	rs16917237	-0.326	0.140	-0.179	0.609	-0.470	0.269
MTCH2	rs10838738	-0.212	0.245	0.353	0.226	0.684	0.057
GNB3	rs5443	-0.027	0.879	-0.002	0.994	-0.140	0.699
PLIN	rs894160	0.155	0.403	-0.216	0.468	-0.289	0.433
FTO	rs6499640	0.015	0.931	0.047	0.866	-0.237	0.503
	rs7206010	-0.007	0.967	0.033	0.908	-0.165	0.644
	rs9935401	-0.315	0.077	-0.409	0.157	-0.357	0.316
	rs9939609	-0.230	0.197	-0.299	0.298	-0.289	0.415
MAF	rs1424233	-0.123	0.477	-0.447	0.113	-0.786	**0.022**
MC4R	rs1673482	-0.062	0.729	-0.240	0.405	-0.662	0.059
	rs17700144	-0.124	0.551	-0.384	0.242	-0.444	0.262
	rs17782313	0.035	0.858	-0.124	0.691	-0.305	0.425
	rs502933	0.000	1.000	-0.076	0.804	-0.425	0.245
NPC1	rs1805081	-0.093	0.612	-0.223	0.451	-0.487	0.178
KCTD15	rs11084753	0.328	0.069	-0.117	0.682	-0.298	0.389
	rs29941	0.137	0.449	0.080	0.782	0.017	0.961
HTR2C	rs6318*	-0.117	0.653	-0.274	0.532	-0.659	0.246

Beta estimates (kg) and p-values are shown; an additive genetic model was assumed; adjustment for age, sex, height, country, intervention and baseline fat mass was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Comparing the results from the logistic regression with the results from the linear regression there is little evidence for consistency. None of the results from logistic regression was really confirmed by the linear model.

5.3.1.4 Results from genetic analyses – delta waist circumference

To test whether the genotyped polymorphisms are associated with delta waist circumference at various time points, Kruskal-Wallis test, logistic and linear regression as well as different adjustment models were calculated. Analogue to the outcomes delta weight and fat mass a selection of results from the fully adjusted model were reported in the main text.

In **appendix O** results from the Kruskal-Wallis test (p-values) are shown for delta waist circumference after six and twelve months in both datasets (completers and BCF). Four loci were significant (p≤0.05): *NEGR1*, *TMEM18*, *ADIPOQ*, *KCTD15* (p-values not very different from 0.05). After adjustment for multiple testing no p-value stayed statistically significant.

Table 5-17: Results from logistic regression concerning delta waist circumference after two, six and twelve months

Locus	SNP	Delta waist (2 months)		Delta waist (6 months)		Delta waist BCF (6 months)		Delta waist (12months)		Delta waist BCF (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	0.895	0.428	0.952	0.763	1.105	0.467	0.964	0.835	1.287	0.073
NEGR1	rs2568958	0.808	0.079	0.849	0.240	0.848	0.164	0.770	0.091	0.878	0.276
	rs2815752	0.808	0.079	0.849	0.240	0.848	0.164	0.770	0.091	0.878	0.276
SDCCAG8	rs10926984	1.013	0.944	0.973	0.895	0.833	0.311	1.053	0.818	1.122	0.524
	rs12145833	1.062	0.741	0.902	0.622	0.798	0.216	0.974	0.907	1.078	0.680
	rs2783963	1.094	0.606	0.959	0.837	0.839	0.311	1.167	0.485	1.197	0.302
SEC16B, RASAL2	rs10913469	0.905	0.504	1.100	0.568	0.904	0.489	0.973	0.879	0.799	0.130
INSIG2	rs11684454	1.103	0.433	1.113	0.464	1.113	0.387	0.995	0.976	0.781	**0.049**
TMEM18	rs7561317	1.217	0.232	0.850	0.372	0.729	**0.054**	0.714	0.087	0.782	0.131
ADIPOQ	rs17300539	1.005	0.984	0.936	0.808	0.939	0.780	0.580	0.068	0.928	0.744
PPARG	rs1801282	0.802	0.231	0.729	0.135	0.909	0.596	0.668	0.069	0.711	0.062
SFRS10, ETV5, DGKG	rs7647305	1.182	0.244	1.367	0.063	1.095	0.519	1.319	0.135	1.267	0.098
UCP1	rs45539933	1.702	**0.034**	1.091	0.746	0.803	0.369	1.371	0.285	0.726	0.194
ADRB2	rs12654778	1.316	**0.032**	1.031	0.830	1.237	0.092	1.057	0.719	1.012	0.926
PCSK1	rs12186664	0.951	0.699	0.974	0.857	0.912	0.466	1.307	0.088	0.917	0.496
PRL	rs4145443	1.072	0.547	0.963	0.780	1.041	0.725	0.943	0.686	1.204	0.109
TNKS-MSRA	rs13278851	1.249	0.219	0.825	0.358	1.105	0.578	0.931	0.745	1.141	0.469
	rs17150703	1.264	0.203	0.726	0.135	0.967	0.852	0.805	0.335	1.025	0.892
	rs516175	1.283	0.141	0.836	0.361	1.035	0.837	0.767	0.212	1.021	0.904
TRHR	rs7832552	1.203	0.133	0.967	0.809	0.970	0.804	1.372	**0.038**	0.972	0.818
ADRA2A	rs1800544	0.952	0.708	0.758	0.070	0.951	0.697	0.972	0.867	1.096	0.481
PFKP	rs17132175	1.145	0.511	1.033	0.893	1.120	0.577	1.136	0.630	1.087	0.683
PTER	rs10508503	1.438	0.135	1.380	0.237	1.145	0.567	0.874	0.647	0.846	0.485
BDNF	rs16917237	0.963	0.801	0.999	0.996	0.956	0.756	0.946	0.759	0.886	0.408
MTCH2	rs10838738	1.064	0.613	1.044	0.761	0.982	0.881	1.026	0.865	0.902	0.401
GNB3	rs5443	0.947	0.659	0.979	0.880	1.061	0.620	0.971	0.851	1.051	0.680
PLIN	rs894160	1.093	0.483	0.932	0.625	1.021	0.868	0.898	0.495	1.069	0.595
FTO	rs6499640	1.014	0.910	1.037	0.784	1.187	0.142	1.040	0.787	0.987	0.912
	rs7206010	1.055	0.654	1.068	0.626	1.198	0.124	1.126	0.429	0.991	0.937
	rs9935401	1.088	0.489	0.932	0.616	0.843	0.154	1.026	0.866	0.955	0.700
	rs9939609	1.069	0.586	0.970	0.827	0.867	0.231	1.021	0.888	0.978	0.853
MAF	rs1424233	1.111	0.370	1.146	0.317	1.078	0.520	0.854	0.281	1.135	0.281
MC4R	rs1673482	0.954	0.703	0.972	0.839	0.796	0.059	0.994	0.968	0.793	0.058
	rs17700144	0.942	0.673	0.814	0.196	0.746	**0.038**	1.005	0.978	0.772	0.068
	rs17782313	1.038	0.778	0.891	0.445	0.750	**0.032**	1.099	0.559	0.776	0.060
	rs502933	1.012	0.925	1.068	0.648	0.860	0.224	1.012	0.940	0.871	0.271
NPC1	rs1805081	1.008	0.948	1.210	0.175	1.083	0.511	0.980	0.892	1.102	0.428
KCTD15	rs11084753	1.081	0.524	0.938	0.643	1.136	0.288	0.902	0.491	0.940	0.604
	rs29941	1.130	0.320	1.187	0.222	1.243	0.073	0.967	0.825	1.003	0.981
HTR2C	rs6318*	1.122	0.528	1.208	0.374	1.060	0.748	1.222	0.391	1.175	0.376

Both datasets (completers and BCF) were analyzed for delta waist circumference after six and twelve months; odds ratios (ORs) and p-values for lower waist circumference loss are shown; variables were dichotomized according to their median (≤ and >); waist circumference loss after twelve months (BCF) were dichotomized as < and ≥; an additive genetic model was assumed; adjustment for age, sex, height, country, intervention and baseline waist circumference was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

For logistic regression analysis delta waist circumference was dichotomized by the time point-specific median. In **appendix T** the age- and sex-adjusted results are shown for delta waist circumference after two (completers), six and twelve months (completer and BCF analysis) and are similar to the results from the fully adjusted model (age, sex, height, country, intervention, and baseline waist circumference) shown in **table 5-17**. All investigated SNPs showed no significant results for an association with delta waist circumference. For polymorphisms showing a trend the p-values ranged from 0.032 to 0.054 (**Table 5-17**).

Table 5-18: Results from linear regression concerning delta waist circumference after two, six and twelve months

Locus	SNP	Delta waist (2 months)		Delta waist (6 months)		Delta waist (12 months)	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	-0.054	0.863	-0.112	0.791	0.369	0.516
NEGR1	rs2568958	-0.254	0.342	-0.232	0.519	-0.771	0.116
	rs2815752	-0.254	0.342	-0.232	0.519	-0.771	0.116
SDCCAG8	rs10926984	0.264	0.515	-0.080	0.882	0.720	0.321
	rs12145833	0.202	0.619	-0.266	0.627	0.223	0.759
	rs2783963	0.278	0.475	0.179	0.731	0.922	0.193
SEC16B, RASAL2	rs10913469	-0.083	0.800	-0.386	0.374	-0.348	0.551
INSIG2	rs11684454	0.194	0.486	0.175	0.645	-0.481	0.324
TMEM18	rs7561317	0.252	0.493	-0.804	0.092	-0.914	0.149
ADIPOQ	rs17300539	-0.471	0.357	-1.006	0.156	-1.551	0.094
PPARG	rs1801282	-0.258	0.519	-0.723	0.186	-0.603	0.385
SFRS10, ETV5, DGKG	rs7647305	0.197	0.540	0.432	0.318	0.297	0.614
UCP1	rs45539933	0.655	0.240	0.282	0.686	0.978	0.292
ADRB2	rs12654778	0.542	0.056	0.401	0.284	0.363	0.457
PCSK1	rs12186664	-0.071	0.805	0.149	0.693	0.412	0.404
PRL	rs4145443	0.278	0.282	-0.376	0.279	-0.029	0.950
TNKS-MSRA	rs13278851	0.590	0.142	-0.051	0.926	0.176	0.804
	rs17150703	0.661	0.107	-0.235	0.670	-0.195	0.787
	rs516175	0.541	0.152	0.075	0.884	-0.181	0.791
TRHR	rs7832552	0.468	0.087	0.120	0.735	0.933	**0.053**
ADRA2A	rs1800544	-0.008	0.977	-0.228	0.565	-0.097	0.859
PFKP	rs17132175	0.114	0.804	0.226	0.717	0.563	0.501
PTER	rs10508503	0.856	0.117	0.920	0.192	0.171	0.856
BDNF	rs16917237	-0.247	0.451	0.373	0.394	-0.005	0.993
MTCH2	rs10838738	-0.289	0.292	0.100	0.785	0.024	0.961
GNB3	rs5443	-0.123	0.651	0.294	0.430	0.085	0.865
PLIN	rs894160	-0.047	0.867	0.122	0.745	-0.457	0.366
FTO	rs6499640	0.012	0.964	0.413	0.234	-0.223	0.641
	rs7206010	0.069	0.795	0.546	0.121	0.018	0.971
	rs9935401	-0.034	0.898	-0.186	0.613	-0.194	0.688
	rs9939609	-0.062	0.817	-0.099	0.785	-0.041	0.932
MAF	rs1424233	0.113	0.665	0.178	0.616	-0.290	0.533
MC4R	rs1673482	0.237	0.386	-0.249	0.493	0.031	0.948
	rs17700144	0.133	0.673	-0.414	0.319	0.177	0.743
	rs17782313	0.308	0.304	-0.201	0.611	0.220	0.672
	rs502933	0.274	0.325	-0.067	0.861	0.163	0.738
NPC1	rs1805081	-0.086	0.753	0.304	0.407	-0.421	0.385
KCTD15	rs11084753	0.337	0.213	-0.074	0.837	-0.508	0.286
	rs29941	0.385	0.158	0.500	0.166	-0.101	0.834
HTR2C	rs6318*	-0.080	0.847	0.136	0.805	0.268	0.715

Beta estimates (cm) and p-values are shown; an additive genetic model was assumed; adjustment for age, sex, height, country, intervention and baseline waist circumference was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Analogue to the outcomes delta weight and fat mass, in the linear regression-based model delta waist circumference was only analyzed in completers. In **appendix R** the age- and sex-adjusted results from the linear regression analysis are shown for delta waist circumference after two, six and twelve months and were similar to the results from the fully adjusted model (age, sex, height, country, intervention, and baseline waist circumference) as shown in **table 5-18**. None of the SNPs showed a significant association with delta waist circumference in the linear regression-based model.

Comparing the results from the logistic regression with the results from the linear regression there was consistency because neither the logistic nor the linear regression showed a significant association with delta waist circumference.

5.3.2 LOGIC study

5.3.2.1 Results from genetic analyses – delta weight

To test whether the genotyped polymorphisms are associated with delta weight at various time points, Kruskal-Wallis test, logistic and linear regression, mixed effect models as well as different adjustment models were calculated. Due to the multiple analysis approaches and the number of SNPs (N=44) especially results from the fully adjusted model with p-value ≤ 0.05 are mentioned.

In **appendix U** results from the Kruskal-Wallis test (p-values) are shown for delta weight after four and six weeks as well as after four and six weeks together (if six weeks value was not available, four weeks value was used). Concerning delta weight significant p-values (≤0.05) were found for *SDCCAG8*, *ADRA2A*, *MTCH2*, *FTO*, and *HTR2C*. Often the p-values were not very different from 0.05. After adjustment for multiple testing (p≤0.001) all p-values lost significance.

For logistic regression analysis delta weight was dichotomized by the time point-specific median. In **appendix V** the age- and sex- (if necessary duration of stay) adjusted results from the logistic regression analysis are shown for delta weight after four or six weeks and after four or six weeks together. The results from the fully adjusted model (age, sex, height, duration of stay (if necessary), baseline weight) are shown in **table 5-19**.
In the age, sex and duration of stay adjusted analysis, the *NEGR1* polymorphisms rs2568958 and rs2815752 showed an OR of 1.526 (CI: 1.024, 2.273, p=0.038) and of 1.532 (CI: 1.029, 2.280, p=0.036), respectively, for an association with lower delta weight in the four and six weeks together analysis (**Appendix V**). This association was abolished in the fully adjusted model (**Table 5-19**).

The loci *SFRS10 ETV5 DGKG*, *ADRB2*, *PCSK1*, *PRL*, *TRHR*, *MTCH2*, and *MAF* showed a marginally significant association with delta weight in the fully adjusted model (p-values from 0.015 to 0.050), but not after adjustment for multiple testing.

Table 5-19: Results from logistic regression concerning delta weight after four or six weeks or after 4 four and six weeks together

Locus	SNP	Delta weight (4 weeks)		Delta weight (6 weeks)		Delta weight (4 or 6 weeks)	
		OR	**p-value**	**OR**	**p-value**	**OR**	**p-value**
LEPR	rs1805134	1.193	0.549	0.707	0.383	1.427	0.329
NEGR1	rs2568958	1.049	0.835	0.794	0.454	1.120	0.689
	rs2815752	1.049	0.834	0.794	0.454	1.121	0.688
SDCCAG8	rs10926984	1.068	0.854	2.277	0.101	1.021	0.964
	rs12145833	1.090	0.807	2.352	0.088	1.010	0.983
	rs2783963	1.067	0.858	1.798	0.241	0.806	0.633
SEC16B, RASAL2	rs10913469	0.614	0.092	0.488	0.064	0.749	0.403
INSIG2	rs11684454	0.709	0.168	0.751	0.394	0.601	0.098
TMEM18	rs7561317	0.635	0.121	1.014	0.971	0.626	0.156
ADIPOQ	rs17300539	0.638	0.243	1.573	0.379	0.703	0.430
PPARG	rs1801282	0.730	0.382	0.845	0.741	0.660	0.355
SFRS10, ETV5, DGKG	rs7647305	1.149	0.636	1.008	0.985	2.171	**0.035**
UCP1	rs45539933	1.392	0.476	0.746	0.637	1.009	0.987
ADRB2	rs12654778	0.763	0.229	0.929	0.805	0.533	**0.033**
PCSK1	rs12186664	1.874	**0.015**	1.479	0.264	0.699	0.260
PRL	rs4145443	1.138	0.570	0.485	**0.027**	1.005	0.986
IL6	rs1554606	1.057	0.810	0.787	0.431	1.087	0.778
TNKS-MSRA	rs13278851	0.908	0.808	0.548	0.208	0.926	0.870
	rs17150703	0.857	0.701	0.555	0.231	0.869	0.771
	rs516175	0.829	0.589	0.657	0.298	0.715	0.412
TRHR	rs7832552	0.973	0.915	0.681	0.248	0.460	**0.031**
ADRA2A	rs1800544	1.193	0.474	1.631	0.187	1.399	0.279
PFKP	rs17132175	0.737	0.438	0.552	0.243	0.577	0.260
PTER	rs10508503	1.363	0.518	1.135	0.858	0.684	0.512
BDNF	rs16917237	1.362	0.233	0.801	0.542	0.742	0.369
MTCH2	rs10838738	0.931	0.765	0.505	**0.029**	0.793	0.434
MTNR1B	rs10830963	1.199	0.455	1.016	0.962	1.078	0.813
UCP2	rs659366	0.647	0.056	0.843	0.584	0.802	0.420
GNB3	rs5443	1.053	0.820	0.882	0.669	1.335	0.285
PLIN	rs894160	0.861	0.539	1.175	0.624	0.776	0.400
FTO	rs6499640	1.120	0.631	0.951	0.873	1.185	0.572
	rs7206010	1.139	0.579	0.971	0.924	1.180	0.580
	rs9935401	1.325	0.198	1.375	0.296	1.115	0.684
	rs9939609	1.298	0.230	1.337	0.337	1.145	0.609
MAF	rs1424233	0.641	**0.050**	0.669	0.185	0.799	0.395
SH2B1	rs7498665	0.780	0.254	1.344	0.304	0.758	0.310
MC4R	rs1673482	0.927	0.744	1.039	0.899	0.913	0.745
	rs17700144	1.012	0.962	1.189	0.610	1.155	0.647
	rs17782313	1.033	0.894	1.082	0.810	1.337	0.351
	rs502933	0.980	0.930	1.041	0.892	0.949	0.851
NPC1	rs1805081	1.170	0.476	0.794	0.462	0.774	0.352
KCTD15	rs11084753	1.095	0.719	1.226	0.550	1.632	0.117
	rs29941	0.971	0.902	1.022	0.948	1.247	0.469
HTR2C	rs6318*	1.204	0.657	1.503	0.458	1.987	0.192

Odds ratios (ORs) and p-values for lower loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age, sex, height, and baseline weight was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls

For linear regression delta weight was log-transformed. In **appendix W** the age- and sex- (if necessary duration of stay) adjusted results from linear regression are shown for delta weight after four or six weeks and after four or six weeks together. In **table 5-20** the results from the fully adjusted model (age, sex, height, duration of stay (if necessary), baseline weight) are shown.

Table 5-20: Results from linear regression concerning delta weight after four or six weeks or after four and six weeks together

Locus	SNP	Delta weight (log) (4 weeks)		Delta weight (log) (6 weeks)		Delta weight (log) (4 or 6 weeks)	
		beta	p-value	beta	p-value	beta	p-value
LEPR	rs1805134	-0.002	0.897	-0.013	0.537	-0.015	0.393
NEGR1	rs2568958	-0.031	**0.034**	-0.016	0.370	-0.032	**0.029**
	rs2815752	-0.031	**0.037**	-0.015	0.384	-0.032	**0.031**
SDCCAG8	rs10926984	-0.001	0.964	-0.007	0.784	0.004	0.823
	rs12145833	0.001	0.974	-0.006	0.821	0.005	0.784
	rs2783963	0.003	0.889	-0.002	0.926	0.007	0.722
SEC16B, RASAL2	rs10913469	-0.039	**0.036**	-0.050	**0.020**	-0.042	**0.025**
INSIG2	rs11684454	-0.018	0.229	-0.037	**0.042**	-0.022	0.139
TMEM18	rs7561317	-0.038	**0.043**	-0.040	0.065	-0.034	0.071
ADIPOQ	rs17300539	0.011	0.636	0.019	0.460	0.032	0.170
PPARG	rs1801282	-0.005	0.823	-0.032	0.248	-0.015	0.481
SFRS10, ETV5, DGKG	rs7647305	0.006	0.756	0.033	0.125	0.018	0.314
UCP1	rs45539933	0.013	0.668	-0.039	0.268	0.003	0.906
ADRB2	rs12654778	-0.014	0.336	-0.006	0.700	-0.013	0.352
PCSK1	rs12186664	0.024	0.114	-0.001	0.944	-0.003	0.859
PRL	rs4145443	0.018	0.208	0.005	0.781	0.012	0.393
IL6	rs1554606	-0.008	0.600	0.024	0.151	-0.002	0.868
TNKS-MSRA	rs13278851	-0.004	0.869	-0.021	0.442	-0.013	0.602
	rs17150703	0.000	0.988	-0.022	0.440	-0.008	0.739
	rs516175	0.011	0.606	-0.006	0.801	0.000	0.992
TRHR	rs7832552	-0.004	0.815	-0.032	0.107	-0.013	0.401
ADRA2A	rs1800544	-0.001	0.962	0.010	0.628	0.001	0.952
PFKP	rs17132175	-0.035	0.163	-0.084	**0.004**	-0.031	0.199
PTER	rs10508503	0.027	0.355	0.014	0.704	0.016	0.587
BDNF	rs16917237	0.000	0.987	-0.009	0.639	-0.012	0.463
MTCH2	rs10838738	-0.008	0.591	-0.021	0.212	-0.004	0.765
MTNR1B	rs10830963	0.025	0.106	0.002	0.907	0.012	0.430
UCP2	rs659366	-0.036	**0.010**	-0.015	0.398	-0.029	**0.035**
GNB3	rs5443	0.009	0.527	0.003	0.877	0.017	0.239
PLIN	rs894160	-0.017	0.268	-0.004	0.831	-0.006	0.691
FTO	rs6499640	0.012	0.399	0.005	0.779	0.016	0.252
	rs7206010	0.012	0.420	0.006	0.700	0.016	0.274
	rs9935401	0.021	0.124	0.022	0.187	0.021	0.111
	rs9939609	0.021	0.123	0.024	0.148	0.022	0.103
MAF	rs1424233	-0.027	**0.044**	-0.019	0.243	-0.022	0.091
SH2B1	rs7498665	-0.002	0.871	0.006	0.731	0.006	0.669
MC4R	rs1673482	-0.012	0.392	-0.012	0.464	-0.010	0.473
	rs17700144	0.005	0.733	0.003	0.878	0.004	0.805
	rs17782313	0.004	0.764	0.006	0.732	0.007	0.649
	rs502933	-0.009	0.499	-0.012	0.477	-0.008	0.581
NPC1	rs1805081	-0.017	0.230	-0.005	0.782	-0.021	0.119
KCTD15	rs11084753	-0.015	0.336	0.006	0.769	-0.018	0.257
	rs29941	-0.008	0.587	-0.005	0.809	-0.016	0.283
HTR2C	rs6318*	0.073	**0.005**	0.076	**0.012**	0.072	**0.005**

Beta estimates and p-values are shown; an additive genetic model was assumed; adjustment for age, sex, height, and baseline weight was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls; log=logarithmized

In the age- and sex-adjusted model (**Appendix W**) three loci showed an association with delta weight with a p-value of at least 0.008: *SFRS10 ETV5 DGKG* (beta=0.105, CI: 0.033, 0.177, p=0.004 for lower delta weight after six weeks), *ADRB2* (beta=-0.061, CI: -0.106, -0.016, p=0.008 for higher delta weight after four weeks), and *HTR2C* (beta=0.146, CI: 0.063, 0.228, p=0.0006 for lower delta weight after four weeks; beta=0.139, CI: 0.041, 0.237, p=0.006 for lower delta weight after six weeks; beta=0.140, CI: 0.060, 0.219, p=0.0007 for lower delta weight after four or six weeks (adjusted also for duration of stay)). After adjustment for multiple testing, the association between *HTR2C* and delta weight remained significant (p=0.0006, p=0.0007).

In the fully adjusted model (**Table 5-20**) all of these results lost statistical significance after adjustment for multiple testing (*SFRS10 ETV5 DGKG*: beta=0.033, CI: -0.009, 0.076, p=0.125 for delta weight after six weeks; *ADRB2*: beta=-0.014, CI: -0.041, 0.014, p=0.336 for delta weight after four weeks; *HTR2C* beta=0.073, CI: 0.023, 0.123, p=0.005 for delta weight after four weeks; beta=0.076, CI: 0.017, 0.135 p=0.012 for delta weight after six weeks; beta=0.072, CI: 0.022, 0.121, p=0.005 for delta weight after four or six weeks).
All other loci which showed a marginally significant association with delta weight in the fully adjusted model (*NEGR1*, *SEC16B RASAL2*, *INSIG2*, *TMEM18*, *PFKP*, *UCP2*, *MAF*), were also not significant after adjustment for multiple testing (p≤0.001).

In addition a linear mixed effect model was calculated taking into account delta weight (log-transformed) at five or seven different time points. Results from two different adjustment models (age, sex / age, sex, height) are shown in **table 5-21**.

The *SEC16B RASAL2* polymorphism showed a significant association with higher delta weight (beta=-0.061, SE: 0.028, p=0.033, seven time points) in the fully adjusted model, but not in the age- and sex-adjusted model. For SNP rs12654778 (*ADRB2*) an age- and sex-adjusted beta estimate of -0.067 (SE: 0.025, p=0.006) or of -0.071 (SE: 0.026, p=0.005) was observed for delta weight including five or seven time points, respectively. There was no significant result in the fully adjusted model. *UCP2* showed in both adjustment approaches an association with delta weight including five time points (beta=-0.055, SE: 0.025, p=0.026; beta=-0.046, SE: 0.020, p=0.023). The *HTR2C* polymorphism was associated with lower delta weight in both adjustment as well as delta weight approaches with a p-value of at least 0.0002. For the given beta estimates the SE is as following: beta=0.181, SE: 0.044; beta=0.183 SE: 0.047; beta=0.149, SE: 0.038; beta=0.149, SE: 0.039. For the *HTR2C* gene the associations in the mixed effect model remained statistically significant after adjustment for multiple testing.

Table 5-21: Results from mixed effect model concerning delta weight at five (0 to 4 weeks) and seven (0 to 6 weeks) time points

Locus	SNP	Delta weight (log) 0 to 4 weeks (five time points) *adjusted for age and sex*		Delta weight (log) 0 to 6 weeks (seven time points) *adjusted for age and sex*		Delta weight (log) 0 to 4 weeks (five time points) *adjusted for age, sex, height*		Delta weight (log) 0 to 6 weeks (seven time points) *adjusted for age, sex, height*	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	-0.006	0.863	-0.011	0.749	-0.017	0.525	-0.019	0.496
NEGR1	rs2568958	-0.008	0.760	-0.023	0.386	-0.005	0.826	-0.020	0.383
	rs2815752	-0.007	0.793	-0.022	0.409	-0.004	0.848	-0.019	0.396
SDCCAG8	rs10926984	0.037	0.294	0.046	0.211	-0.004	0.897	0.008	0.803
	rs12145833	0.038	0.279	0.047	0.203	-0.002	0.936	0.009	0.777
	rs2783963	0.050	0.167	0.056	0.134	0.003	0.911	0.012	0.702
SEC16B, RASAL2	rs10913469	-0.029	0.381	-0.038	0.262	-0.051	0.062	-0.061	**0.033**
INSIG2	rs11684454	0.016	0.542	0.013	0.627	0.006	0.784	0.003	0.880
TMEM18	rs7561317	-0.024	0.482	-0.030	0.384	-0.031	0.255	-0.037	0.200
ADIPOQ	rs17300539	-0.013	0.750	-0.025	0.571	-0.013	0.713	-0.022	0.543
PPARG	rs1801282	0.054	0.163	0.065	0.107	-0.004	0.892	0.007	0.838
SFRS10, ETV5, DGKG	rs7647305	0.045	0.169	0.042	0.212	0.028	0.291	0.025	0.372
UCP1	rs45539933	0.086	0.103	0.069	0.206	0.032	0.468	0.013	0.779
ADRB2	rs12654778	-0.067	**0.006**	-0.071	**0.005**	-0.035	0.091	-0.038	0.075
PCSK1	rs12186664	-0.007	0.803	-0.001	0.959	0.007	0.762	0.013	0.596
PRL	rs4145443	0.037	0.140	0.031	0.228	0.015	0.465	0.007	0.727
IL6	rs1554606	-0.034	0.178	-0.042	0.107	-0.001	0.974	-0.009	0.695
TNKS-MSRA	rs13278851	0.027	0.534	0.005	0.916	0.019	0.600	-0.003	0.936
	rs17150703	0.013	0.765	0.002	0.973	0.015	0.675	0.004	0.923
	rs516175	0.070	0.064	0.052	0.181	0.045	0.148	0.028	0.387
TRHR	rs7832552	0.027	0.346	0.030	0.316	0.001	0.958	0.002	0.934
ADRA2A	rs1800544	0.028	0.323	0.039	0.181	0.025	0.270	0.036	0.138
PFKP	rs17132175	-0.044	0.313	-0.051	0.262	-0.041	0.252	-0.048	0.208
PTER	rs10508503	0.023	0.652	0.034	0.529	0.032	0.457	0.044	0.321
BDNF	rs16917237	0.026	0.377	0.026	0.397	0.026	0.283	0.025	0.331
MTCH2	rs10838738	-0.017	0.508	-0.027	0.284	-0.017	0.411	-0.030	0.169
MTNR1B	rs10830963	-0.018	0.518	-0.016	0.574	0.002	0.940	0.004	0.879
UCP2	rs659366	-0.055	**0.026**	-0.047	0.067	-0.046	**0.023**	-0.038	0.075
GNB3	rs5443	0.014	0.583	0.022	0.415	0.016	0.458	0.022	0.327
PLIN	rs894160	-0.028	0.302	-0.032	0.257	-0.042	0.062	-0.044	0.061
FTO	rs6499640	-0.017	0.501	-0.028	0.292	-0.008	0.699	-0.019	0.394
	rs7206010	-0.019	0.462	-0.030	0.248	-0.008	0.699	-0.020	0.362
	rs9935401	0.018	0.444	0.019	0.455	0.030	0.131	0.029	0.168
	rs9939609	0.017	0.487	0.017	0.500	0.028	0.149	0.027	0.192
MAF	rs1424233	0.001	0.964	-0.013	0.604	-0.012	0.529	-0.024	0.239
SH2B1	rs7498665	0.013	0.587	0.007	0.778	0.015	0.454	0.008	0.690
MC4R	rs1673482	-0.015	0.544	-0.034	0.188	-0.016	0.436	-0.032	0.127
	rs17700144	-0.006	0.812	-0.015	0.594	-0.003	0.877	-0.011	0.621
	rs17782313	-0.017	0.497	-0.031	0.242	-0.009	0.679	-0.021	0.332
	rs502933	-0.012	0.612	-0.030	0.233	-0.013	0.536	-0.029	0.177
NPC1	rs1805081	-0.004	0.855	0.001	0.960	-0.009	0.647	-0.003	0.878
KCTD15	rs11084753	-0.035	0.212	-0.033	0.257	-0.036	0.119	-0.033	0.171
	rs29941	-0.022	0.423	-0.008	0.787	-0.022	0.327	-0.010	0.687
HTR2C	rs6318*	0.181	**<.0001**	0.183	**<.0001**	0.149	**<.0001**	0.149	**0.0002**

Beta estimates and p-values are shown; an additive genetic model was assumed; two different adjustment models were calculated (age, sex / age, sex, height); p-values ≤ 0.05 are bold/grey; *) only analyzed in girls; log=logarithmized

Comparison of the results between the linear regression and the linear mixed effect model revealed consistency. The *SEC16B RASAL2*, the *UCP2*, and the *HTR2C* locus showed a significant association with delta weight in both approaches. Minor alleles of the SNPs within *SEC16B* (*RASAL2*) and near *UCP2* gene were associated with greater delta weight and the minor allele of the SNP within *HTR2C* gene was associated with lower delta weight, whereas the *HTR2C* association was also significant after adjustment for multiple testing.

In addition to the single SNP analysis, a cumulative analysis was performed as described in the methods part (**Chapter 4.5.5**). The number of subjects for a specific number of risk alleles (≤6 to ≥14) is shown in **figure 5-4**. The mean/median (s.d./IQR) of delta weight after four and six weeks or four and six weeks together in the different GPS categories is given in **table 5-22**. A graphical illustration is shown in **figure 5-5**. These descriptive results give no hint for a linear association or trend.

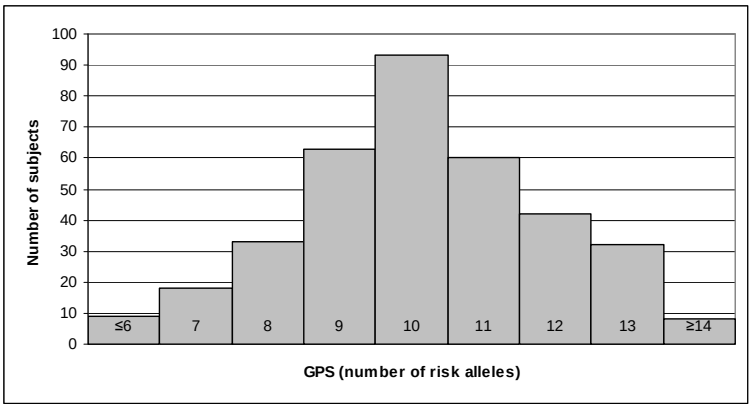


Figure 5-4: Number of subjects in the different GPS categories is shown

Table 5-22: Means (s.d.) and medians (IQR) of delta weight at different time points are shown for the specific GPS categories

Parameter	GPS (number of risk alleles)								
	≤6	7	8	9	10	11	12	13	≥14
	mean (s.d.) median (IQR)								
Delta weight (4 weeks)	-8.57 (2.12) -8.40 (3.19)	-7.73 (1.99) -7.50 (2.90)	-8.14 (2.69) -7.10 (2.90)	-7.59 (2.24) -7.50 (2.70)	-8.09 (2.77) -7.65 (3.00)	-8.59 (3.63) -7.40 (5.40)	-8.55 (3.02) -8.85 (3.85)	-8.64 (3.05) -8.80 (3.80)	-8.33 (2.35) -8.10 (2.10)
Delta weight (6 weeks)	-9.83 (2.04) -10.30 (4.00)	-10.13 (2.76) -10.40 (4.30)	-10.97 (3.89) -10.50 (6.70)	-9.69 (3.18) -9.40 (4.90)	-10.39 (3.57) -9.50 (4.20)	-12.19 (4.54) -11.30 (7.50)	-11.89 (3.21) -12.00 (3.90)	-11.25 (3.36) -11.30 (3.50)	-11.14 (3.89) -11.50 (2.10)
Delta weight (4 or 6 weeks)	-9.15 (1.90) -8.60 (2.80)	-8.78 (2.82) -8.00 (3.30)	-9.91 (3.95) -9.15 (6.10)	-8.65 (3.13) -8.15 (4.10)	-9.40 (3.60) -9.10 (4.10)	-10.13 (4.76) -9.10 (7.60)	-10.15 (4.05) -10.95 (6.35)	-10.00 (3.72) -9.60 (5.70)	-10.11 (3.64) -9.50 (4.60)

The lowest and the highest mean weight at the specific time point is grey

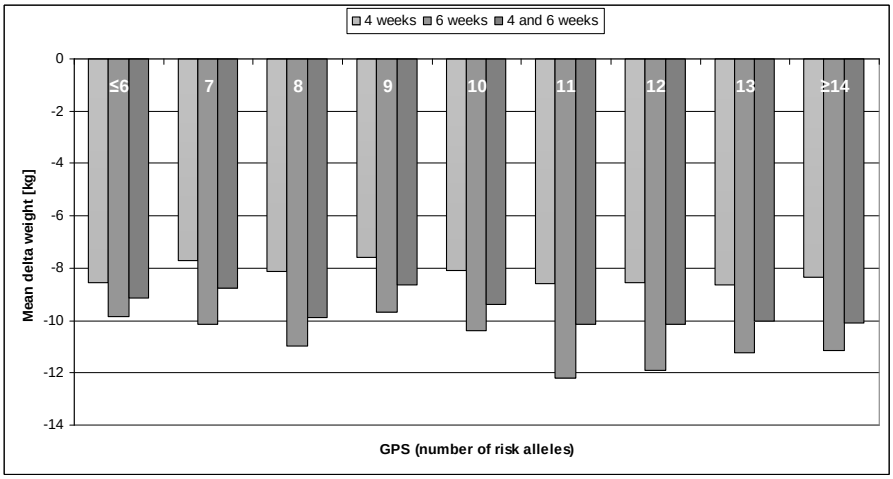


Figure 5-5: Graphical illustration of mean delta weight after four, six and four and six weeks together in the different GPS categories

To test whether there is a statistically significant association between the number of risk alleles (GPS) and delta weight a linear regression with different adjustment approaches was performed. Therefore, delta weight was log-transformed. There were no significant results after adjustment for multiple testing (**Table 5-23**). The association between number of risk alleles and delta weight after six weeks (p=0.021) was abolished after adjustment for height and baseline weight.

Table 5-23: Results from linear regression concerning delta weight after four or six weeks or after four and six weeks together

Parameter	*adjusted for age and sex*		*adjusted for age, sex, height, baseline weight*	
	beta	**p-value**	**beta**	**p-value**
Delta weight (log) (4 weeks)	-0.008	0.398	0.001	0.866
Delta weight (log) (6 weeks)	-0.026	**0.021**	-0.007	0.325
Delta weight (log) (4 or 6 weeks)*	-0.006	0.501	0.002	0.679

Beta estimates and p-values are shown; an additive genetic model was assumed; p-values ≤ 0.05 are bold/grey; *also adjusted for duration of stay; log=logarithmized

5.3.2.2 Results from genetic analyses – delta BMI-SDS

To test whether the genotyped polymorphisms are associated with delta BMI-SDS at various time points, Kruskal-Wallis test, logistic and linear regression models as well as different adjustment models were calculated. Analogue to the outcome delta weight a selection of results from the fully adjusted model were reported in the main text.

In **appendix U** results from the Kruskal-Wallis test (p-values) are shown for delta BMI-SDS after four and six weeks and after four and six weeks together. Concerning delta BMI-SDS there were significant p-values for two loci (*NEGR1*, *PFKP*). After adjustment for multiple testing the p-values for the two *NEGR1* SNPs and an association with delta BMI-SDS after four and six weeks together remained borderline significant (p=0.002).

For logistic regression analysis delta BMI-SDS was dichotomized by the time point-specific median. In **appendix V** the age- and sex- (if necessary duration of stay) adjusted results are shown for delta weight after four or six weeks and after four or six weeks together and are similar to the results from the fully adjusted model (age, sex, height, duration of stay (if necessary), baseline BMI-SDS) shown in **table 5-24**.

For lower BMI-SDS loss four loci showed a significant p-value (*TMEM18*: OR=0.491, CI: 0.254, 0.947, p=0.034; *PFKP*: OR=0.371, CI: 0.560, 0.858, p=0.029; *MAF*: OR=0.652, CI: 0.447, 0.951, p=0.026; *HTR2C*: OR=3.404, CI: 1.301, 8.910, p=0.013). After adjustment for multiple testing none of the results remained statistically significant (**Table 5-24**).

Table 5-24: Results from logistic regression concerning delta BMI-SDS after four or six weeks or after four and six weeks together

Locus	SNP	Delta BMI-SDS (4 weeks)		Delta BMI-SDS (6 weeks)		Delta BMI-SDS (4 or 6 weeks)	
		OR	**p-value**	**OR**	**p-value**	**OR**	**p-value**
LEPR	rs1805134	1.009	0.970	0.978	0.941	1.127	0.643
NEGR1	rs2568958	0.826	0.330	0.787	0.351	0.705	0.103
	rs2815752	0.833	0.349	0.793	0.363	0.704	0.103
SDCCAG8	rs10926984	1.221	0.456	1.042	0.912	1.558	0.126
	rs12145833	1.253	0.398	1.087	0.822	1.580	0.114
	rs2783963	1.279	0.361	1.145	0.720	1.444	0.201
SEC16B, RASAL2	rs10913469	1.004	0.988	1.190	0.582	0.700	0.182
INSIG2	rs11684454	0.726	0.112	0.779	0.329	0.823	0.357
TMEM18	rs7561317	0.785	0.341	0.491	**0.034**	0.772	0.355
ADIPOQ	rs17300539	0.744	0.353	0.735	0.412	1.170	0.637
PPARG	rs1801282	0.788	0.408	0.807	0.577	0.809	0.500
SFRS10, ETV5, DGKG	rs7647305	1.108	0.668	1.707	0.094	1.304	0.305
UCP1	rs45539933	0.944	0.882	0.518	0.187	0.822	0.628
ADRB2	rs12654778	0.972	0.881	1.247	0.374	0.760	0.188
PCSK1	rs12186664	1.134	0.539	0.922	0.769	0.956	0.840
PRL	rs4145443	1.085	0.663	0.972	0.907	1.033	0.873
IL6	rs1554606	0.983	0.929	1.363	0.191	1.069	0.733
TNKS-MSRA	rs13278851	1.005	0.988	0.547	0.139	0.856	0.663
	rs17150703	0.999	0.997	0.501	0.103	0.935	0.851
	rs516175	0.956	0.871	0.669	0.251	0.790	0.442
TRHR	rs7832552	1.057	0.793	0.657	0.138	0.704	0.128
ADRA2A	rs1800544	0.922	0.701	1.267	0.409	0.943	0.797
PFKP	rs17132175	0.704	0.305	0.371	**0.029**	1.101	0.795
PTER	rs10508503	1.025	0.949	0.695	0.502	0.912	0.824
BDNF	rs16917237	1.164	0.483	0.869	0.608	0.845	0.467
MTCH2	rs10838738	1.207	0.342	0.789	0.340	1.078	0.716
MTNR1B	rs10830963	1.098	0.644	0.861	0.563	0.917	0.697
UCP2	rs659366	0.885	0.509	0.965	0.886	0.806	0.294
GNB3	rs5443	1.019	0.922	0.795	0.341	1.154	0.479
PLIN	rs894160	0.996	0.985	1.005	0.984	0.996	0.984
FTO	rs6499640	1.120	0.563	1.148	0.573	1.443	0.083
	rs7206010	1.086	0.677	1.167	0.528	1.442	0.086
	rs9935401	1.035	0.848	1.340	0.229	1.302	0.176
	rs9939609	0.997	0.985	1.301	0.274	1.312	0.163
MAF	rs1424233	0.774	0.151	1.000	1.000	0.652	**0.026**
SH2B1	rs7498665	0.915	0.627	0.907	0.672	0.730	0.122
MC4R	rs1673482	1.126	0.519	0.990	0.966	0.841	0.374
	rs17700144	1.064	0.753	1.043	0.864	0.948	0.793
	rs17782313	1.145	0.482	1.063	0.799	1.006	0.978
	rs502933	1.128	0.508	1.000	1.000	0.843	0.377
NPC1	rs1805081	0.902	0.568	0.729	0.204	0.888	0.545
KCTD15	rs11084753	1.211	0.365	1.080	0.779	0.983	0.939
	rs29941	1.399	0.099	0.964	0.891	1.187	0.436
HTR2C	rs6318*	1.183	0.608	3.404	**0.013**	1.772	0.120

Odds ratios (ORs) and p-values for lower loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age, sex, height, and baseline BMI-SDS was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls

In **appendix X** the age- and sex- (if necessary duration of stay) adjusted results from linear regression are shown for delta BMI-SDS after four or six weeks and after four or six weeks together. In **table 5-26** the results from the fully adjusted model (age, sex, height, duration of stay (if necessary), baseline BMI-SDS) are shown.

Table 5-26: Results from linear regression concerning delta BMI-SDS after four or six weeks or after four and six weeks together

Locus	SNP	Delta BMI-SDS (4 weeks)		Delta BMI-SDS (6 weeks)		Delta BMI-SDS (4 or 6 weeks)	
		beta	p-value	beta	p-value	beta	p-value
LEPR	rs1805134	0.003	0.710	-0.011	0.366	-0.007	0.458
NEGR1	rs2568958	-0.013	**0.049**	-0.010	0.350	-0.014	0.075
	rs2815752	-0.012	**0.052**	-0.010	0.358	-0.014	0.078
SDCCAG8	rs10926984	0.005	0.561	-0.004	0.807	0.007	0.514
	rs12145833	0.006	0.521	-0.003	0.838	0.007	0.486
	rs2783963	0.008	0.377	0.005	0.752	0.011	0.296
SEC16B, RASAL2	rs10913469	-0.010	0.214	-0.021	0.099	-0.015	0.117
INSIG2	rs11684454	-0.006	0.342	-0.017	0.117	-0.008	0.314
TMEM18	rs7561317	-0.010	0.196	-0.021	0.103	-0.014	0.159
ADIPOQ	rs17300539	-0.003	0.779	0.004	0.780	0.007	0.545
PPARG	rs1801282	-0.004	0.681	-0.019	0.248	-0.013	0.261
SFRS10, ETV5, DGKG	rs7647305	0.005	0.538	0.016	0.209	0.011	0.247
UCP1	rs45539933	0.002	0.895	-0.031	0.133	-0.007	0.634
ADRB2	rs12654778	-0.009	0.143	-0.007	0.510	-0.011	0.132
PCSK1	rs12186664	0.009	0.170	-0.006	0.569	-0.003	0.740
PRL	rs4145443	0.007	0.259	0.007	0.441	0.006	0.413
IL6	rs1554606	-0.006	0.319	0.005	0.631	-0.004	0.603
TNKS-MSRA	rs13278851	-0.003	0.772	-0.009	0.565	-0.008	0.547
	rs17150703	-0.003	0.807	-0.011	0.514	-0.007	0.589
	rs516175	0.002	0.839	-0.004	0.801	-0.002	0.875
TRHR	rs7832552	-0.000	0.964	-0.016	0.169	-0.006	0.490
ADRA2A	rs1800544	0.002	0.757	0.008	0.525	0.003	0.708
PFKP	rs17132175	-0.015	0.159	-0.048	**0.006**	-0.023	0.073
PTER	rs10508503	0.009	0.452	0.018	0.426	0.007	0.652
BDNF	rs16917237	0.001	0.897	-0.002	0.878	-0.005	0.531
MTCH2	rs10838738	-0.004	0.504	-0.011	0.284	-0.003	0.740
MTNR1B	rs10830963	0.009	0.161	-0.003	0.784	0.004	0.639
UCP2	rs659366	-0.009	0.138	-0.001	0.934	-0.007	0.327
GNB3	rs5443	0.001	0.832	-0.004	0.710	0.004	0.572
PLIN	rs894160	-0.006	0.339	-0.004	0.734	-0.002	0.793
FTO	rs6499640	0.005	0.389	0.007	0.474	0.010	0.168
	rs7206010	0.005	0.416	0.008	0.412	0.010	0.179
	rs9935401	0.011	0.059	0.019	**0.050**	0.015	**0.039**
	rs9939609	0.010	0.074	0.019	**0.049**	0.014	**0.044**
MAF	rs1424233	-0.016	**0.005**	-0.017	0.077	-0.019	**0.007**
SH2B1	rs7498665	-0.003	0.614	0.001	0.946	0.002	0.773
MC4R	rs1673482	-0.002	0.792	-0.005	0.579	-0.003	0.675
	rs17700144	0.004	0.529	0.004	0.717	0.003	0.655
	rs17782313	0.006	0.369	0.008	0.416	0.007	0.354
	rs502933	-0.001	0.828	-0.006	0.547	-0.002	0.736
NPC1	rs1805081	0.001	0.852	0.004	0.696	-0.002	0.762
KCTD15	rs11084753	-0.005	0.474	0.003	0.812	-0.006	0.425
	rs29941	-0.001	0.924	0.000	0.985	-0.004	0.609
HTR2C	rs6318*	0.029	**0.003**	0.042	**0.006**	0.035	**0.002**

Beta estimates and p-values are shown; an additive genetic model was assumed; adjustment for age, sex, height, and baseline BMI-SDS was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls

In the age- and sex-adjusted model (**Appendix X**) the *PFKP* locus showed an association with higher delta BMI-SDS after six weeks (beta=-0.061, CI: -0.105, -0.018, p=0.006) which lost statistical significance after adjustment for multiple testing. All other loci showed an association with a p-value not very different from 0.05 or no significant association at all.
In the fully adjusted model (**Table 5-26**) the association concerning the *PFKP* locus and higher delta BMI-SDS after six weeks was confirmed (beta=-0.048, CI: -0.081, -0.014, p=0.006). Furthermore, two other loci showed a significant association. Polymorphism rs1424233 (*MAF*) was associated with higher delta BMI-SDS after four weeks (beta=-0.016, CI: -0.027, -0.005, p=0.005) and in the combined analysis of four and six weeks (beta=-0.019, CI: -0.032, -0.005, p=0.007). For *HTR2C* locus an association with lower delta BMI-SDS after four, six, and four and six weeks together was observed (beta=0.029, CI: 0.010, 0.048, p=0.003; beta=0.042, CI: 0.012, 0.071, p=0.006; beta=0.035, CI: 0.013, 0.057, p=0.002), respectively. After adjustment for multiple testing all associations lost statistical significance.

The comparison of the results from the logistic regression to the results from the linear regression showed some consistency because in both approaches three loci (*PFKP*, *MAF*, *HTR2C*) showed an association with delta BMI-SDS – without statistically significance after adjustment for multiple testing. The minor allele was associated with greater BMI-SDS loss in the case of *PFKP* and *MAF* locus and with lower BMI-SDS loss in the case of the *HTR2C* locus.

5.3.3 Summary and comparison of results

In both studies for most of the SNPs the results seem to be rather random. One SNP in each study give little evidence for a more robust association with a certain trait over different time points (*MC4R* in the WW study and *HTR2C* in the LOGIC study).

Comparing the different statistical approaches (e.g. linear, logistic, adjustment models) in each single study cohort the observed association results are in agreement to each other concerning effect direction and size.

Considering only p-values with at least ≤0.009 and delta weight as outcome, there was no SNP which showed an association in both the WW and the LOGIC study, except the *ADRB2* polymorphism which was associated with higher probability for lower weight loss in the LOGIC study (logistic regression) and with higher weight loss in the WW study (mixed effect model).

5.4 Lifestyle factors (Holzapfel C et al. 2010b)

The genotyped polymorphisms were checked by Fisher´s exact test for deviation from HWE. Three SNPs (rs10789336 (*NEGR1*), rs7498665 (*SH2B1*), rs11084753 (*KCTD15*)) violated HWE (p<0.05). One SNP (rs10938397 (*GNPDA2*)) was not genotyped successfully. For all analyzed SNPs, genotyping success rate was 94 percent. **Table 5-27** summarizes the associations between polymorphisms and BMI (model 1).

Table 5-27: Results concerning the SNP-BMI association

Locus	SNP	Minor allele	MAF [%]	Overall			Men			Women		
				N	beta	p-value	N	beta	p-value	N	beta	p-value
NEGR1	rs10789336	G	39	11290	-0.035	0.54	5650	-0.053	0.44	5640	-0.022	0.80
TMEM18	rs6548238	T	17	11687	-0.418	**$1.22x10^{-8}$**	5856	-0.350	$1.03x10^{-4}$	5831	-0.475	**$3.30x10^{-5}$**
MTCH2	rs10838738	G	33	11771	-0.064	0.27	5916	-0.111	0.12	5855	-0.015	0.87
FTO	rs9935401	A	41	11701	0.290	**$2.85x10^{-7}$**	5875	0.206	**$2.82x10^{-3}$**	5826	0.364	**$4.08x10^{-5}$**
MC4R	rs17700144	A	23	11693	0.101	0.13	5863	0.157	0.06	5830	0.067	0.52
SH2B1	rs7498665	G	39	11683	0.145	**$9.83x10^{-3}$**	5851	0.043	0.53	5832	0.236	**$7.89x10^{-3}$**
KCTD15	rs11084753	A	33	11814	0.012	0.83	5922	-0.045	0.52	5892	0.076	0.41

Beta estimates (kg/m²) and p-value from linear regression of SNP on outcome BMI, adjusted for age, sex, and survey are given for overall and gender-specific analyses. An additive genetic model was assumed; p-values ≤ 0.05 are bold/grey; MAF=minor allele frequency

Significant results were detected for rs6548238 near the *TMEM18* gene and rs9935401 within the *FTO* gene. Results were similar for men and women. Polymorphism rs7498665 (*SH2B1*) showed a borderline significant association using a two-sided test. Applying a one-sided test for the direction reported by *Willer C et al.* (Willer CJ et al. 2009), the association reached significance (0.145 kg/m², $p=4.92x10^{-3}$), but was not pronounced in men. None of the other polymorphisms showed a significant association with BMI. Gene-gene interaction tests (*TMEM18* SNP with each other SNP or *FTO* SNP with each other SNP) showed no statistically significant associations with BMI (p-values between 0.03 and 0.93) after adjustment for multiple testing.

Table 5-28: Results concernint the association between lifestyle factors and BMI

Lifestyle factor	**Overall** (N=12297)		**Men** (N=6200)		**Women** (N=6103)	
	beta	p-value	beta	p-value	beta	p-value
High carbohydrate score	-0.422	**$3.19x10^{-7}$**	-0.282	**$5.18x10^{-3}$**	-0.465	**$2.91x10^{-4}$**
High fat score	-0.265	**$1.82x10^{-3}$**	-0.179	0.08	-0.284	**0.04**
High alcohol consumption	-0.477	**$3.19x10^{-7}$**	0.099	0.35	-1.228	**$1.15x10^{-14}$**
Ever smoking	-0.273	**$7.26x10^{-4}$**	0.230	**0.02**	-0.495	**$9.57x10^{-5}$**
High physical activity	-0.861	**$5.08x10^{-28}$**	-0.657	**$6.85x10^{-12}$**	-1.052	**$1.84x10^{-17}$**

Beta estimates (kg/m²) and p-values from linear regression of lifestyle factors on outcome BMI, adjusted for age, sex, survey, and all lifestyle factors are shown for the effects of high (≥ median) versus low (reference) carbohydrate/fat score, high (men: ≥ 40 g/d / women: ≥ 20 g/d) versus low (reference) alcohol consumption, ever versus never smokers (reference), high (scores 1 and 2) versus low (reference) physical activity; p-values ≤ 0.05 are bold/grey

There were significant associations between lifestyle factors and BMI (model 2) both in the "single lifestyle factor model" (p-values from 8.10×10^{-4} to 6.77×10^{-31}; data not shown) and in the "multiple lifestyle factor model" (p-values from 1.82×10^{-3} to 5.08×10^{-28}; **Table 5-28**).

High carbohydrate score, high fat score, high alcohol consumption, smoking, and high physical activity were significantly associated with decreased BMI. High fat score was associated with decreased BMI ("single lifestyle factor model": -0.432 kg/m², $p=1.00 \times 10^{-7}$), but less strongly associated when adjusting for carbohydrate score ("multiple lifestyle factor model": -0.265 kg/m², $p=1.82 \times 10^{-3}$). The association of all investigated lifestyle factors with BMI was stronger among women compared to men. There were significant differences between men and women for the association of fat score (p-value for gender difference=3.76×10^{-4}), alcohol consumption ($p=1.78 \times 10^{-17}$), smoking ($p=2.52 \times 10^{-10}$) and physical activity ($p=4.08 \times 10^{-6}$) with BMI, but not for carbohydrate score (p=0.06).

There was no evidence for association between genetic variants and lifestyle factors (model 3) although polymorphisms rs6548238 (*TMEM18*) as well as rs11084753 (*KCTD15*) showed a trend towards an association with fat intake (**Table 5-29**).

Polymorphism rs9935401 (*FTO*) showed a trend towards an association with smoking and rs10789336 (*NEGR1*) was weakly associated with alcohol consumption (**Table 5-29**). Gene-environment interaction tests showed no significant association with BMI (data not shown). A trend was seen for the interaction rs9935401 and alcohol consumption (-0.411 kg/m², $p=2.64 \times 10^{-3}$). The more complex interaction terms including *TMEM18* SNP, *FTO* SNP and one lifestyle factor showed no significant associations (p>0.05).

Table 5-29: Results concerning the association between SNPs and lifestyle factors

Locus	SNP	Carbohydrate score		Fat score		Alcohol consumption		Smoking behaviour		Physical activity	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Overall											
NEGR1	rs10789336	1.033	0.22	0.999	0.96	0.903	**$1.50x10^{-3}$**	0.979	0.45	0.973	0.31
TMEM18	rs6548238	1.035	0.32	1.081	**0.03**	1.009	0.83	0.951	0.17	1.027	0.45
MTCH2	rs10838738	1.020	0.47	1.018	0.54	0.979	0.53	0.969	0.28	1.036	0.21
FTO	rs9935401	0.989	0.69	1.003	0.91	0.954	0.14	0.936	**0.02**	0.975	0.34
MC4R	rs17700144	1.042	0.19	0.998	0.95	0.985	0.69	1.035	0.29	1.005	0.89
SH2B1	rs7498665	1.020	0.46	0.974	0.35	0.996	0.89	1.031	0.27	0.962	0.16
KCTD15	rs11084753	1.039	0.16	1.066	**0.03**	1.006	0.86	1.016	0.59	0.988	0.67
Men											
NEGR1	rs10789336	1.058	0.13	0.952	0.21	0.933	0.10	1.037	0.37	0.995	0.89
TMEM18	rs6548238	1.065	0.20	1.027	0.61	0.967	0.55	0.945	0.28	0.995	0.93
MTCH2	rs10838738	1.030	0.45	1.067	0.11	0.911	**0.03**	0.937	0.12	1.050	0.22
FTO	rs9935401	1.016	0.68	1.020	0.62	0.961	0.35	0.934	0.09	0.953	0.21
MC4R	rs17700144	1.031	0.50	0.969	0.51	0.938	0.21	1.036	0.47	1.006	0.90
SH2B1	rs7498665	1.046	0.24	0.965	0.36	1.008	0.85	1.047	0.25	0.980	0.60
KCTD15	rs11084753	1.047	0.23	1.089	**0.03**	0.994	0.89	1.001	0.97	1.005	0.89
Women											
NEGR1	rs10789336	1.038	0.32	1.049	0.23	0.867	**$3.85x10^{-3}$**	0.932	0.07	0.953	0.21
TMEM18	rs6548238	1.041	0.41	1.137	**0.01**	1.062	0.34	0.941	0.24	1.059	0.25
MTCH2	rs10838738	1.020	0.61	0.971	0.48	1.067	0.20	0.992	0.83	1.018	0.65
FTO	rs9935401	0.966	0.36	0.988	0.77	0.943	0.24	0.941	0.12	0.995	0.90
MC4R	rs17700144	1.023	0.61	1.025	0.59	1.043	0.46	1.019	0.69	1.003	0.94
SH2B1	rs7498665	1.034	0.38	0.987	0.75	0.982	0.71	1.028	0.48	0.944	0.13
KCTD15	rs11084753	0.996	0.93	1.041	0.34	1.026	0.61	1.031	0.46	0.971	0.46

Odds ratios (ORs) and p-values from logistic regression are shown. An additive genetic model was assumed; lifestyle factors were dichotomized with higher versus lower (reference) scores for carbohydrate and fat intake, alcohol consumption, and physical activity, with smoking versus never smoking (reference); adjustment for age, sex, and survey was done; p-values ≤ 0.05 are bold/grey

Results presenting lifestyle factors as covariates in the genotype-outcome (BMI) association model (model 4) are shown in **table 5-30**.

Table 5-30: Results from linear regression concerning an association between SNP and BMI

Locus	SNP	beta	p-value	beta	p-value	beta	p-value	beta	p-value	beta	p-value	beta	p-value
NEGR1	rs10789336	-0.033	0.56	-0.038	0.50	-0.042	0.45	-0.035	0.53	-0.040	0.48	-0.049	0.38
TMEM18	rs6548238	-0.414	**$1.63x10^{-8}$**	-0.410	**$2.15x10^{-8}$**	-0.417	**$1.33x10^{-8}$**	-0.421	**$9.57x10^{-9}$**	-0.412	**$1.66x10^{-8}$**	-0.407	**$2.29x10^{-8}$**
MTCH2	rs10838738	-0.068	0.25	-0.068	0.24	-0.066	0.26	-0.067	0.25	-0.057	0.33	-0.063	0.27
FTO	rs9935401	0.290	**$2.65x10^{-7}$**	0.292	**$2.25x10^{-7}$**	0.289	**$3.05x10^{-7}$**	0.286	**$4.03x10^{-7}$**	0.287	**$3.10x10^{-7}$**	0.278	**$6.97x10^{-7}$**
MC4R	rs17700144	0.104	0.12	0.099	0.14	0.101	0.13	0.102	0.13	0.101	0.13	0.107	0.11
SH2B1	rs7498665	0.144	**0.01**	0.139	**0.01**	0.141	**0.01**	0.147	**$9.10x10^{-3}$**	0.135	**0.02**	0.133	**0.02**
KCTD15	rs11084753	0.018	0.76	0.020	0.74	0.012	0.83	0.013	0.83	0.007	0.90	0.020	0.72
Lifestyle factor		**Carbohydrate score**		**Fat score**		**Alcohol consumption**		**Smoking behaviour**		**Physical activity**		**All lifestyle factors**	

Beta estimates (kg/m²) and p-values are shown; an additive genetic model was assumed; adjustment for age, sex, survey, and one or all of the lifestyle factor was done; p-values ≤ 0.05 are bold/grey

Adjustment for carbohydrate score, fat score, or alcohol consumption did not change the associations between genotype and BMI. Including the covariate smoking slightly lowered the p-value for the association of rs6548238 (*TMEM18*) on BMI. Gender-specific analysis provided similar results (data not shown).

6 Discussion

6.1 Genotyping

Due to cost/performance ratio Sequenom increased plexing possibilities to 40 SNPs. The here investigated SNPs have been genotyped in very high plexes (23-plex, 33-plex, 35-plex, 37-plex) with some other SNPs not analyzed in this work. That could be one reason why some SNPs failed genotyping or have not fulfilled the genotyping quality (not all data shown). The peak intensity depends on masses and decreases with the power of three. The laser energy decreases for low masses and increases for high masses which influences the detection of the heterozygous genotype. Nevertheless, the here analyzed SNPs fulfilled all quality controls.

For most of the genetic loci one SNP – the best described SNP in the literature or a proxy SNP – was investigated. For six loci (*NEGR1*, *SDCCAG8*, *TNKS-MSRA*, *FTO*, *MC4R*, *KCTD15*) at least two SNPs were genotyped. In all cases the SNP selection was not suitable for gene coverage and it cannot be excluded that other SNPs, not in LD with those investigated here, are associated with the outcome. The selection strategy used in this work was focused to genotype as many as possible loci in the WW and LOGIC studies and so one SNP per locus was regarded as sufficient. For the mediator analysis the SNPs were selected according to the publication by *Willer C et al.* (Willer CJ et al. 2009). The advantage of this selection strategy is having information about a lot of different loci which might give a better picture about the general association between genetic factors and loss of anthropometric traits during intervention. Compared to this selection strategy, the gene covering strategy has the intention to get all genetic information within or near one locus. It has to be considered that up to now, this approach is only an attempt to cover most or all of the genetic information of one locus because some genetic information (e.g. rare variants) are currently missed. Due to new technologies sequencing the whole genome the gaps will be completely closed and full gene coverage might be possible.

Polymorphisms of four loci (*ADIPOQ*, *UCP1*, *PFKP*, *PTER*) in the WW study and of five loci (*ADIPOQ*, *UCP1*, *TNKS-MSRA*, *PFKP*, *PTER*) in the LOGIC study have a MAF lower than ten percent which leads to a very small number or either to no subjects (WW study: *PTER*; LOGIC study: *UCP1*, *PTER*) homozygous for the minor allele. This may result in a non representative group for the seldom homozygous genotype and in false positive findings.

6.2 Association with anthropometric traits in the Weight Watchers (WW) study

6.2.1 Delta weight in the two intervention groups (Jebb S et al., in preparation)

After twelve months weight loss treatment the WW group achieved greater weight loss than the GP group. All analysis strategies confirmed these results. These findings are discussed in the enclosed abstract (**Appendix I**).

6.2.2 Genetic analyses concerning delta weight, fat mass and waist circumference

The data underscore that participation in a weight loss intervention programme leads to weight, fat mass and waist circumference loss, whereas there was a considerable inter individual variation in weight loss. The investigated genetic factors have a minor contribution to BMI in the general population or to weight loss in intervention studies. The findings in the WW study give no evidence for an association between the investigated genetic loci and delta weight, fat mass or waist circumference after adjustment for multiple testing, whereas there is a borderline significant finding for *ADRB2* and *MC4R* (p=0.002). Multiple testing is a major problem in association studies, bearing mischief of false positive associations by chance when performing a large amount of tests. However, corrected significance levels could also lead to false negative or positive associations. After adjustment for multiple testing p-values ≤ 0.002 were regarded as statistically significant. In the WW study none of the observed p-values was notably low. Therefore, all association results with a p-value ≤ 0.05 should be observed as trends.

Considering p-values ≤ 0.009 there were some loci showing an association with the main outcome delta weight in the fully adjusted model: four (*SFRS10 ETV5 DGKG*, *ADRB2*, *PTER*, *MC4R*) or three (*NEGR1*, *SFRS10 ETV5 DGKG*, *MC4R*) loci in the logistic regression analysis with dichotomization of delta weight according to median or percent weight loss, respectively, and two loci (*NEGR1*, *MC4R*) in the linear regression model as well as in the linear mixed model. The *HTR2C* polymorphism showed an association in the sex- and age-adjusted model, but not in the fully adjusted model.

SFRS10 ETV5 DGKG

In the WW study the minor allele T of polymorphism rs7647305 near *SFRS10 ETV5 DGKG* showed an association with a higher probability for lower weight loss in both logistic regression approaches, whereas the association was not consistent concerning the time point of weight loss measurement. This finding could not be confirmed in both linear regression analyses, but the beta estimates go into the same direction as the ORs. The latest one was confirmed by the Kruskal-Wallis test showing no difference in weight loss across genotypes. There were no associations between the *SFRS10 ETV5 DGKG* locus and

delta fat mass or waist circumference. In the published genome-wide association study the major allele C of the here investigated SNP was associated with higher BMI and obesity risk in Caucasians (Thorleifsson G et al. 2009). Also in children the major allele at rs7647305 was associated with greater BMI-SDS (Elks CE et al. 2010). In a Japanese and Chinese population the *SFRS10 ETV5 DGKG* locus was not or marginally associated with obesity (Cheung CY et al. 2010; Hotta K et al. 2009; Ng MC et al. 2010).

If risk allele C carriers have a higher BMI – as shown in the literature, it is on the one hand unexpected that the non risk allele carriers tend to lose less weight in the WW study. On the other hand one could argue that persons with the risk genotype are predicted to gain weight if specific environmental factors exist and these persons could be considered as "responders". Regarding the weight loss programme as specific environment the formerly risk allele carriers are the "responders" and lose more weight. The results support that genetically predisposed obese individuals are responsive to weight loss promoting exposures. A similar phenomenon was seen in the unpublished meta-analysis concerning the *FTO* locus in which physical activity attenuates the effect of the genetic variant on BMI (unpublished data, Kilpelainen T et al., MRC Cambridge). This let assume that environmental factors may overcome genetic factors.

All analyses were adjusted for initial weight, fat mass, or waist circumference, respectively, in order to consider the baseline value as a confounder.

The investigated SNP rs7647305 is located about 7.4 kilobases (kb) upstream of the *ETV5* gene. ETV5 is a transcription factor that is widely expressed, predominantly in brain and placenta, and to a lesser degree in lung, pancreas, and heart (Monte D et al. 1994). The SNP is also located about 30.7 kb downstream of the *DGKG* gene which is expressed in brain and the retina (Goto K et al. 1994; Kai M et al. 1994). Furthermore, rs7647305 is located about 178.5 kb upstream of *SFRS10* gene for which expression in brain is reported (Nayler O et al. 1998). Due to the fact that energy homeostasis is regulated via the hypothalamus (Schwartz MW et al. 2000; Schwartz MW and Porte D, Jr. 2005) and the *SFRS10*, the *ETV5* and the *DGKG* gene are expressed in the brain, an association between this locus and weight loss is suggested but not investigated so far. Based on the given p-values in the WW study it should be concluded that there is no association between the *SFRS10 ETV5 DGKG* locus and delta weight, fat mass and waist circumference, whereas there are some hints – mentioned above – which should be followed in larger study samples or in meta-analyses.

ADRB2

Patients carrying the minor allele A had a higher risk for lower weight loss after two months than carriers of the major allele. This was seen in the logistic regression approach with dichotomization according to the median. All other statistical models gave no evidence for an association after adjustment for multiple testing. There was also no association with delta fat mass and waist circumference. These negative findings were also confirmed by the Kruskal-Wallis test. The here investigated SNP rs12654778 is in LD with the SNP rs1042713 (Arg16Gly, r^2=0.9). The rs1042713 mutant allele seems to alter ADRB2 function by changing the amino acid sequence at the 16th amino acid position of ADRB2 protein where the amino acid glycine is replaced with the amino acid arginine.

Regarding the fact that the logistic regression in which information gets lost because of dichotomization is not so meaningful as the linear regression and that the p-value is borderline (p=0.002), the likely negative finding is in line with the results from *Ruiz JR et al.* (Ruiz JR et al. 2010b). A descriptive association between carriers of the Glu allele (rs1042714) and a greater reduction in body weight was observed, but not for Arg16Gly. In both studies a false negative finding could not be excluded due to sample size.

Two studies investigating further weight gain of obese subjects or weight regain after weight reduction, respectively, observed that persons with weight gain had a higher frequency of the Gly16 allele (Kawaguchi H et al. 2006; Masuo K et al. 2005), whereas *Pereira AC et al.* reported an association between the Arg16 allele and an increased risk of obesity (Pereira AC et al. 2003). In a recent study it was observed that the Arg16Gly variant is a genetic modifier of DASH diet responsiveness. It was associated with greater blood pressure reduction to the DASH diet (Sun B et al. 2010).

Considering the function of ADRB2 receptor in lipid mobilization, the reported association with obesity (Bengtsson K et al. 2001; Pereira AC et al. 2003) might be due to reduced lipolysis (Arner P 2001). Nevertheless, the results concerning an association between Arg16Gly and obesity are inconsistent. There are some bigger studies with much more power which show no association between the *ADRB2* locus and obesity (Gjesing AP et al. 2007; Gjesing AP et al. 2009; Haworth CM et al. 2008; Jalba MS et al. 2008).

It should be concluded that there is no association between *ADRB2* and weight loss in the WW study and replication in a larger study population and meta-analysis is required.

PTER

The minor allele T of polymorphism rs10508503 near *PTER* was associated with lower delta weight in both logistic regression approaches and there was a borderline association with lower weight loss after two and six months in the linear regression analysis. Concerning delta fat mass and waist circumference no association was observed. Furthermore, the Kruskal-Wallis test showed no differences except for delta weight after six months (p=0.033). In the WW study no persons were homozygous for the minor allele. There are no data in the literature concerning this locus and weight reduction, therefore a direct comparison to published data is not possible. The recent genome-wide association study by *Meyre D et al.* showed an association for the major allele C of rs10508503 and obesity (Meyre D et al. 2009), whereas the other two genome-wide association studies – also published in 2009 – did not identify *PTER* as an obesity locus (Thorleifsson G et al. 2009; Willer CJ et al. 2009). In the Auckland Birthweight Collaborative study the major allele C of rs10508503 near *PTER* was associated with being small for gestational age (SGA), whereas the association lost significance after adjustment for multiple testing (Morgan AR et al. 2010). *PTER* is ubiquitously expressed with the highest expression in CD34+ bone marrow, B lymphoblasts and kidney (Meyre D et al. 2009). The investigated polymorphism is also located near *C10orf97* gene. C10ORF97 is a member of the caspase-associated recruitment domain (CARD) family of proteins having a role in apoptosis (Liu B et al. 2002). C10ORF97 seems to be ubiquitously expressed, more strongly in the brain, in particular in the hypothalamus (Meyre D et al. 2009).

Due to the limited and also inconsistent data about *PTER* and the not low p-values in the WW study, it should be concluded that the associations in the WW study are false positive.

MC4R

In all statistical analyses (logistic, linear, and linear mixed model) at least one out of the four investigated *MC4R* SNPs showed an association with delta weight which was confirmed by the Kruskal-Wallis test. Furthermore, there was an association with delta fat mass and a borderline association with delta waist circumference, both in the logistic regression model. The linear regression model showed no association at all with delta fat mass or waist circumference. All given ORs and beta estimates with a p-value ≤ 0.009 suggest that the minor alleles are associated with greater weight loss. The four SNPs correspond to two LD blocks (rs1673482/rs502933 and rs17700144/rs17782313).

In a genome-wide association study the C allele of rs502933 (in LD with rs1673482) and the G allele of rs477181 (in LD with rs1673482) were associated with higher waist circumference (Chambers JC et al. 2008). The minor allele of rs1673482 was associated with greater weight loss (p<0.009) in the WW study, whereas the polymorphism rs502933 was marginally

associated (p<0.05). The significance was not consistent over all time points and statistical approaches and only the association with delta weight after twelve months (rs1673482, p=0.002, BCF analysis) remained marginally significant after adjustment for multiple testing.
In the second LD block (rs17700144/rs17782313) the minor allele C of rs17782313 was associated with higher BMI in genome-wide association studies (Loos RJ et al. 2008; Meyre D et al. 2009; Willer CJ et al. 2009) and the minor allele A of rs17700144 was associated with extreme obesity in children and adolescents (Scherag A et al. 2010). In the WW study the minor allele A of rs17700144 was associated with greater weight loss, whereas the association was not consistent over all time points and statistical approaches and lost statistical significance after adjustment for multiple testing. There were similar results for delta fat mass and no significant association for delta waist circumference. The other polymorphism in this LD block gave a similar picture, whereas one association remained marginally significant after adjustment for multiple testing (delta weight after twelve months, rs17782313, p=0.002, BCF analysis).
It seems that also for the *MC4R* locus the "responder hypothesis" might be true. Carriers of the risk alleles tended to lose more weight in the WW study, except for rs502933 and rs1673482 (proxy SNP to rs477181), for which the major alleles predicted higher BMI and the minor alleles greater weight loss. Due to the inconsistency in the WW study and the marginally significant association after adjustment for multiple testing the findings give only a hint and should not be overestimated.
There are many studies showing a strong association between *MC4R* polymorphisms and obesity and obesity-related traits (Meyre D et al. 2009; Scherag A et al. 2010; Thorleifsson G et al. 2009; Willer CJ et al. 2009; Zobel DP et al. 2009). A few studies with small sample size investigated an association between the *MC4R* locus and weight loss which found no significant associations (**Table 6-1**).

Table 6-1: Studies investigating *MC4R* locus and weight loss

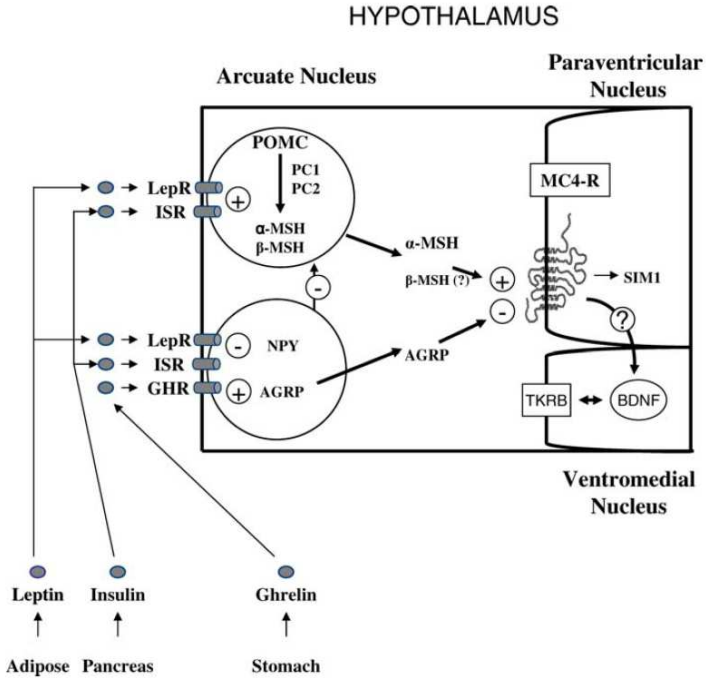
Study design	Sample	Polymorphism	Results	Reference
"TULIP" study; lifestyle intervention	242 adults	rs17782313	no association with changes in body weight or fat distribution after lifestyle intervention	(Haupt A et al. 2009a)
"Obeldicks" study; lifestyle intervention	9 children with mutations; 46 without mutations	*MC4R* mutations leading to reduced receptor function	no difference in weight loss between groups; weight loss maintenance failed in children with mutations	(Reinehr T et al. 2009a)
"SOS" study; bariatric surgery	1,443 adults	17 polymorphisms	no association with weight loss or weight regain	(Sarzynski MA et al. 2010)
lifestyle intervention	538 adults	rs17782313 rs12970134	no association between *MC4R* and the percentage of BMI change	(Cha S et al. 2009)

TULIP=Tübingen Lifestyle Intervention Program; SOS=Swedish obese subjects intervention study

However, some studies suggesting that *MC4R* polymorphisms are associated with dietary intake (Heid IM et al. 2008; Pichler M et al. 2008; Qi L et al. 2008; Stutzmann F et al. 2009) and a trend for an association between *MC4R* SNPs and intake of energy from whole grains was seen (Hasselbalch AL et al. 2010). Furthermore, the *MC4R* gene may affect eating behaviour (Valladares M et al. 2010), whereas no association was found with dietary energy intake (Tenesa A et al. 2009). Linkage findings also mapped carbohydrate intake and physical activity to the region on chromosome 18 containing the *MC4R* gene (Cai G et al. 2006).

The MC4R is a G protein coupled receptor which is expressed in the hypothalamus and plays, as part of the melanocortinergic pathway, a crucial role in energy homeostasis (**Figure 6-1**). Neurons which lie in the ARC of the hypothalamus express either POMC or both AgRP and NPY. POMC-derived alpha- and beta-melanocyte sitmulating hormones (MSH) are potent agonists of MC4R, whereas AgRP is an antagonist. Energy intake and fat storage lead to an increase in leptin which stimulates POMC neurons and inhibits AgRP neurons. Thereby MC4R signalling increases and food intake is suppressed. A negative energy balance decreases leptin and thereby POMC-expressing neurons are inactivated and AgRP expression is stimulated. MC4R signalling decreases and food intake is stimulated (O'Rahilly S et al. 2004).

Figure 6-1: Schematic view over hypothalamic sensing to energy-related signals with MC4R as a key regulator. Signals from peripheral tissues are fundamental to the regulation of energy homeostasis. POMC neurons in the ARC are activiated by leptin and insulin and produce alpha-MSH which activates the MC4R receptor. Activation of MC4R results in satiety signalling. NPY and AgRP act as inhibitors of MC4R signalling.



AGRP = agouti-related protein; alpha-MSH = melanocyte sitmulating hormone; BDNF = brain-derived neurotrophic factor; GHR = ghrelin receptor; ISR = insulin receptor; LepR = leptin receptor; NPY = neuropeptide Y; PC1 and 2 = proconvertase 1 and 2; POMC = proopiomelanocortin; SIM1 = single-minded homolog 1; TRKB = tyrosine kinase receptor; (Mutch DM and Clement K 2006)

The *MC4R* gene represents a compelling biological candidate for an effect on body weight, as the MC4R receptor plays an important role in energy homeostasis and rare mutations in the gene are the leading cause of monogenic obesity in humans (Farooqi IS et al. 2003), and similar phenotypes are seen in murine models of Mc4r disruption (Huszar D et al. 1997).
Taken together, the *MC4R* gene is due to its biological background a plausible candidate gene for obesity and this association is shown in many studies. Concerning all other phenotypes like energy intake investigated so far the results are inconsistent. For the outcome weight loss no significant association is reported up to now.

NEGR1

The minor alleles G and C of *NEGR1* SNPs rs2568958 and rs2815752, respectively, were associated with higher percent weight loss in the logistic regression model and with greater weight loss after twelve months in the linear regression approaches. There was a borderline association with delta fat mass in the logistic regression and no association at all with delta waist circumference. This is the first study concerning this locus and weight reduction, therefore a direct comparison to published data is not possible. The recent genome-wide association studies have identified both polymorphisms as genetic variants for BMI by finding an association between the major alleles and higher BMI (Thorleifsson G et al. 2009; Willer CJ et al. 2009). These findings have not been replicated in Chinese and Japanese studies (Cheung CY et al. 2010; Hotta K et al. 2009; Ng MC et al. 2010) and in a small sample there was no association between the *NEGR1* locus and being SGA (Morgan AR et al. 2010). The *NEGR1* risk allele carriers showed higher BMI in genome-wide association studies as well as lower weight loss in the WW study. This could suggest that persons carrying the risk allele might be predisposed to gain weight, but not to lose weight.
The NEGR1 protein is a member of the IgLON family of cell adhesion molecules and plays a role in the development of the CNS. In rats *Negr1* is primarily expressed in the brain (Funatsu N et al. 1999). The involvement in neural development may suggest an influence on the development and function of brain regions having a role in the regulation of eating.

Other genes

All other investigated loci showed no association ($p>0.009$) with delta weight, fat mass or waist circumference despite they were biological candidate genes or BMI-related genetic loci as illustrated in the introduction part (**Chapter 1.2**). For most of these loci no data concerning weight loss are available. These negative findings are in line with the publication by *Sorensen TIA et al.* in which 26 candidate genes showed no significant association with ten-week weight loss. The authors concluded that obesity-related genes like *PCSK1*, *UCP2*, *PPARG2*, *ADIPOQ*, *IL-6*, *TNFalpha* play a minor role, if any, in modulating weight changes induced by a moderate hypo-energetic diet (Sorensen TI et al. 2006).

FTO

None of the four investigated *FTO* polymorphisms representing two LD blocks was associated with delta weight, fat mass or waist cirucumference. Negative results concerning an association between the *FTO* gene and weight loss are rather consistent (**Table 6-2**).

Table 6-2: Studies investigating *FTO* locus and weight loss

Study design	Sample	Polymorphism	Results	Reference
"TULIP" study; lifestyle intervention	204 adults	rs8050136	no association with weight change	(Haupt A et al. 2008)
"SOS" study; bariatric surgery	1,443 adults	rs16945088 and 24 further *FTO* polymorphisms	minor allele carriers of rs16945088 lost less weight	(Sarzynski MA et al. 2010)
lifestyle intervention	538 adults	14 *FTO* SNPs and their haplotypes	association of one SNP (rs7206790) with percentage BMI loss in a subgroup analysis (N=48)	(Cha S et al. 2009)
"Obeldicks" study; lifestyle intervention	280 children	combination of risk alleles of *INSIG2* and *FTO* (rs7566605 and rs9939609)	combination of risk alleles were associated with lowest overweight reduction	(Reinehr T et al. 2009b)
"DPS" study; long-term lifestyle intervention	502 adults	rs9939609	weight reduction was not modified by the *FTO* genotype	(Lappalainen TJ et al. 2009)
"Obeldicks" study; lifestyle intervention	207 children	rs9939609	no association with weight loss	(Mueller TD et al. 2008)
exercise programme	234 women	rs8050136	no genotype by exercise interaction on weight loss; comparable weight loss across the genotypes; exercise at or above the recommendations was associated with greater weight loss in homozygotes for the minor allele	(Mitchell JA et al. 2010)
"NUGENOB" study; dietary intervention	764 adults (with drop outs)	rs9939609	no association with delta weight	(Grau K et al. 2009)
"HERITAGE" Family study; exercise	481 adults	rs8050136	carriers of C allele showed greater fat mass and percent body fat losses than AA homozygotes	(Rankinen T et al. 2010a)
"DPP" study; lifestyle intervention	973 adults	rs9939609	no association with change of anthropometric traits	(Franks PW et al. 2008)
lifestyle intervention	109 women	rs17817449 / rs17818902	no association with change of anthropometric traits	(Dlouha D et al. 2010)

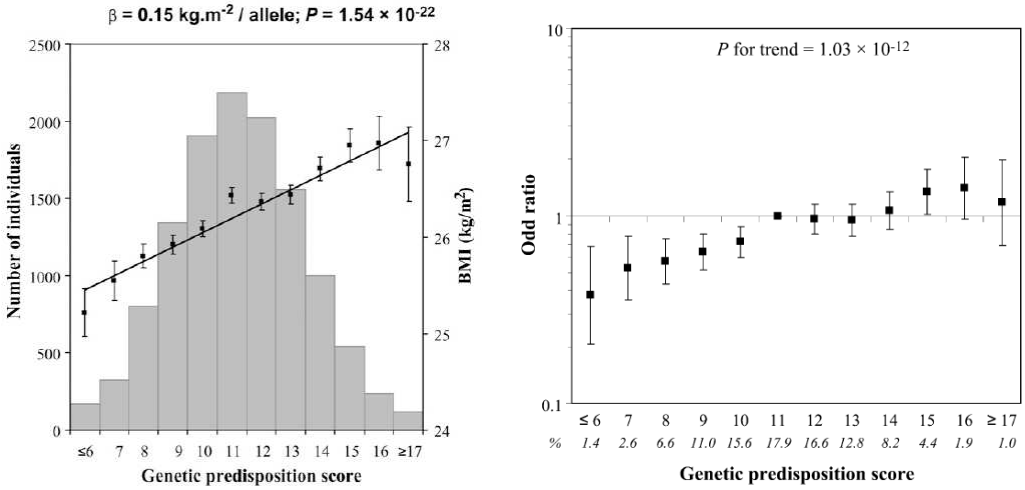
TULIP=Tübingen Lifestyle Intervention Program; DPS=Finnish Diabetes Prevention Study; SOS=Swedish obese subjects intervention study; NUGENOB=Nutrient-gene interaction in human obesity: implication for dietary guidelines; HERITAGE=Health, Risk Factors, Exercise Training, and Genetics; DPP=Diabetes Prevention Program

The *FTO* gene codes for an oxygenase involved in DNA methylation (Gerken T et al. 2007). Recently the crystal structure of the FTO protein was identified (Han Z et al. 2010). RNA expression data showed that *FTO* transcripts containing the risk allele were more abundant suggesting that increased expression of *FTO* is associated with increased body mass (Berulava T and Horsthemke B 2010). In another study *FTO* expression in adipose tissue, but not in blood cells, was greater in obese than in normal weight subjects (Lappalainen T et al. 2010). Overexpression of *Fto* in mice leads to an increase in body and fat mass independent of diet. The overexpression results suggest increased energy intake in these mice (Church C et al. 2010). The *FTO* gene is the strongest genetic risk factor of polygenic obesity identified as yet with body mass increase of about 3 kg for homozygous carriers of the risk allele (Dina C et al. 2007; Frayling TM et al. 2007; Scuteri A et al. 2007; Thorleifsson G et al. 2009; Willer CJ et al. 2009). *Fto* deficient mice show significantly reduced adipose tissue and lean body mass and an increased energy expenditure through increased sympathetic nervous system activity. *FTO* is assumed to have a function in energy homeostasis via control of energy expenditure (Church C et al. 2009; Fischer J et al. 2009).

GPS

It is more and more common to create genetic risk scores in order to calculate a cumulative effect. In the WW study there was no evidence for an association in the GPS analysis including nine SNPs. This is the first study calculating a cumulative effect for weight loss. Thus, a direct comparison to published data is not possible. For BMI as well as obesity risk *Li S et al.* calculated the GPS with twelve obesity loci. The GPS was significantly associated with BMI or obesity risk, whereas not every single SNP was associated with the outcome in the investigated sample size (Li S et al. 2010) (**Figure 6-2**).

Figure 6-2: Genetic predisposition score (GPS)



GPS distribution and cumulative effects (mean and SE are shown) of risk alleles from twelve genetic variants on BMI (left panel); odds ratio (OR) and 95% CI for obesity (BMI ≥ 30 kg/m² vs. 18.5 ≤ BMI < 25 kg/m²) in subjects with different GPS (right panel); (Li S et al. 2010)

Furthermore, a cumulative genetic effect on obesity and BMI was observed in Chinese populations (Cheung CY et al. 2010; Ng MC et al. 2010). In a birth cohort the GPS including eight SNPs was associated with greater weight and BMI as well as with childhood overweight and obesity at age of nine years. The score was positively associated with rate of weight gain between birth and age of eleven years (Elks CE et al. 2010).

6.2.3 Strengths and limitations: Weight Watchers (WW) study

The findings in the WW study provide no evidence concerning an association of genetic variants with changes of anthropometric traits. Considering the dimensions of multiple testing carried out, the few statistically significant results, obtained without any adjustment for multiple testing, should be considered as hints to new hypotheses about genetic effects. Larger studies are required to get sufficient power for the calculation of gene-environment interactions and to find out whether the genetic contribution modulates the success of obesity treatment.

The present study is the first study which has investigated the effect of almost all BMI-related loci recently identified by genome-wide association studies in the context of changes of anthropometric traits during intervention. The analysis was extended by loci from candidate gene studies. Furthermore, this work represents a systematic approach by analysing different anthropometric traits at different time points with different statistical models.

The strength of this study was that beside weight also fat mass and waist circumference as more direct measures of obesity were investigated. Weight measurement was done to the nearest 0.1 kg with standard scales. A weight reduction seen on the scale suggests that the person lost weight, but this could also be due to clothes because weight was measured with clothes, or due to physiologic reasons (e.g. full vs. empty bladder, water storage during menses). The twelve months period of intervention and the weight measurement at six time points led to a representative picture of weight reduction. In the linear mixed effect model delta weight at all time points was included, so the random effects of a person were considered. Furthermore, fat mass was measured with a standard scale with high accuracy, but also for fat mass the circumstances mentioned for weight measurement occurred as potential errors. Waist circumference represents a more difficult picture despite standardized measurement because the error of waist measurement is high (Ulijaszek SJ and Kerr DA 1999).

In the WW study few trends for an association with delta weight, but not with delta fat mass or waist circumference were seen. A similar picture was seen in genome-wide association studies. There was evidence of an association with waist circumference for the transcription factor AP-2 beta (*TFAP2B)* and the *TNKS-MSRA* locus, but only week evidence for an association of these loci with BMI (Lindgren CM et al. 2009). None of the genome-wide

significant BMI-related variants showed strong association with waist, hip or waist-to-hip ratio after adjustment for the effect of BMI or weight (Thorleifsson G et al. 2009). However, the BMI-increasing loci were also associated with increased percentage fat mass (Willer CJ et al. 2009). The *MC4R* locus is associated with both BMI and related traits like waist circumference and fat mass (Chambers JC et al. 2008; Loos RJ et al. 2008). Although the very large meta-analyses suffer from measurement errors of anthropometric traits, especially of waist circumference, it is clearly highlighted in the literature, that some genetic factors are associated with BMI, whereas others are associated with waist circumference. The genetic findings in this work are in line with these results that the investigated genetic factors were not associated with different anthropometric parameters.

The clear inclusion criteria as well as the recruitment basis and randomization procedures may have implications for the generalisability of the results. The fact that the majority of participants were women could be regarded as a limitation, but this is a common problem in weight loss trials. The study participants are likely to represent the population seeking dietary treatment for their obesity problem.

Compared to population-based genetic studies with thousands of subjects and to meta-analyses with even larger sample sizes, the here investigated study cohort is rather small and reached not sufficient power for the chosen candidate gene approach. However, the WW study is rather huge and even larger than others investigating genetic effects on weight loss success (**Tables 6-1** and **6-2**). The underlying study is a clinical intervention study designed for the comparison of two different weight loss programmes with weight loss as primary outcome. For this purpose the study represents a large-scale, long-term randomised controlled trial.

In general, the genetic effects from genome-wide association studies are very small and explain only a small proportion of variance. There is a modest chance to replicate their findings in single studies, especially in smaller intervention studies.

The logistic regression analysis was performed in the completer (only persons with data available for the specific time point were included) as well as in the BCF (missing values were replaced by the baseline values) dataset. In the linear regression-based model delta weight was analyzed only in completers because delta weight neither as original nor as log-transformed variable was normally distributed in the BCF dataset. One could choose the approach to add the smallest delta weight (-0.100 kg) to all persons with no weight loss (delta weight = 0), but this was not preferred in the current analysis.

In the completer analysis the sample size is not so large as in the BCF analysis, but in the completer analysis “true” values were analyzed. Nevertheless, the delta weight in the completer analysis might be not representative for the whole study population because it is

likely that persons who dropped out have no weight loss success. In the BCF dataset it is assumed that the drop out persons had no weight loss which is only an assumption and might underestimate the delta weight.
The variation of results between the completer and BCF analysis might be on the one hand due to false positive results seen at the p-values which lost statistical significance after adjustment for multiple testing and on the other hand due to the different datasets (sample size, overestimation of delta, underestimation of delta). Furthermore, the difference between the datasets might be explained by differences in adherence. The WW completers were subjects who did not drop out and fulfilled all visits during the course of the study. This leads to the question why persons dropped out. On the one hand it could be that persons staying shorter were less motivated and compliant than others and on the other hand that these persons were successfull much earlier than others. The latter point is rather unlikely, but can not be excluded. Therefore, it might be possible that the investigated genes may affect a related outcome such as adherence, rather than be directly associated with weight loss.

One big issue in intervention studies is the compliance of participants. However, the WW programme is well standardized in its structure, but it is not clear how participants fulfil the recommendations in daily life. The GP care was standardized according to “usual care” guidelines, but “usual care” can be handled in different ways. The number of WW meetings and GP appointments was not considered in this work. Although the number of meetings/appointments might give a hint for motivation and compliance, it is not a guarantee for adherence of recommendations in daily life. Another possibility to define compliance could be weight loss success (e.g. weight loss > 2 kg). This is on the one hand a rather risky parameter because not only compliance is an important factor for weight loss success. On the other hand compliance could be a valid parameter for weight loss success because compliance/adherence had a very high impact on weight loss (Alhassan S et al. 2008). Due to the fact that the aim was to investigate whether genetic factors are associated with weight loss, it might be an advantage to include all persons in the statistical analysis.

There are many other factors influencing weight loss success (Holzapfel C and Hauner H, in press) which are partly considered in the present analysis. The intervention group has statistical significant effect on weight loss success (Jebb S et al., **Appendix I**). This was considered by adjustment for intervention group. Also height as an important factor for obesity measurement as well as the baseline value of the analyzed parameter was taken into account. A multivariate analysis with considering as many as possible variables affecting the results (e.g. energy intake, physical activity, adherence, medication) would be preferable. Although such an approach is necessary, it is risky and has to be reconsidered because of

the small dataset. Furthermore, it is not common in population genetic analyses to adjust for factors other than sex and age. Beside the consideration of covariates also interaction analysis would be interesting, but for this the sample size is not suitable.

All association analyses were performed in the whole study population and were not restricted to the Caucasian population because the study characteristics as well as genotyping results of the whole (mixed) population were similar to the Caucasian population alone in the WW study. This was not unexpected because the non-Caucasians displayed only a small minority in the whole WW population. Beside the chosen separation – mixed population vs. Caucasian population – it would be interesting to separate the population into Caucasian and non-Caucasian, whereas the non-Caucasians could be again divided into subgroups (e.g. Asians, Africans), and to meta-analyze the results. Despite the interesting value of such an approach it is risky to stratify the subjects according to ethnical background because the non-Caucasian group is very small (N=76). A replication analysis with another population would be necessary to confirm the results.

In conclusion, the data provide no evidence for genetic factors being associated with delta weight, fat mass and waist circumference, whereas some genetic loci showed a trend for an association. The here investigated genetic factors might have a very small effect on weight loss if any and this effect would have no clinical relevance at all. The results should be considered in the context of the study design, main outcome and especially the study population. In order to get an answer to the question whether genetic factors are associated with weight loss induced in clinical trials replication in a greater sample size as well as in a meta-analysis is required. Nevertheless, there are four main explanations which could be concluded from the results in the WW study:

(I) The observed associations are false positive/negative associations and the study was underpowered to detect a true association.
(II) The effects of genetic factors are undetectable small and can not be found in the present study. Factors other than genetic ones are more important for weight loss success or failure.
(III) A different set of genes contributes to inter-individual differences in lifestyle induced weight loss than those investigated here.
(IV) Genetic factors are not associated with weight loss.

6.3 Association with anthropometric traits in the LOGIC study

6.3.1 Genetic analyses concerning delta weight and BMI-SDS

The data underscore that participation in a standardized in-patient weight loss programme leads to weight and BMI-SDS loss in children, whereas the delta strongly differs from child to child. The findings in the LOGIC study give no evidence for an association between the investigated genetic loci and delta weight or BMI-SDS after adjustment for multiple testing ($p \leq 0.001$). None of the observed p-values was notably low. Therefore, all association results with a p-value ≤ 0.05 should be observed as trends. There is a significant finding between the *HTR2C* locus and delta weight ($p<0.0001$) in the mixed effect model.

Considering p-values ≤ 0.009 there were some loci showing an association with the main outcome delta weight in the fully adjusted model: two loci (*PFKP* and *HTR2C*) in the linear regression analysis and two loci (*ADRB2* and *HTR2C*) in the mixed effect model. The *SFRS10 ETV5 DGKG* polymorphism showed an association in the sex- and age-adjusted model, but not in the fully adjusted model. For delta BMI-SDS the *PFKP*, the *MAF*, and the *HTR2C* locus showed an association ($p \leq 0.009$) in the fully adjusted linear regression. All other genes and the GPS showed no association with weight or BMI-SDS loss.

ADRB2

Children carrying the minor allele A had greater weight loss after four and six weeks than carriers of the major allele. This was seen in the sex- and age-adjusted linear mixed model, but significance was lost after correction for height. There was no association with delta BMI-SDS indicating that height might be a confounding factor. These negative findings were also confirmed by the Kruskal-Wallis test showing no differences between *ADRB2* genotypes and changes of anthropometric traits.

The finding in the LOGIC study tends to be in contrast to the WW study in which the minor allele was associated with lower weight loss**.** Due to small sample size in both studies and the borderline associations, a direct comparison is not possible. Considering the associations as false positives, both studies give no evidence for an association. Further details about function and association studies concerning *ADRB2* were already mentioned in the discussion part of the WW study (**Chapter 6.2.2**). It should be concluded that there is no association between *ADRB2* and weight loss in children and replication in a larger study population and meta-analysis is required.

PFKP

The minor allele C of the investigated *PFKP* SNP rs17132175 was associated with greater weight and BMI-SDS loss after six weeks in the linear regression model. In this analysis one child was homozygous for the minor allele, so this is not representative at all and a false positive association cannot be excluded. After adjustment for multiple testing the significance was lost. This is the first study investigating the *PFKP* locus and weight loss, thus a direct comparison to literature is not possible. The investigated SNP is in LD to the SNP rs6602024 whose minor allele A is associated with higher BMI (Scuteri A et al. 2007). Another study also provided evidence for an association between the *PFKP* locus and obesity (Liu YJ et al. 2008), whereas in a study of 18,014 Danes the replication failed (Andreasen CH et al. 2008a). In a very small study the major allele G of rs6602024 was associated with being SGA, whereas the association lost significance after adjustment for multiple testing (Morgan AR et al. 2010). As already mentioned in the introduction part (**Chapter 1.2**) the enzyme phosphofructokinase is the rate-limiting enzyme in glycolysis, thus the *PFKP* gene could alter the balance between glycolysis and glycogen production. The functional importance of the *PFKP* gene as well as the replication of results awaits further validation studies.

MAF

The minor allele G of rs1424233 (*MAF*) was associated with higher delta BMI-SDS after four weeks and after four or six weeks (combined). All further analyses showed no associations, but the ORs and beta estimates went into the same direction. Knowledge about the *MAF* gene and its protein is very limited, but an association with obesity has been described in the recent genome-wide association study. There, the minor allele A of rs1424233 was associated with obesity (Meyre D et al. 2009). In the LOGIC study “G” was – with a frequency of 47.34 percent – the minor allele which corresponds to HapMap. The risk allele carriers from the study published by *Meyre D et al.* may correspond to the children with lower weight loss in the LOGIC study, whereas this should be considered with caution because in the present study the HWE was violated (p=0.034). Due to the fact that the genome-wide association studies by *Willer C et al.* and *Thorleifsson G et al.* have not identified *MAF* as obesity gene the finding by *Meyre D et al.* needs replication (Meyre D et al. 2009; Thorleifsson G et al. 2009; Willer CJ et al. 2009).

The c-MAF transcription factor is ubiquitously expressed and involved in developmental and cellular differentiation processes, for instance in the immune system (Agnello D et al. 2003), pancreas (Tsuchiya M et al. 2006) and adipose tissue (Serria MS et al. 2003).

HTR2C

The minor allele C of the *HTR2C* SNP rs6318 (Cys23Ser) was associated with lower weight loss after four weeks and four and six weeks (combined) in the linear regression model. This was confirmed by the mixed effect model and the Kruskal-Wallis test. Furthermore, an association with lower BMI-SDS loss in the linear regression model was observed. In the LOGIC study five girls were homozygous for the minor allele. This number was reduced in the sub-group analysis and not representative. False positive associations cannot be excluded. After adjustment for multiple testing a significant association remained between rs6318 and delta weight in the mixed effect model ($p \leq 0.0002$).

Teenage girls losing weight and being underweight had a higher frequency of the Ser allele compared to normal-weight girls (Westberg L et al. 2002). In the population-based European Prospective Investigation into Cancer (EPIC) Norfolk study six *HTR2C* polymorphisms were investigated. The T allele of -759C/T SNP was borderline significantly associated with BMI and risk of major depressive disorder (MDD) in a sub-sample of the EPIC Norfolk study. There was no association with rs6318 and a modest validation of the association between -759C/T SNP and BMI in the whole EPIC Norfolk study. The authors concluded that the *HTR2C* locus is unlikely to have a major effect on obesity in the general population (Vimaleswaran KS et al. 2010). In a further study, the Ser23 (rs6318) was more common in underweight subjects (BMI < 20 kg/m²) than in normal weight or overweight persons (Bah J et al. 2010). Subjects with the heterozygous genotype of the polymorphism C-759T of the *HTR2C* gene lost less weight than homozygous carriers of the risk allele during psychological weight loss treatment (Pooley EC et al. 2004).

The *HTR2C* locus was especially investigated in the context of antipsychotic agent-induced weight gain and the development of behavioural and psychological symptoms. In schizophrenic patients *HTR2C* SNPs were associated with antipsychotic-induced weight gain, but not the SNP rs6318 (Opgen-Rhein C et al. 2010). A meta-analysis investigating an association between *HTR2C* gene (-759C/T) and antipsychotic induced weight gain provide slight evidence for an association (De Luca V et al. 2007). The 23Cys allele was associated with olanzapine-induced weight gain in schizophrenic patients (Ujike H et al. 2008).

In humans, serotonin is a monoamine with many diverse central effects, including the process of satiation. The serotonin system is the primary target of several centrally acting drugs for obesity treatment (for example sibutramine) which increase serotonin-receptor signalling and thereby suppress food intake (Leibowitz SF and Alexander JT 1998). The 5HT2C serotonin-receptor subtype is implicated in this process. The knockout of this receptor increases food intake (Nonogaki K et al. 1998). It seems that energy homeostasis requires an intact serotonin signalling. Compared to the phenotype of mice lacking MC4 or leptin receptors, obesity in the serotonin knockout model is modest.

6.3.2 Strengths and limitations: LOGIC study

The findings in the LOGIC study are similar to the WW study. There are no indications in these results of a major genetic effect on the clinical outcomes delta weight or BMI-SDS. The few statistically significant results, obtained without any adjustment for multiple testing, should be considered as hints for new hypotheses. The significant association of the *HTR2C* locus might be a false positive finding which has to be examined in larger studies.

The present study is the first study which has investigated the effect of almost all BMI-related loci recently identified by genome-wide association studies in the context of changes of anthropometric traits during intervention in overweight and obese children. The analysis was extended by loci from candidate gene studies. Furthermore, this work represents a systematic approach by analyzing different anthropometric traits at different time points with different statistical models.

The strength of this study was that weight measurement was done in underwear to the nearest 0.1 kg with always the same standard scale. The repeated weight measurement (every week) during treatment leads to a representative picture of weight reduction. In the linear mixed effect model delta weight at all time points was included, so the random effects of a child were considered. The short intervention period of four or six weeks is a snap-short and not for generalisation, but the programme is very standardized which leads to a rather homogenous group for genetic analysis. The in-patient performance of the programme excludes environmental influences which differs from child to child and might influence weight loss.

The clear inclusion criteria as well as the recruitment basis may have implications for the generalisability of the results. The fact that the majority of children came from broken families with low social status could be regarded as a limitation, but these children are likely to represent the population seeking in-patient therapy for the obesity problem. Another advantage of such a therapy is the very low drop out rate during the in-patient phase. The dataset with very few missing values provides the possibility to analyze “true” values.

In the LOGIC study sample size and power were lower than in the WW study for which these issues were already discussed (**Chapter 6.2.3**).

The analyses were performed for changes of anthropometric traits after four and six weeks, separately or combined. In the combined analysis (adjusted for duration of stay) the power was higher due to increased sample size. Due to separation of both time points the impact of

genetic factors at different weight loss phases was considered. It could be that genetic factors have a different effect in the early and the late weight loss phase, but the distance of two weeks might be too short to find differences. For example it was shown in children that *FTO* was not associated with BMI in the first three years of life. From age three years onwards, BMI differences between genotypes were seen (Rzehak P et al. 2010). The association between *FTO* or *MC4R* SNPs and BMI-SDS strenghtened during childhood and became weaker during adulthood (Hardy R et al. 2010).

Although in in-patient programmes the compliance is a not big issue, it still exists. However, the weight loss programme was very standardized, but there may be factors which can be hardly detected. Although there was a defined physical activity programme integrated into the therapy, the leisure time physical activity varied from child to child. The same could be argued concerning energy intake. Although the daily energy intake was defined, it is difficult to evaluate whether children really ate the whole portion size. Nevertheless, all children lost weight which makes the dataset rather homogeneous. All other factors influencing weight loss and their consideration in the present work were already discussed in the WW study (**Chapter 6.2.3**). Also in the LOGIC study the sample size was too small for sensitivity analyses.

All association analyses were performed in the whole study population and were not restricted to the Caucasian population, due to the same reasons already mentioned for the WW study (**Chapter 6.2.3**). In the LOGIC study 46 non-Caucasian children were included.

In conclusion, the data provide no evidence for genetic factors being associated with delta weight or BMI-SDS in this in-patient weight loss trial, whereas some genetic loci showed a trend for an association. The results should be considered in the context of the study design, main outcome and especially the study population. To get an answer to the question whether genetic factors are associated with weight loss in children, replication in a greater sample size as well as in a meta-analysis is required. The four main explanations for the results from the WW study discussed in **chapter 6.2.3** seem to be also true for the LOGIC study. It has to be concluded that the genetic factors investigated have a very small effect on weight loss in children, if any, and this effect would have no clinical relevance at all.

6.4 Comparison between WW and LOGIC study

In both studies weight loss was induced by a lifestyle-based intervention programme. Data were available at different time points and the same SNPs were genotyped in both studies.

The comparison of results from the WW and the LOGIC study however leads to a rather heterogeneous picture. Most of the loci showed a trend only in one of the two studies. Due to the non-comparable study designs, this inconsistency is not unexpected (**Table 6-3**). For instance the *ADRB2* locus was significant in both studies, but suggested opposite associations in the WW study compared to the LOGIC study.

Table 6-3: Comparison of study design and analysis in the WW and LOGIC study

Parameter	WW study	LOGIC study
Study population	653 adults mean BMI: 31.40 kg/m² heterogeneous (job, medication, ...)	358 children mean BMI-SDS: 2.74 homogeneous
Intervention	two intervention groups lifestyle intervention (WW programme / “usual care”) handled in daily life max. twelve months	one intervention group lifestyle intervention (physical activity / caloric restriction / psychological therapy) in-patient max. six weeks
Genotyping	identical	
Statistical analysis	identical (as far as possible)	
Outcomes	weight, fat mass, waist circumference	weight, BMI-SDS

The study designs led to different adjustment variables in the WW compared to the LOGIC study which made the results not really comparable. In the WW study sample size was bigger than in the LOGIC study, but the cohort was more heterogeneous. The study participants handled their weight loss programme in daily life which might lead to a different picture concerning compliance, motivation, and realization of recommendations. Furthermore, the social and environmental factors differ from individual to individual. In the LOGIC study the social and environmental factors might not influence the outcome very strong because the therapy was carried out in a hospital. Moreover, medication is not a big issue in children. Compared to the WW study in which patiens were followed for twelve months with a visit distance of two to three months, the LOGIC study had an intervention period of four to six weeks with weekly weight measurements. In the context of the clinical outcome weight loss the WW study was – due to handling in daily life – more representative than the LOGIC study, but the LOGIC study was more standardized due to the in-patient intervention which leads to more homogenous phenotype data for genetic analysis. Despite the heterogenous sample the WW study had more power from the statistical point of view to find a genetic association with weight loss. The advantage in cohorts of children is that genetic variants might affect children more than adults, due to more genetic and less environmental influences.

In general overweight and obese children are a meaningful sample even in the context of genetic association studies. For instance, the *SDCCAG8* locus was associated with obesity in extreme obese children, but has never been identified as obesity locus in genome-wide association studies in adults (Meyre D et al. 2009; Scherag A et al. 2010; Thorleifsson G et al. 2009; Willer CJ et al. 2009). The *TNKS-MSRA* locus is reported to be associated with waist circumference in adults, but with obesity in extreme obese children only (Lindgren CM et al. 2009; Scherag A et al. 2010). Nevertheless, there are studies showing an association in adults as well as in children for example for the *FTO* locus and BMI or obesity (Dina C et al. 2007; Frayling TM et al. 2007). Although associations exist in both adults and children, the effect size could be different. For instance, the *MTNR1B* polymorphism rs10830963 seems to have a greater effect on fasting glucose in overweight and obese children than in adults and this association seems to differ among BMI-SDS categories (Holzapfel C et al. 2010c).

6.5 Mediator analysis concerning lifestyle factors (Holzapfel C et al. 2010b)

The mediator analysis investigated the association of obesity-related genetic factors with BMI in the MONICA/KORA study to explore the association of polymorphisms with lifestyle factors related to nutritional intake or energy expenditure, and whether such lifestyle factors could be mediators of the detected SNP-association with BMI.

Data confirm the findings for *TMEM18*, *FTO* and *SH2B1* with BMI in adults. There was no evidence for a direct SNP-lifestyle association, whereas there was weak evidence for an association of the *TMEM18* SNP with fat intake and of the *FTO* SNP with smoking. There was no evidence that lifestyle factors act as mediators within the SNP-BMI association. This was confirmed in another recently published study in which such a mediator analysis was performed for the *FTO* locus (Hubacek JA et al. 2010).

6.5.1 Genetic risk factors

This is the first study positively replicating *TMEM18* (rs6548238) as a locus for obesity in adults in a homogenous study sample apart from the two initial reports from meta-analyses of genome-wide association studies (Thorleifsson G et al. 2009; Willer CJ et al. 2009). It should be noted, however, that there was an overlap of this sample with the gene discovery analysis (Willer CJ et al. 2009) of 13 percent (N=1,600). An association between *TMEM18* variants and BMI was recently reported in children (N=6,078) (Zhao J et al. 2009). In Dutch females (N=1,700) and Swedish adults (N=3,885), the effect of *TMEM18* gene on obesity risk could not be confirmed which might be due to low power (Bauer F et al. 2009; Renstroem F et al. 2009). Regarding *FTO*, replication studies have substantiated a strong association between the *FTO* gene and BMI (Dina C et al. 2007; Scuteri A et al. 2007). Per minor allele of SNP rs9935401 highly correlated with the leading variant in the gene

discovery study (rs9939609), BMI increased by 0.3 kg/m² and OR for obesity by 17 percent. The association between *SH2B1* (rs7498665) locus could already be replicated in Swedish adults (Renstroem F et al. 2009), but failed replication in other reports (Bauer F et al. 2009; Zhao J et al. 2009), also most likely due to limited power.

An association of the other obesity-related loci reported with BMI by *Willer C et al.* (*NEGR1*, *MTCH2*, *MC4R*, and *KCTD15)* could not be confirmed in this MONICA/KORA sample which could be due to a limited power for the small associations of these variants despite the substantial sample size. It could also be due to violation of HWE for the *NEGR1* and *KCTD15* SNP genotypes which might have derived from these SNPs being within or near copy number variations (CNVs) as already described for *NEGR1* (Willer CJ et al. 2009).

6.5.2 Lifestyle risk factors

The data in this work are in line with a predominant association of lifestyle factors on BMI which was more pronounced in women. The strongest association was found for high versus low physical activity which is similar to previous reports (Hu FB et al. 2003; Meisinger C et al. 2005). The picture for dietary variables is more complex: high carbohydrate intake was associated with decreased BMI. This might point towards a beneficial or anti-obesogenic effect of a high carbohydrate diet. However, this view is disrupted by the lack of association of low fat intake with decreased BMI. A reason for the more difficult pattern of dietary variables could be a high measurement error in these variables: firstly, quantitative assessment of food intake is difficult and – independently of the method used – associated with a high error rate of up to 75 percent (Carroll RJ et al. 1995). Secondly, the intake of healthy foods might often be overestimated and that of fat-containing foods underestimated due to ignoring hidden fats (e.g. in salad dressings). Measurement error could even be differential between obese and non-obese subjects due to a different intentional or unintentional attempt of more obese persons to underreport the amount of food or fat intake (Braam LA et al. 1998; Price GM et al. 1997).

Most notably, high fat intake score was associated with a lower BMI which was to some part confounded by the association between higher carbohydrate intake score and lower BMI. This points towards a close relationship between lifestyle factors and the need to view these as a system rather than studying them separately.

The data in this work are in line with previous studies showing a significantly lower BMI in smokers compared to never smokers (Albanes D et al. 1987; Molarius A et al. 1997) and an inverse relation between alcohol consumption and BMI. Interestingly, the inverse alcohol-BMI relation is only seen in women which might be due to the different selection of alcoholic beverages between men and women (Colditz GA et al. 1991; Williamson DF et al. 1987).

6.5.3 Genetic associations on BMI mediated through lifestyle factors?

There was neither evidence in this sample that lifestyle factors were mediators of the association between genotypes and BMI, nor was there a clear direct association between genotype and lifestyle factors. This could be due to low power – considering the small effects and the potentially high assessment error in lifestyle factors – or due to a real lack of an association.

Beside the deliberate hypothesis of a potential role in the CNS, the physiological role of the genetic variants within obesity-related loci is not clear. For *FTO,* mouse models were developed in order to study their role in physiological systems like energy homeostasis (Church C et al. 2009; Fischer J et al. 2009). For the *TMEM18* gene no functional studies are available and the SH2B protein has a role in leptin signalling (Ren D et al. 2005). For *FTO* and *MC4R* there is diverging evidence from association studies that they have an effect on energy intake and expenditure. A Danish study revealed that low physical exercise might accentuate the effect of the *FTO* gene on body weight (Andreasen CH et al. 2008b). In contrast, in Swedish and Finnish adults there was no significant interaction between physical activity and the *FTO* variant rs9939609 on BMI (Jonsson A et al. 2009). There are findings that genetic variants (*FTO*, *MC4R*) influence dietary intake (Cecil JE et al. 2008; Pichler M et al. 2008; Qi L et al. 2008; Timpson NJ et al. 2008) and satiety (den Hoed M et al. 2009; Wardle J et al. 2008), but there are also studies in which no association between genetic variants near the *MC4R* and energy or dietary intake could be detected (Bauer F et al. 2009; Tenesa A et al. 2009).

It is a great opportunity to investigate human data in a large homogeneous study in the attempt to learn about associations between SNPs and energy intake or expenditure. Up to know only one study addresses the same focus in a substantially smaller sample (N=1,700) (Bauer F et al. 2009). The results from this work underscore that attempts to seek replication for the reported obesity-loci requires a substantial sample size that not even this study with more than 12,000 subjects fulfills completely. The results indicate that the SNP-BMI association cannot easily be pinpointed to lifestyle factors by epidemiological studies.

6.5.4 Strengths and limitations: mediator analysis

It is a strength of this study that a large homogenous population-based and well-phenotyped cohort being representative for the general population of Augsburg was analyzed. Furthermore, the mediator analysis is a systematic approach to examine the potential mediator role of lifestyle factors in the relationship between genetic variants and obesity. Limitations of this data are the missing information of total energy intake as well as the lack of information on absolute carbohydrate and fat intake in gram which may have given a better insight into real dietary habits. Total energy intake adjustment may lead to a more

precise association between nutritional factors and BMI. Despite the large sample of over 12,000 subjects, the limited power and the violation of HWE in three of the investigated polymorphisms also need to be considered as limitations.

6.5.5 Conclusion: mediator analysis

In conclusion, the data provide evidence for genetic (*TMEM18*, *FTO, SH2B1*) and environmental (dietary habits, alcohol consumption, smoking, physical activity) factors being associated with BMI in a large homogenous population-based study. There is great value in attempting to support pathways with epidemiological data, but there were no clear associations of the polymorphisms with lifestyle factors directly nor were lifestyle factors clear mediators of the genetic association with BMI.

6.5.6 Meta-analysis: *FTO* and physical activity

Meanwhile the MONICA/KORA dataset which was analyzed in the mediator analysis was included in a meta-analysis investigating whether physical activity attenuates the effect of the *FTO* gene on obesity. Therefore, data from 34 studies were meta-analyzed including up to 176,834 men and women. The *FTO* rs9939609 variant or any proxy (r^2>0.8) was analyzed using an additive genetic model. Physical activity was treated as a dichotomous variable (sedentary vs. active). The physical activity categorisation was harmonised across cohorts. Each cohort performed interaction analyses by including the physical activityx*FTO* interaction term in the regression model and adjusting for age and sex. The interaction terms (beta estimate, SE) were meta-analyzed using the random effects inverse variance method. The minor A allele of rs9939609 increased BMI and risk of obesity less in the active group (beta=0.31±0.02 kg/m²/per-allele; OR=1.23/per-allele, CI: 1.19-1.26, respectively) than in the inactive group (beta=0.44±0.05 kg/m²/per-allele; OR=1.30/per-allele, CI: 1.25-1.35, respectively). P-value for interaction was 0.006 and 0.019 for BMI and obesity risk, respectively. It was shown that physical activity attenuates the effect of *FTO* on obesity suggesting that increasing physical activity is particularly important for individuals who are genetically predisposed to obesity (unpublished data, Kilpelainen T et al., MRC Cambridge).
Compared to the mediator analysis described in this thesis the meta-analysis found an interaction between physical activity and *FTO* on BMI and obesity risk. These data give hints that very large sample sizes with harmonised measurements of physical activity are necessary to detect a gene-physical activity interaction which cannot be identified with small cohorts. The same might be true for the other lifestyle factors investigated in the mediator analysis.

6.6 “Missing heritability”

Although in this work all genetic factors which can be assumed to be genetic factors for obesity in the general population were analyzed, there was no strongly significant association with weight loss or lifestyle factors, nor were lifestyle factors mediators in the SNP-BMI association. Other factors (e.g. adherence) or other genetic loci might have a greater effect on weight loss and lifestyle factors than the here explored genetic factors.

The discrepancy between the heritability estimated as 70 percent (Stunkard AJ et al. 1990) from twins raised apart as compared to the BMI variance of less than one percent explained by currently known genetic factors (Thorleifsson G et al. 2009; Willer CJ et al. 2009) is subject of intensive debates in genetic science. In the largest meta-analysis of genome-wide association studies new genetic variants with very small effects on BMI were identified but the additional variance explained by them is negligible (Speliotes EK et al. 2010). The remaining “missing heritability” is widely discussed (Gibson G 2010; Manolio TA et al. 2009). Wrong heritability estimates from family data could be one explanation for this discrepancy. Furthermore, the causal variants might not be tagged by the used SNP chips in the current genome-wide association studies or the effect sizes are likely too small for identification. Alternative approaches have been proposed to identify genetic effects that may have a larger contribution to the variation in obesity. These include examining the role of other sources of genetic variation such as rare variants, structural variants (e.g. CNVs), and epigenetic modification. Moreover, intermediate phenotypes closer to biological pathways and complex interactions (gene-gene, gene-environment) will bring more light into “missing heritability”.

Starting genetic association studies the aim was to identify genetic variants which are usable for risk prediction and individual therapy. The last years have shown and teached that the identified genetic variants are more a starting point for future studies in order to find new disease mechanisms and pathways than a good tool for risk prediction (Lango H et al. 2008; Meigs JB et al. 2008). Followed by the “missing heritability” the predictive value of genetic profiling is still limited. This might be due to the facts that (i) not enough variants were identified, (ii) the outcome is only partly heritable, (iii) genetic architecture of disease development is complex and not fully-understood, and (iv) the genetic contribution is very small and “context”-specific (e.g. age). The problematic issues of risk prediction were already addressed (Janssens AC and Van Duijn CM 2008; Janssens AC and Van Duijn CM 2009) and new gene discoveries may not evidently improve the prediction of complex diseases.

Major environmental conditions (“drivers”) predisposing to the development of obesity or to weight loss success are energy intake and expenditure on the background of a genetic predisposition (“modulator”). The promise is to include genetic determinants as well as much as possible “other” predictors (e.g. social, physiological) into a prediction score to develop prevention strategies.

The translation of the knowledge from genetic association studies into benefits for patients requires a lot of additional work, especially functional studies. The complexity of body weight regulation requires a systems biology approach including expression data, epigenetic studies, and other “omics” technologies as well as lifestyle factors (**Figure 6-3**).

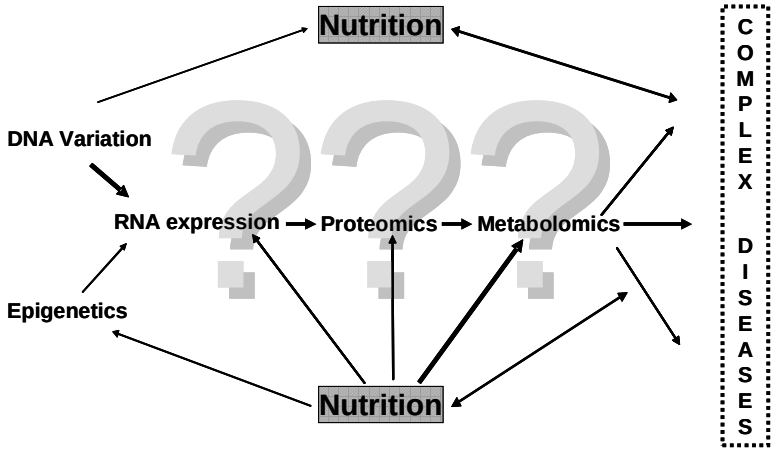


Figure 6-3: Systems biology approach in order to get a better understanding of disease-related pathways

In the case of complex diseases the first approach to combine genotyping and metabolic characterization was made by *Gieger C et al.* (Gieger C et al. 2008). Metabolites are small molecules (e.g. lipids, sugars) which are intermediates and products of metabolism. The identification of genetic variants in genes coding for enzymes where the corresponding metabotype matches biochemical pathways suggests that genetic variants induce differentiations in the metabolic phenotypes. Genetically determined metabotypes may subscribe the reaction to lifestyle intervention. A study by *Illig T et al.* showed that the variance in metabolite concentrations explained by the identified loci is much higher (up to 36 percent) than the variance for complex traits (Illig T et al. 2010). The study of metabolomics might be used to identify baseline characteristics which predict weight loss outcomes. Also gene expression analysis as well as proteomic studies are needed to identify novel regulatory pathways. Epigenetics and gene expression may play a role in response to caloric restriction (Bouchard L et al. 2010; Marquez-Quinones A et al. 2010).

Epigenetics is the study of heritable changes in gene expression that are not caused by changes in the DNA sequence and that provides a plausible link between the environment and alterations in gene expression that might lead to disease phenotypes. *Heijmans BT et al.* showed that individuals who were prenatally exposed to famine during the Dutch Hunger Winter had, six decades later, less DNA methylation of the imprinted insulin-like growth factor 2 (*IGF2*) gene compared with their unexposed, same-sex siblings (Heijmans BT et al. 2008). Furthermore, studies have indicated that certain transient environmental influences can produce persistent changes in epigenetic marks that have life-long phenotypic consequences (Gluckman PD et al. 2009; Gluckman PD and Hanson MA 2008; Zeisel SH 2009). It would be interesting what the effect of weight loss might be on “DNA metabolism” or whether epigenetic factors affect weight loss.

7 Future projects

Association studies on genetic variations within a certain gene locus deliver insight in a small part of genetic susceptibilities. Knowledge about disease physiology is limited and does often not lead to suitable hypotheses providing the chance to find new genetic loci. Genome-wide association studies provide a possibility to investigate most of the genetic information over the whole genome without *a priori* hypotheses. Hence, the offered genome-wide technologies may currently miss some important genetic information. Hopefully, the 1,000 genome project will bring new insights into the genetic field by identification of new sequence variants. In addition larger study samples combined with meta-analyses are needed to detect small effects of gene variation in complex diseases. Furthermore, intermediate phenotypes closer to the molecular basis will show stronger associations by excluding some confounders. Association studies themselves are only the first step on a long way to understand mechanisms which are behind detected associations between gene variants and disease-related parameters. Furthermore, analysis should not only be focused on single gene loci. Gene-gene and gene-environment interactions may play a major role and also "other" phenotypes e.g. social factors should be investigated (Holzapfel C et al. 2010a).
In the following concrete projects considering some aspects mentioned in the discussion part of this work already started or in the planning phase will be described (**Figure 7-1**).

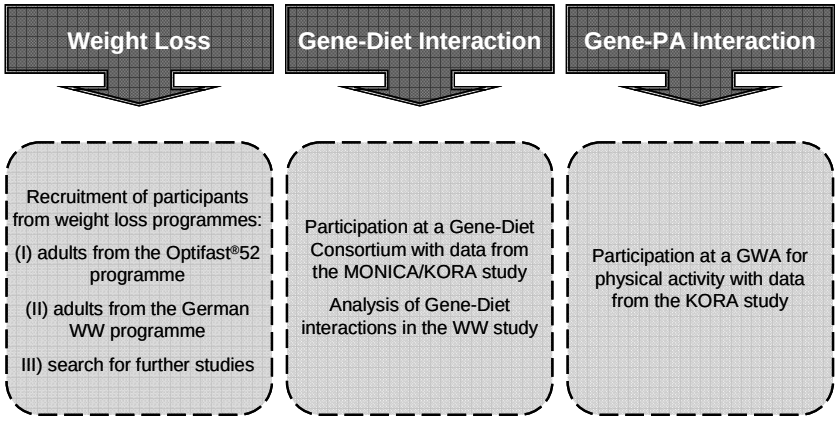


Figure 7-1: Overview about future projects concerning the genetic contribution to weight loss as well as gene-environment interactions; PA=physical activity

Concerning the genetic contribution to weight loss success larger study samples will be recruited in order to have more statistical power, to make a genome-wide scan and to meta-analyze the data. In the context of gene-environment interactions the MONICA/KORA data will be included in two international consortia

(i) investigating the interactions between SNPs and specific types of dietary fat and whether types of dietary fat may mitigate the genetic effect on plasma lipids
(ii) identifying loci for moderate and vigorous physical activity as well as for the time spent sedentary through meta-analyses of genome-wide association studies.

The “MetaboChip” consists of about 180,000 SNPs and represents a fine-mapping of known genetic loci related to complex traits, especially cardio-metabolic traits. These chip data would be a meaningful and cheap approach – compared to genome-wide scan –to repeat the mediator analysis with much more genotype data. Also in the context of weight loss the “MetaboChip” could bring more light into the dark. Furthermore, the offered “Methylation Chip” generates a genome-wide profile of DNA methylation. This would be a promising tool to investigate the association between epigenetic factors and body weight.

The identification and analysis of intermediate phenotypes closer to the pathway steps would lead to stronger associations and to a better insight into disease pathways. Nevertheless, the investigation of “other” phenotypes than complex diseases might give a new picture and some hints for confounding factors. In the context of weight loss adherence could be such an “other” outcome. For an association with social factors as “other” outcome a meta-analysis of genome-wide association data from the KORA study and the “Study of Health in Pomerania” (SHIP) is in progress.

Finally, it is important to perform new studies using best phenotyping strategies in order to find out how participants with a specific genetic risk respond to defined challenges (lipid intake, “westernized meal”, etc.). This is already done in the context of T2DM in the “Virtual Diabetes Institute (VID)” and is planned also for obesity-related genes. Therefore, a new study cohort will be recruited in the region of Freising. For expression analysis, it is worthwhile to collect RNA samples, in particular from fat cells. In the context of body weight it would be great to have genome-wide expression as well as whole transcriptome sequencing data at different time points. For metabolomic phenotyping serum samples should be collected according to highly standardized protocols at different weight loss time points in order to get insight into metabolic changes. This might be very interesting especially in persons with a high amount of weight loss under very standardized conditions (e.g. participants of the Optifast®52 programme).

In Germany a very large population-based cohort – the Helmholtz Cohort – with 200,000 Germans and a follow-up of 20,000 Germans is planned. The feasability study will start by the end of 2010. Participants will be very well phenotyped and a huge biobank will be established. A similar study “UK biobank” recruiting more than 500,000 people is done in England. These cohorts will be the largest ones and will provide an impressive amount of data for research. These cohorts will have power for detailed statistical analysis as well as for identification of new associations. They will help to understand the diseases of the 21st Century from an epidemiological perspective.

Beside these large cohorts also intervention studies with best phenotyping and detailed biosampling are necessary. Bringing all technologies in a “system biology” approach together might elucidate the puzzle from the past decades.

8 Conclusion

In both weight loss studies, the results do not provide evidence to future optimisation of dietary treatment of obesity by tailoring the diet to the individual patients according to genotypes that may predict the outcome of the treatment. The results gave tentative hints that some polymorphisms may modulate weight loss, but this needs to be confirmed and further explored in future studies. However, the observed effects of the investigated SNPs might be moderate and might have a very small contribution if any to the individual variation in weight loss. Nevertheless this is an important result showing that these very small effects might not have clinical relevance. The results should be considered in the context of the study designs, main outcome and especially the study populations. In order to get an answer to the question whether genetic factors are associated with weight loss induced in clinical trials replication in a greater sample size as well as in a meta-analysis is required.

The results from the mediator analysis provide evidence for genetic (*TMEM18*, *FTO, SH2B1*) and environmental (dietary habits, alcohol consumption, smoking, physical activity) factors being associated with BMI in the MONICA/KORA study. There were no associations of the investigated SNPs with lifestyle factors nor were lifestyle factors mediators within the SNP-BMI association. The performed meta-analysis gives evidence that physical activity attenuates the effect of the *FTO* gene on BMI and obesity.

The evaluation of the findings from this work in other intervention settings will provide better evidence on whether genetic information can be used to predict individual responses to specific weight loss programmes. Furthermore, it is important to find out whether genetic factors are associated with lifestyle factors and how lifestyle factors modulate the genetic predisposition. Based on such data in the combination with results from “omics” technologies and animal studies, new strategies for a personalized and more successful treatment of obesity might be developed.

Publications

A Original papers

1. **Holzapfel C**, Klopp N, Grallert H, Huth C, Gieger C, Meisinger C, Strassburger K, Giani G, Wichmann HE, Laumen H, Hauner H, Herder C, Rathmann W, Illig T. *Genetic variants in the leukemia-associated Rho guanine nucleotide exchange factor (ARHGEF12) gene are not associated with T2DM and related parameters in Caucasians (KORA study)*. Eur J Endocrinol 2007 157:R1-5.

2. **Holzapfel C**, Baumert J, Grallert H, Müller AM, Thorand B, Khuseyinova N, Herder C, Meisinger C, Hauner H, Wichmann HE, Koenig W, Illig T, Klopp N. *Genetic variants in the USF1 gene are associated with low-density lipoprotein cholesterol levels and incident type 2 diabetes mellitus in women: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002*. Eur J Endocrinol 2008 159:407-16.

3. **Holzapfel C**, Gedrich K, Karg G, Lang O, Döring A. *Self-assessment of one´s state of health and body mass index: results from the MONICA/KORA project Augsburg.* Ernährungsumschau 2008 10:584-91. Article in German.

4. Singmann P, Baumert J, Herder C, Meisinger C, **Holzapfel C**, Klopp N, Wichmann HE, Klingenspor M, Rathmann W, The KORA Group, Illig T, Grallert H. *Gene-gene interaction between APOA5 and USF1: Two candidate genes for the metabolic syndrome.* Obesity facts 2009 2:235-242.

5. **Holzapfel C**, Grallert H, Huth C, Wahl S, Fischer B, Döring A, Rücker IM, Hinney A, Hebebrand J, Wichmann HE, Hauner H, Illig T, Heid IM. *Genes and lifestyle factors in obesity: results from 12 462 subjects from MONICA/KORA*. Int J Obes 2010 34:1538-1545.

6. **Holzapfel C**, Grallert H, Baumert J, Thorand B, Döring A, Wichmann HE, Hauner H, Illig T, Mielck A. *First investigation of two obesity-related loci (TMEM18, FTO) concerning their association with educational level as well as income: the MONICA/KORA study*. J Epidemiol Community Health (in press).

7. Mueller A, **Holzapfel C**, Hauner H, Crosby RD, Engel SG, Mühlhans B, Kolotkin RL, Mitchell JE, Horbach T, de Zwaan M. *Psychometric evaluation of the German version of the Impact of Weight on Quality of Life-Lite (IWQOL-Lite) questionnaire*. Exp Clin Endocrinol Diabetes (in press).

8. **Holzapfel C**, Siegrist M, Rank M, Langhof H, Grallert H, Baumert J, Klopp N, Wolfarth B, Illig T, Hauner H, Halle M. *Association of a melatonin receptor 1B (MTNR1B) gene variant with fasting glucose and HOMA-B in children and adolescents of high BMI-SDS groups.* Eur J Endocrinol (in press).

B Reviews

1. **Holzapfel C**, Hauner H. *Ernährungstherapeutische Konzepte bei Adipositas*. Gastroenterologe 2008 3:383-90.

2. **Holzapfel C**, Hauner H. *Gewichtsreduktion bei Adipositas – welche Rolle spielen die Gene?* Dtsch Med Wochenschr 2009 134:644-9.

3. **Holzapfel C**, Hauner H. *Gewichtsreduktion bei Adipositas - welche Rolle spielen die Gene?* BDI aktuell 2009, Nr. 7.

4. **Holzapfel C**, Hauner H. Refresher: Der Einfluss von Genen auf die Gewichtsreduktion. ZKM 2009 3:26-27.

5. **Holzapfel C**, Hauner H. *Diabetes – State of the Art*. Kompendium Ernährungsmedizin 2009, Nr. 1.

6. **Holzapfel C**, Hauner H. *Gewichtserhaltung nach Gewichtsreduktion – wie der Körper sein Gewicht verteidigt.* Dtsch Med Wochenschr (in press).

C Others

1. Interview with **Holzapfel C**, Hauner H. Liegt Diabetes an den Genen? Diabetiker Ratgeber 11/2009.

2. **Holzapfel C**, Hauner H. Diäten - was hilft wirklich beim Abnehmen? Druckpunkt, Ausgabe 3-4/2009.

3. **Holzapfel C**, Skurk T. 25. Jahrestagung der Deutschen Adipositas-Gesellschaft gemeinsam mit der Herbsttagung der Deutschen Diabetes-Gesellschaft in Berlin, 05. bis 07. November 2009: *Gewichtsreduktion: Welche Programme sind evidenzbasiert*. Diabetes Congress Report, 3/2010.

4. **Holzapfel C**, Hauner H. Homepage Kompetenznetz Adipositas (www.kn-adipositas.de): Patienteninformation.

Contributions

A Original publications

1. **Holzapfel C***, Grallert H*, Huth C, Wahl S, Fischer B, Döring A, Rückert IM, Hinney A, Hebebrand J, Wichmann HE, Hauner H, Illig T, Heid IM. *Genes and lifestyle factors in obesity: results from 12,462 subjects from MONICA/KORA*. Int J Obes 2010 34:1538-1545.

Holzapfel C has performed together with Heid IM the statistical analyses of the dataset and wrote the manuscript. The second first author Grallert H performed the experimental parts of the study: assay design, genotyping and quality control.
* authors contributed equally

2. **Holzapfel C**, Grallert H, Baumert J, Thorand B, Döring A, Wichmann HE, Hauner H, Illig T, Mielck A. *First investigation of two obesity-related loci (TMEM18, FTO) concerning their association with educational level as well as income: the MONICA/KORA study*. J Epidemiol Community Health (in press).

Holzapfel C has performed together with Baumert J the statistical analyses of the dataset and wrote the manuscript.

3. Mueller A, **Holzapfel C**, Hauner H, Crosby RD, Engel SG, Mühlhans B, Kolotkin RL, Mitchell JE, Horbach T, de Zwaan M. *Psychometric evaluation of the German version of the Impact of Weight on Quality of Life-Lite (IWQOL-Lite) questionnaire*. Exp Clin Endocrinol Diabetes (in press).

Holzapfel C provided the data of the Munich dataset (WW study) and recruited the controls from Munich.

4. **Holzapfel C**, Siegrist M, Rank M, Langhof H, Grallert H, Baumert J, Klopp N, Wolfarth B, Illig T, Hauner H, Halle M. *Association of a melatonin receptor 1B (MTNR1B) gene variant with fasting glucose and HOMA-B in children and adolescents of high BMI-SDS groups.* Eur J Endocrinol (in press).

Holzapfel C was responsible for the genetic analyses (sample selection, SNP selection, biobanking, quality controls, statistical analyses) and wrote the manuscript.

5. Jebb SA, Ahern AL, Olson AD, Aston LM, **Holzapfel C**, Stoll J, Simpson A, Pearson S, Fuller N, Caterson I, Hauner H. *Referral to a commercial weight management programme enhances weight loss achieved in primary care*. In preparation.

Holzapfel C was involved in designing the study. Together with the co-author Stoll J she managed the German part of the study: recruitment of doctors and patients, data and sample collection, performing of BIA measurement, quality control.

6. Kilpelainen T, …, **Holzapfel C**, Autenrieth C, et al. Physical activity attenuates the effect of the *FTO* gene on obesity; a meta-analysis of 176,834 adults. In preparation.

Holzapfel C managed together with the co-author Autenrieth C the participation of the MONICA/KORA study in this meta-analysis and was involved in the statistical analyses.

B Reviews

1. **Holzapfel C**, Hauner H. *Ernährungstherapeutische Konzepte bei Adipositas*. Gastroenterologe 2008 3:383-90.

Holzapfel C performed the literature search and wrote the manuscript.

2. **Holzapfel C**, Hauner H. *Gewichtsreduktion bei Adipositas – welche Rolle spielen die Gene?* Dtsch Med Wochenschr 2009 134:644-9.

Holzapfel C performed the literature search and wrote the manuscript.

3. **Holzapfel C**, Hauner H. *Gewichtserhaltung nach Gewichtsreduktion – wie der Körper sein Gewicht verteidigt.* Dtsch Med Wochenschr (in press).

Holzapfel C performed the literature search and wrote the manuscript.

Appendix

Appendix A: Metabolic parameters involved in energy homeostasis

CCK as the typical satiety signal (Chandra R and Liddle RA 2007; Raybould HE 2007) is secreted after meal and activates receptors which transmit satiety signals through the vagus nerve to the brain (Moran TH et al. 1997; Schwartz GJ and Moran TH 1994). CCK influences the secretion of pancreatic enzymes and gallbladder contraction, inhibits gastric emptying, increases gut motility and gastric acid secretion, and reduces portion sizes as well as duration of meals (Ballinger A et al. 1995; Gibbs J et al. 1973; Grider JR 1994; Kissileff HR et al. 1981; Liddle RA et al. 1985; Moran TH et al. 1994; Moran TH 2000; Schwartz GJ et al. 1997; Smith GP et al. 1981). The long-term effect of CCK on the body weight is transmitted via the interaction with other signals like leptin which elevates the effect of CCK (Matson CA et al. 2000).

GLP-1 is an incretine hormone from the proglucagon cleavage and is postprandially secreted. GLP-1 acts on glucose metabolism by stimulation of insulin secretion and inhibition of glucagon release (Drucker DJ and Nauck MA 2006). In animals and humans GLP-1 reduces food intake (Donahey JC et al. 1998; Gutzwiller JP et al. 1999b; Tang-Christensen M et al. 1996; Turton MD et al. 1996). Furthermore, GLP-1 reduces appetite and increases satiety (Gutzwiller JP et al. 1999a; Toft-Nielsen MB et al. 1999). Intravenous GLP-1 infusion lowers hunger and energy intake and delays gastric emptying in obese men (Naslund E et al. 1999). A meta-analysis of the effect of GLP-1 on energy intake confirmed a dose dependent reduction of energy intake and the decreased gastric emptying (Verdich C et al. 2001a). GLP-1-receptor agonists are effective for the treatment of diabetes mellitus type 2 and cause weight loss (Drucker DJ and Nauck MA 2006; Mafong DD and Henry RR 2008). A prandial subcutaneous injection of GLP-1 resulted in a weight loss of 0.55 kg after five days (Naslund E et al. 2004). In another study, a weight reduction of 1.9 kg was seen after six weeks (Zander M et al. 2002).

PYY postprandially rises and is stimulated via a neural reflux as well as via nutrients within the ileum itself (Adrian TE et al. 1985a; Fu-Cheng X et al. 1995; Fu-Cheng X et al. 1997). Administration of PYY leads to delayed gastric and gallbladder emptying (Adrian TE et al. 1985b; Allen JM et al. 1984; Hoentjen F et al. 2001). Furthermore, PYY as a satiety signal has an effect on appetite and reduces food intake (Batterham RL et al. 2002; Batterham RL and Bloom SR 2003). PYY secretion is proportional to the caloric intake of meals, the higher the caloric intake, the larger the PYY response (Degen L et al. 2005; Le Roux CW et al. 2006). In obese humans circulating PYY levels are lower suggesting that low PYY levels may have a causative role in the development of obesity (Batterham RL et al. 2003; Le Roux CW et al. 2006).

Ghrelin is an orexigenic gastrointestinal hormone and is postprandially decreased (Ariyasu H et al. 2001; Cummings DE et al. 2001; Tschop M et al. 2001). Ghrelin is regulated by both caloric intake and circulating nutritional signals like glucose. Gastric distension seems to be no regulator because the ingestion of water did not decrease ghrelin levels in rats (Tschop M et al. 2000). Ghrelin levels correlate with hunger scores (Cummings DE et al. 2004) and an intravenous infusion or injection of ghrelin leads to an increase of food intake in humans (Wren AM et al. 2001). The inverse correlation between ghrelin levels and fat mass leads to an increase of ghrelin levels after weight reduction (Cummings DE et al. 2002; Hansen TK et al. 2002). In obese persons postprandial ghrelin levels seem to be changed because compared to lean subjects in obese persons there is no such rapid postprandial decrease of ghrelin which might contribute to the development of obesity (English PJ et al. 2002).

The peptide hormone **leptin** which is secreted by adipose tissue is positively correlated with fat mass (Maffei M et al. 1995) and has a central role in the regulation of food intake. Leptin enters the blood-brain barrier in proportion to the plasma level (Schwartz MW et al. 1996). Food deprivation or energy restriction is associated with a quick decrease of leptin levels (Maffei M et al. 1995). Circulating leptin levels thus reflect both energy stores and food intake. Leptin decreases food intake and increases energy expenditure (Halaas JL et al. 1995; Jeon JY et al. 2003; Jorgensen JO et al. 1998; Kennedy A et al. 1997; Pelleymounter MA et al. 1995). Obese persons have increased leptin levels (Maffei M et al. 1995) which suggests a leptin resistance. Recombinant leptin administration has only shown modest effects on body weight in obese humans (Fogteloo AJ et al. 2003; Heymsfield SB et al. 1999). Leptin levels affect the hypothalamo-pituitary control of the gonadal, adrenal, and thyroid axes (Ahima RS et al. 1996; Chehab FF et al. 1996), and the immune response (Lord GM et al. 1998). Weight loss decreases leptin level (Weigle DS et al. 1997) which leads to a decrease of energy expenditure. Low dose leptin administration during weight loss maintained the energy expenditure (Rosenbaum M et al. 2002; Rosenbaum M et al. 2005).

Insulin is produced by the pancreas and positively associated with fat mass. A positive energy balance increases and a negative one decreases insulin levels (Bagdade JD et al. 1967; Woods SC et al. 1974). The satiety hormone insulin is transported to the blood-brain barrier in proportion to the circulating insulin (Baura GD et al. 1993) and unlike leptin levels, insulin secretion increases rapidly after a meal (Polonsky KS et al. 1988). Insulin and leptin interact with neuronal factors and modulate the sensitivity of satiety signals. Food deprivation leads to a decreased insulin/leptin signal and more food is necessary in order to reach enough satiety. If body weight increases, the insulin/leptin signal increases and this leads to an increased sensitivity of satiety signals which is associated with a decreased energy intake. The role of this effect is unknown because the homeostatic system allows large energy storage and obesity (Woods SC and D'Alessio DA 2008).

Appendix B: Studies for weight maintenance

Table B-1: Overview of studies for weight maintenance (Holzapfel C and Hauner H, in press)

Subjects	Initial weight loss	Weight maintenance programme	Duration	Weight	Reference
1032 adults (37% men)	At least 4 kg	a) Monthly personal contact b) Interactive technology-based intervention c) Self-directed control	30 months	a) +4.0 kg b) +5.2 kg c) +5.5 kg Imputation analysis	(Svetkey LP et al. 2008)
103 women	7.6 ± 2.6 kg	a) Cognitive behavioural treatment b) like a) plus food monitoring accuracy programme c) like b) plus reduced energy density eating programme	18 months	41.9% regained lost weight completer analysis	(Lowe MR et al. 2008b)
135 women	Weight loss programme of 4 months	a) Internet-based programme (website) b) Self-directed group	12 months	a) +0.4 ± 5.0 b) +0.6 ± 4.0 BCF analysis	(Cussler EC et al. 2008)
699 WW "lifetime members" (95.3% women)	Weight goal (BMI 20-25 kg/m²) achieved with WW programme (weight loss 10.9 ± 7.6 kg)	„Lifetime member" status: WW programme for free, as long weight is within 0.9 kg of goal weight	a) 1 year b) 2 years c) 5 years a) 1 year b) 2 years c) 5 years	a) 79.8% b) 71.0% c) 50.0% maintained ≥ 5% weight loss a) 26.5% b) 20.5% c) 16.2% remained below the goal weight	(Lowe MR et al. 2008a)
795 adults	≥ 13.6 kg	No weight loss programme, but registration in "National Weight Control Registry"	a) 1 year b) 2 years c) 3 years	a) +2.3 ± 4.7 b) +4.3 ± 7.4 c) +5.7 ± 8.7 Intention to treat	(Phelan S et al. 2007)
1002 WW "lifetime members" (96% women)	Weight goal (BMI 20-25 kg/m²) achieved with WW programme one to five years ago (weight loss 12.2 ± 8.4 kg)	„Lifetime member" status: WW programme for free, as long weight is within 0.9 kg of goal weight	5 years	56.8% maintained ≥ 5% weight loss 79.6% remained below initial weight	(Lowe MR et al. 2001)

BCF = baseline carried forward

Appendix C: Weight Watchers (WW) weight loss programme

The multidisciplinary WW programme is based on nutrition, physical activity, behaviour, and WW meetings (**Figure C-1**). The main aim is long-time weight loss success by lifestyle change.

Figure C-1: The multidisciplinary WW weight loss programme

A balanced diet with 55 to 60 percent of total energy intake from carbohydrates, 20 to 30 percent from fat, and 15 to 20 percent from protein is recommended. The programme is based on an individual calculation of *POINTS* regarding age, sex, weight, height, and daily energy expenditure (**Figure C-2**). *POINTS* are a specific measurement of the energy and macronutrient content of foods and resulted in a specific *POINTS* number per food. Participants can individually select and eat food for their allowed *POINTS* number to eat.

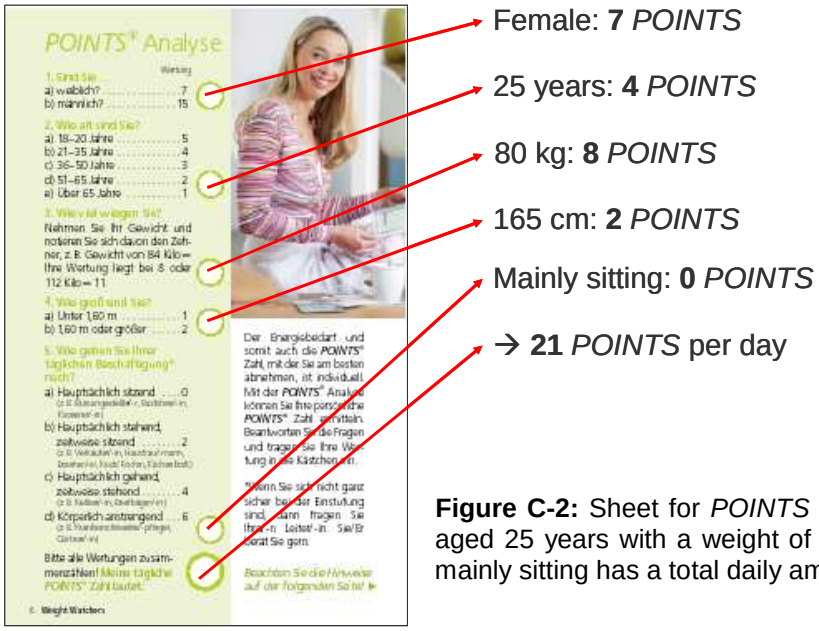


Figure C-2: Sheet for *POINTS* analysis. For example, a woman aged 25 years with a weight of 80 kg and height of 165 cm and mainly sitting has a total daily amount of 21 *POINTS* to eat.

Fruits and vegetables have a *POINTS* value of zero and two small pieces of chocolate have a *POINTS* value of three (**Figure C-3**). There are 18 food items (e.g. whole grain noodles, potatoes, fish) which are called *SuperFlex* and have a fixed *POINTS* value independent of portion size (**Figure C-3**). For example, the *SuperFlex* portion pasta has four *POINTS,* side dishes like chicken and oil have to be calculated separately (**Figure C-3**).

Figure C-3: Examples for food items and *POINTS* values

On the left examples of *POINTS* values; on the right (above) 18 *SuperFlex* food items with a fixed *POINTS* value and (below) an example of a *SuperFlex* portion

The programme *FlexPOINTS* makes possible to calculate *POINTS* on a portion level as usual or on *SuperFlex* basis (**Figure C-4**). *SuperFlex* allows the participant to eat for a fixed *POINTS* value from *SuperFlex* foods until he is satisfied. *SuperFlex* foods are healthy foods with a low energy density within their category and a satiable effect (protein-rich, fibre-rich).

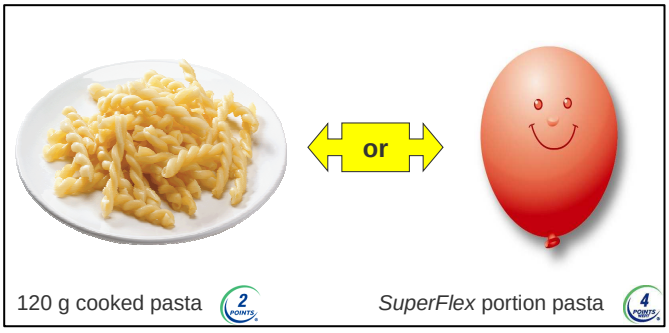


Figure C-4: Calculation of *POINTS* on a portion level (120 g cooked pasta for two *POINTS*) or on *SuperFlex* basis (cooked pasta until the participant is satisfied for four *POINTS*)

Furthermore, there are six "fit rules" for a healthy lifestyle including the following criteria (**Figure C-5**): (1) five servings of fruits and vegetables, (2) adequate drinks, (3) „good" lipids, (4) adequate calcium, (5) variety of food, (6) physical activity

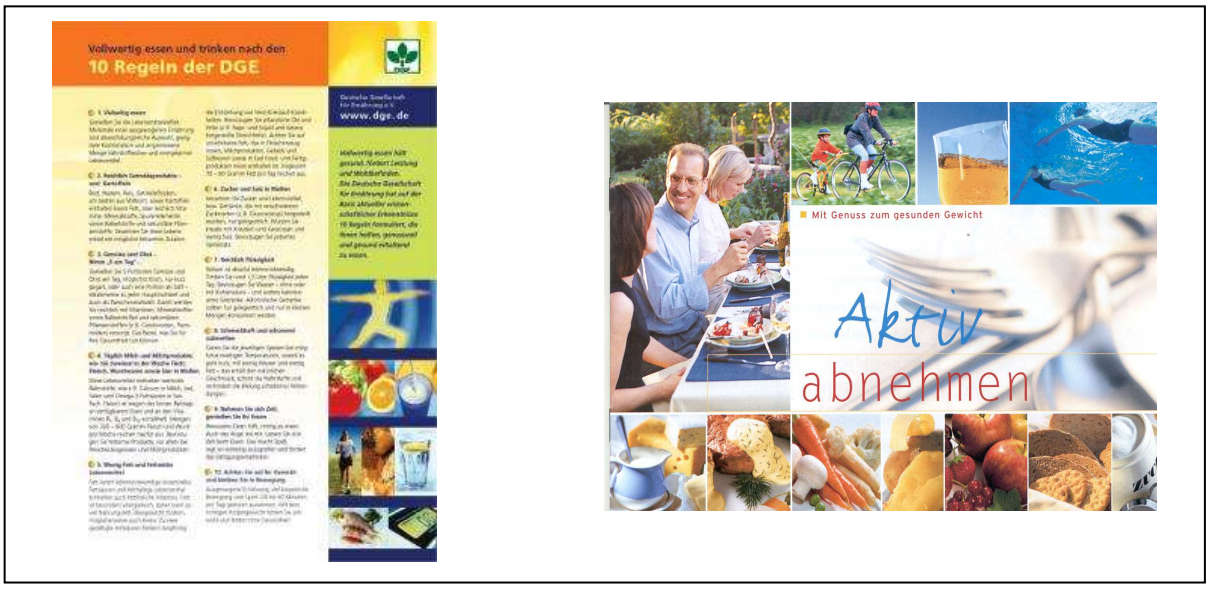
Figure C-5: Six "fit rules" for a healthy lifestyle

This WW programme was strongly revised and at 15th of November 2009 the new WW programme started in Germany. The main changes are the update of *POINTS* calculation for each food as well as for each participant (*ProPOINTS*). This update did not touch our study because last patients finished the first study year in January 2010.

Appendix D: Weight loss advice provided by general practitioners (GPs)

The standard GP care was not defined in detail. Every GP could give his own advice regarding weight loss and healthy lifestyle according to national guidelines. Suggestion of another specific weight loss programme and the prescription of weight loss medications were not allowed. As information material concerning a healthy balanced diet the study team provided the “Zehn Regeln” from the German Society of Nutrition (Deutsche Gesellschaft für Ernährung (DGE), Bonn, Germany), the weight loss brochure “Aktiv abnehmen” from the “Centrale Marketing-Gesellschaft der deutschen Agrarwirtschaft” (Centrale Marketing-Gesellschaft der deutschen Agrarwirtschaft mbH i.L. (CMA), Bonn, Germany), and a list of websites like www.aid.de or www.ernaehrung.de (**Figure D-1**).

Figure D-1: Provided material for the GP care



On the left the “Zehn Regeln” for a healthy balanced diet and on the right the brochure “Aktiv abnehmen”

Appendix E: Schedule of study procedures – Weight Watchers (WW)

In **table E-1** all measured biochemical parameters are listed.

Table E-1: Overview about measured biochemical parameters

Month	**-1**	**0**	**2**	**6**	**12**
Visit	**Screening**	**Visit A**	**Visit B**	**Visit D**	**Visit F**
Fasting glucose	+	+	+	+	+
Fasting insulin	+	+		+	+
Lipid parameters*	+	+	+	+	+
hsCRP		+		+	+
HbA1c**	+	+		(+)	(+)
Liver function***		+		+	+
Kidney function****		+			
TSH	+				
Total protein		+		+	+
Serum collection		+		+	+
EDTA collection		+			

*Total cholesterol, triglycerides, HDL, LDL cholesterol; **measured at screening and visit A; if patient was diabetic HbA1c was also measured at visits D and F; *** GOT, GPT, GGT, bilirubin, alkaline phosphatase; ****serum creatinine

Screening visit (-1 month)

Every potentially suitable participant was invited by GP to take part in the study. The GP discussed the study with the participant and completed the preliminary screening questionnaire. If a participant met the inclusion criteria, the GP completed a brief medical history. The GP gave the participant a copy of the information for participants and one original of the signed participant consent form.

To determine the participants` eligibility for randomisation, data on height, weight, BMI, waist circumference, BP, radial pulse rate, blood samples (glucose, insulin, full lipid profile, HbA1c, TSH) and concomitant medications was collected. Once a participant was deemed eligible for randomisation, he was allocated to a treatment group and was contacted to make an appointment for the baseline visit A. The participants got a pedometer (WW^{TM}, Weight Watchers GmbH, Düsseldorf, Germany) as well as a diet diary and were asked to record their dietary intake for four days and the number of steps per day for seven days prior to the baseline visit A. Furthermore, the three questionnaires were handed out.

Visit A (month 0 – baseline and randomisation)

The participants were advised which group they have been allocated to. Those allocated to the WW group were given vouchers to attend weekly meetings at the WW location of their choice and to use the internet portal “eSource”. The GP group received weight loss advice from the GP. All participants were asked to attend their first session with WW or their GP

within two weeks of the baseline visit. The following measurements occurred at baseline visit A: height, weight, BMI, waist circumference, BIA, BP, radial pulse rate, ECG, laboratory parameters (glucose, insulin, full lipid profile, hsCRP, HbA1c, liver function test, kidney function test, DNA and serum collection). Any changes and additions to concomitant medications were recorded. The four day diet diary, the seven day pedometer record and the three questionnaires were collected. Patients were asked for their ethnicity (patient and the four grandparents).

Visit B (month 2)

The following measurements occurred at this visit: weight, BMI, waist circumference, BIA, BP, radial pulse rate, and laboratory parameters (glucose, full lipid profile). Any changes and additions to concomitant medications were recorded and the compliance diary was reviewed.

Visit C (month 4) and visit E (month 9)

The following measurements occurred at these visits: weight, BMI, waist circumference, BIA, BP, and radial pulse rate. Any changes and additions to concomitant medications were recorded and the compliance diary was reviewed. The four day diet diary, the seven day pedometer record and the three questionnaires were handed out.

Visit D (month 6) and visit F (month 12)

The following measurements occurred at these visits: weight, BMI, waist circumference, BIA, BP, radial pulse rate, laboratory parameters (glucose, insulin, full lipid profile, hsCRP, (HbA1c) and serum collection). Any changes and additions to concomitant medications were recorded and the compliance was reviewed. The four day diet diary, the seven day pedometer record and the three questionnaires were collected. In addition, it was asked for change of smoking habits (visit F). Furthermore, a questionnaire concerning the satisfaction with the study and its intervention was asked (visit F).

Follow-up visit G (month 18) and visit H (month 24)

The following measurements occurred at these visits: weight, BMI, waist circumference, BIA, BP, and radial pulse rate. Any changes and additions to concomitant medications were recorded. The four day diet diary, the seven day pedometer record and the three questionnaires were handed out. Participants were asked to send the sheets per post to the study team. In addition, a questionnaire was asked to determine weight control method used by the participant within the last six months.

Appendix F: Intervention and procedures – LOGIC study

Energy intake is calculated according to gender and height (**Table F-1**) leading to a energy deficiency of about 500 kcal per day.

Table F-1: Calculation of energy intake according to gender and height

Boys		**Girls**	
Height [cm]	**Energy intake / day** [kcal]	**Height** [cm]	**Energy intake / day** [kcal]
< 140	1,250	< 145	1,250
140 - 160	1,500	145 - 165	1,500
> 160	1,800	> 165	1,800

The physical activity part consists of eleven hours per week and includes for example swimming and walking (**Table F-2**).

Table F-2: Overview of the physical activity part

Unit	**Hours / week**
Physical activity within group	1.5
Therapeutic physical activity	2.0
Swimming	3.0
Guided walking tour	3.0
Strength	1.5

Figure F-1: Study flow chart from start until ten years of follow-up

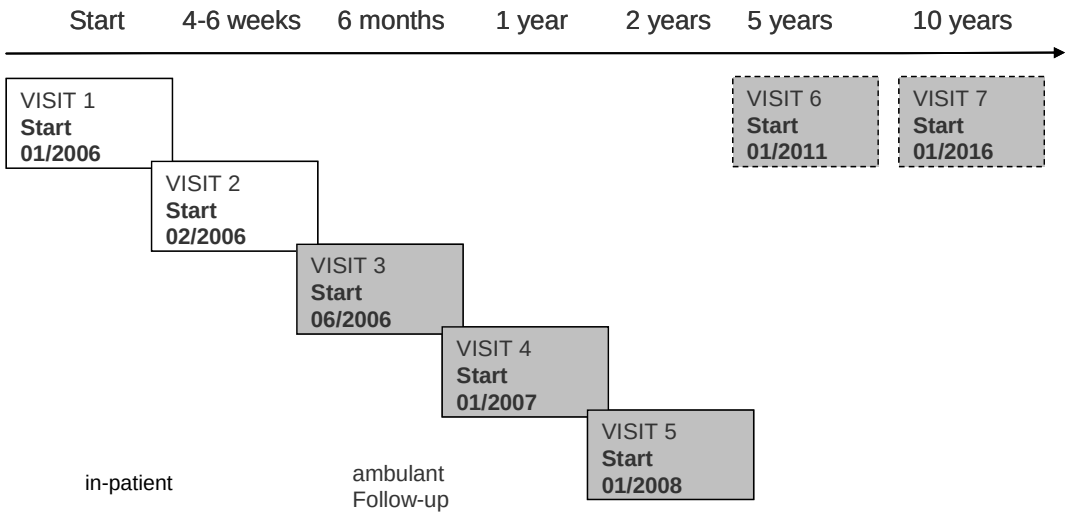


Table F-3: Data collection from visit 1 to visit 7

VISIT	VISIT 1	VISIT 2	VISIT 3	VISIT 4	VISIT 5	VISIT 6	VISIT 7
Time point	baseline	after intervention	6 months follow-up	1 year follow-up	2 years follow-up	5 years follow-up	10 years follow-up
Setting	in-patient		ambulatory			stationary	
Anthropometry*	+	+	+	+	+	+	+
Pubertal status (Tanner)	+		+	+	+	+	+
Biochemical parameters**	+	+				+	+
Collection of EDTA	+						
Questionnaire (parents)	+						
Quality of life (questionnaire)	+	+	+	+	+	+	+
Nutritional behaviour (questionnaire)	+	+	+	+	+	+	+
Physical activity (questionnaire)	+	+	+	+	+	+	+
Pedometer****	+					+	+

*Height, weight, waist circumference; **total cholesterol, HDL and LDL cholesterol, triglycerides, ureic acid, glucose, insulin, TSHbasal, TNFalpha, leptin, adiponektin multimer, resistin, pro-insulin, RBP-4, hsCRP, and IL-6; ****collected only in a subgroup of 200 children

Visit 1 (baseline)

The study team discuss the study with the child and its parent and ask the parent to give written informed consent. Data on height, weight, waist circumference, BP, and pubertal status is collected. Fasting blood samples are taken (lipids, ureic acid, glucose, insulin, TSHbasal, TNFalpha, leptin, adiponektin multimer, resistin, pro-insulin, RBP-4, hsCRP, IL-6) and EDTA blood is stored for genetic analysis. A subgroup of children gets a pedometer (Omron, Walking Style Pro HJ-720IT). Furthermore, a standardized questionnaire for parents and three questionnaires for the child are handed out.

Visit 2 (four or six weeks after intervention)

The following measurements occurre at this visit: height, weight, waist circumference, and BP. Fasting blood samples are taken (lipids, ureic acid, glucose, insulin, TSHbasal, TNFalpha, leptin, adiponektin multimer, resistin, pro-insulin, RBP-4, hsCRP, IL-6). Furthermore, three questionnaires are handed out.

Follow-up visit 3 (six months), 4 (one year), and 5 (two years)

These follow-up visits are performed according to standardized protocol by the supervised medical doctor at the city of the child. The following measurements occur at these visits: weight, waist circumference, BP, and pubertal status. Any changes and additions to concomitant medications are recorded. Furthermore, three questionnaires are handed out.

Follow-up visit 6 (five years) and 7 (ten years)

These follow-up visits are in-patient during a stay of three days. The following measurements occur at these visits: weight, waist circumference, BP, and pubertal status. Fasting blood samples are taken (lipides, ureic acid, glucose, insulin, TSHbasal, TNFalpha, leptin, adiponektin multimer, resistin, pro-insulin, RBP-4, hsCRP, IL-6). A subgroup of children gets a pedometer. Furthermore, three questionnaires are handed out.

Appendix G: Materials

Equipment

Gel electrophoresis

Documentation system, UVT-40 M Transilluminator	Peqlab, Erlangen, Germany
Documentation system, E.A.S.Y. 429 K Camera	Herolab, Wiesloch, Germany
Gel device, gel combs	BIO-RAD, Munich, Germany
Gel tray, Sub-Cell®GT Sys	BIO-RAD, Munich, Germany
Gadget, Power-Pac 300	BIO-RAD, Munich, Germany
Microwave, Micromaxx®	Medion, Essen, Germany
Microwave, Privileg 8020	Privileg
Incubation oven	Memmert, Schwabach, Germany

Centrifuges

Microcentrifuge	NeoLab, Heidelberg, Germany
Small centrifuge, Sigma 2-16 / 2-5	Sigma, Osterode, Germany
Refrigerated centrifuge, Rotanta 46 RS / 460 RS	Hettich, Tuttlingen, Germany
Refrigerated centrifuge, Sigma 4K15	Sigma, Osterode, Germany

Pipetting systemes

Pipettes	Eppendorf, Hamburg, Germany Gilson, Middleton (WI), USA STARLAB, Merenschwand, Switzerland Rainin, Mettler-Toledo, Greifensee, Switzerland
Serological pipettes	Greiner Bio-One, Frickenhausen, Germany
Multi-channel pipettes	Brand, Wertheim, Germany Capp A/S, Odense, Denmark
Multimek™ 96 automated 96 channel pipettor	Beckman Coulter, Krefeld, Germany
Pipetting robot, Genesis RSP 150	Tecan, Crailsheim, Germany
Pipetting robot, TeMo 96/384	Tecan, Crailsheim, Germany
Multi-channel pipetting robot, Aquarius™	Tecan, Crailsheim, Germany

Spotter

Nanodispenser, Mass ARRAY™ Nanodispenser	Sequenom, Hamburg, Germany

PCR multicycler

DNA Engine Tetrad	MJ Research, now Bio-RAD, Munich, Germany

Other

Vortex, MS2 Minishaker	IKA, Staufen, Germany
Ice maschine	Ziegra, Isernhagen, Germany
Mass spectrometer, Autoflex® Sequenom™ Bruker Daltonics®	Sequenom, Hamburg, Germany Bruker Daltonics, Bremen, Germany
Erlenmeyer flask	Schott Duran, Mainz, Germany
Scale, 572 precision balance	Kern&Sohn, Balingen, Germany
Shaker, Titramax 100	Heidolph, Darmstadt, Germany
Rotator, Roto-Shake Genie®	Scientific Industries, New York, USA
Ultrapure water purification system, Milli-Q®	Millipore, Schwalbach, Germany
Photometer, Genios®	Tecan, Crailsheim, Germany
Thermal mixer, Thermomixer Comfort	Eppendorf, Hamburg, Germany
Nanodrop® ND-1000 Spectrophotometer Nanodrop® ND-8000 Spectrophotometer	Thermo Fisher Scientific Inc., Wilmington, USA

Software and databases

Software for genotyping processes

Gemini 3.2, pipetting software	Tecan, Crailsheim, Germany
Nomalisation WorklistMaker	Tecan, Crailsheim, Germany
Xflour4	Tecan, Crailsheim, Germany
Nanodispenser software	Sequenom, Hamburg, Germany
Spectro Typer RT	Sequenom, Hamburg, Germany
Spectro DESIGNER	Sequenom, Hamburg, Germany
MassARRAY - Software, version 4.0	Sequenom, Hamburg, Germany

Online databases for SNP selection

Ensembl	www.ensembl.org
NCBI	www.ncbi.nlm.nih.gov
HapMap	www.hapmap.org

Statistical software

Haploview	www.broad.mit.edu/mpg/haploview
SAS 9.1	SAS Institute Inc., Cary, USA
Quanto 1.2.4	University of Southern California, Los Angeles, USA; http://hydra.usc.edu/gxe

Buffer, solutions, reagents, and enzymes

DNA extraction

Red blood cell (RBC) lysis buffer (pH 7.4)	NH_4Cl (155 mM) $KHCO_3$ (20 mM) Na_2EDTA (0.1 mM)
SE buffer (pH 8.0)	NaCl (75 mM) Na_2EDTA (25 mM)
NaCl solution (saturated)	NaCl (~ 6 M)
TE buffer (pH 8.0)	Tris/HCl (10 mM) EDTA (1 mM)
SDS solution	SDS (20 %)

Agarose gel electrophoresis

6x Loading Dye Solution	Fermentas, St. Leon-Rot, Germany
DNA Agarose	Biozym Diagnostik GmbH, Oldendorf, Germany
Ethidiumbromid	Merck, Darmstadt, Germany
GeneRuler 100 bp DNA-Ladder plus	Fermentas, St. Leon-Rot, Germany
pUC Mix Marker Nr. 8	Fermentas, St. Leon-Rot, Germany
Tris Borat EDTA buffer (TBE)	Sigma-Aldrich, Osterode, Germany

PCR

dNTP mix (25 mM)	Fermentas, St. Leon-Rot, Germany
$MgCl_2$ (25mM) / buffer with $MgCl_2$ (10x)	Qiagen, Hilden, Germany

SNP detection

3-point calibrant	Sequenom, Hamburg, Germany
SpectroClean™	Sequenom, Hamburg, Germany
iPLEX SAP buffer	Sequenom, Hamburg, Germany
iPLEX gold buffer	Sequenom, Hamburg, Germany
iPLEX termination mix	Sequenom, Hamburg, Germany

Enzymes

Proteinase K (Nr. 124568)	Merck, Darmstadt, Germany
HotStar Taq DNA polymerase (5 U/µl)	Qiagen, Hilden, Germany
SAP (1 U/µl)	Sequenom, Hamburg, Germany
Thermosequenase	Amersham, Freiburg, Germany
iPlex Enzym	Sequenom, Hamburg, Germany

Others

Water, LiChrosolu®	Merck, Darmstadt, Germany
Ficoll 400	AppliChem, Darmstadt, Germany
Clean Resin, SpectroClean™	Sequenom, Hamburg, Germany

Expendable items

Silizium-Chip, SpectroCHIP	Sequenom, Hamburg, Germany
Adhesive PCR film	ABgene, Epsom, England
Dimple Platten (384/6mg)	Sequenom, Hamburg, Germany
Eppendorf-Cup (1.5 ml)	Eppendorf, Hamburg, Germany
Falcon Tube (14 ml, 15 ml, 50 ml)	Becton Dickinson, Franklin Lakes, USA
Microplate 96V	Roth, Karlsruhe, Germany
PCR 384 plate, Thermo-Fast®384	ABgene, Epsom, England
96 plate, Thermo-Fast®96	ABgene, Epsom, England
Pipette tips	Molecular BioProducts, San Diego, USA
	Gilson, Lewis Center, USA

	STARLAB, Merenschwand, Switzerland
	Biozym Diagnostik GmbH, Oldendorf, Germany
	Axygen Scientific, Inc, Union City (CA), USA
	Rainin, Mettler-Toledo, Greifensee, Switzerland
Tape pads	Qiagen, Hilden, Germany

Appendix H: Used primers

Table H-1: Primer sequences (forward, reverse, extension) used for PCR and extension reaction in the WW and LOGIC study. Together with other polymorphisms these SNPs were genotyped in a 37plex.

Locus	SNP #	Primer	Sequence
NEGR1	rs2815752	Forward	ACG TTG GAT GAA CTC GGA AGA CAG CTG AAC
		Reverse	ACG TTG GAT GTT CCT CTA GGT ACT AGG CTG
		Extension	CCC AAC TTT CTT CTC AAC
NEGR1	rs2568958	Forward	ACG TTG GAT GAA AGA CTA CAC TCC CAC TCC
		Reverse	ACG TTG GAT GTT TCT AAG TCA GCC TGG GTC
		Extension	TCT CCC ACT CCA GTT TCT
KCTD15	rs29941	Forward	ACG TTG GAT GAA AGC TAG ACA AGC AGA GCC
		Reverse	ACG TTG GAT GAG GAA CGA GCC CCC AAC TCT
		Extension	GTC TCT GCA GAC CTA GGA
TRHR	rs7832552	Forward	ACG TTG GAT GGC CTT GAC CTC AAA GGA ATG
		Reverse	ACG TTG GAT GAC AAC AAG AGT CAA GCA CCC
		Extension	GAA TGT GAT AGT GTG AGG TA
LEPR	rs1805134	Forward	ACG TTG GAT GCT TCC CTC ATT ACA GAT GTC
		Reverse	ACG TTG GAT GTC CTT CTT ATA GAT GCA GTG
		Extension	TGC CAC CTA AAA TTC TGA CAA G
IRS1	rs1801278	Forward	ACG TTG GAT GAT GGT CAT GTA GTC ACC CCG
		Reverse	ACG TTG GAT GTC GAG ATG GGC AGA CTG GG
		Extension	GTC GGC CTG CAA ATG CTA GCA GCC C
TNKS-MSRA	rs17150703	Forward	ACG TTG GAT GCA GTT CCT CTG AAG TTG TGC
		Reverse	ACG TTG GAT GGC CAA TCT GAT GGT TTG GAG
		Extension	GCT ATG AAG TTG TGC AAT AAG CAA G
FTO	rs6499640	Forward	ACG TTG GAT GGC TTT CTG CCT CAA TCT ATC
		Reverse	ACG TTG GAT GGA ACT GAT GGT AGA GTA TTT C
		Extension	TTG GAA GGA ACA GGG TTT CTC TGA A
BDNF	rs16917237	Forward	ACG TTG GAT GCA ACT TCC TAC CAC CAT TAC
		Reverse	ACG TTG GAT GCC CAA TTC AAA ATC CCA AGG
		Extension	ATT ACT ACC ACC ATT ACA TAC TTC TG
SDCCAG8	rs2783963	Forward	ACG TTG GAT GAC TGC CTA GCA CTT ACA ATG
		Reverse	ACG TTG GAT GAG AAT GCA TAT CAC ACT GCC
		Extension	CCT CGC ACT TAC AAT GTT ATG ATT AAC
PTER	rs10508503	Forward	ACG TTG GAT GTT GCA GTC AGA CTT AAA GCG
		Reverse	ACG TTG GAT GAC AGT TCT GGT GTC GAG TTC
		Extension	GAC GTA AAG CGT CTA TTA TGC ATC ACG

Table H-2: Primer sequences (forward, reverse, extension) used for PCR and extension reaction in the WW and LOGIC study. Together with other polymorphisms these SNPs were genotyped in a 37plex.

Locus	SNP #	Primer	Sequence
PFKP	rs17132175	Forward	ACG TTG GAT GCT GAA GAA AGA GCG AAA AAC C
		Reverse	ACG TTG GAT GTA GGA TGC GGA ACT GTG ATG
		Extension	GCG AAA AAC CTT TTC CA
PLIN	rs894160	Forward	ACG TTG GAT GAG AAA TTG ACT GAG CAA GGG
		Reverse	ACG TTG GAT GAA GGA GTC TCT GTT TGT GGG
		Extension	CTG AGG CAC ATT CTA AAC
FTO	rs9939609	Forward	ACG TTG GAT GTT CTA GGT TCC TTG CGA CTG
		Reverse	ACG TTG GAT GTC CCA CTC CAT TTC TGA CTG
		Extension	TTG CGA CTG CTG TGA ATT T
PPARG	rs1801282	Forward	ACG TTG GAT GTG TAT CAG TGA AGG AAT CGC
		Reverse	ACG TTG GAT GCA AAC CCC TAT TCC ATG CTG
		Extension	AGG GAA GGA ATC GCT TTC TG
UCP2	rs659366	Forward	ACG TTG GAT GAA ACG CAC GTG TTT GTC CCG
		Reverse	ACG TTG GAT GTT TAA TTG GCT GAC CCG TCC
		Extension	GCC CGT GTT GGC TGT TCA CGC
IL6	rs1554606	Forward	ACG TTG GAT GGC AGC CAG AGA GGG AAA AG
		Reverse	ACG TTG GAT GAT GTT TAA AAC TCC CAC AGG
		Extension	GGG AAA GAG AGG GAA AAG GCC CTG
GNPDA2	rs10938397	Forward	ACG TTG GAT GCG ATA ATA ATG CTA AGA AC
		Reverse	ACG TTG GAT GCA TTA GTA TTG TAC ACA CAC C
		Extension	TCC TGC TAA GAA CAT TCT TGA AAA C
SH2B1	rs7498665	Forward	ACG TTG GAT GTG TTT CCG GAG TGT CCA AGG
		Reverse	ACG TTG GAT GCG CAT CCC CAT TGA AGA GG
		Extension	TGC TTA GAG GGG ATG AAC TGT CCC TG
NPC1	rs1805081	Forward	ACG TTG GAT GCT CAA CAC AAT TCC TTT CTG
		Reverse	ACG TTG GAT GAG CCT TTG GTG GCA TTG TTC
		Extension	TTC TTC CTT TCT GTA GAT TTT CCA GTC C

Table H-3: Primer sequences (forward, reverse, extension) used for PCR and extension reaction in the WW and LOGIC study. Together with other polymorphisms these SNPs were genotyped in a 35plex.

Locus	SNP #	Primer	Sequence
ADRA2A	rs1800544	Forward	ACG TTG GAT GCC TGC TGG GAG TTG GCC AT
		Reverse	ACG TTG GAT GTT CTC CCA AGA TCC AGC TTC
		Extension	TTG GCC ATG CAG CTC
TNKS-MSRA	rs516175	Forward	ACG TTG GAT GAC AGT GGC CCT TTG TCT TAC
		Reverse	ACG TTG GAT GGG GAA CAT TGG CTT ACT TTC
		Extension	ACT GCC TAG TTA CCG CA
ADIPOQ	rs17300539	Forward	ACG TTG GAT GTC ATC AGA ATG TGT GGC TTG
		Reverse	ACG TTG GAT GAC CTT GGA CTT TCT TGG CAC
		Extension	AGT TTG GCT TGC AAG AAC C
MC4R	rs17700144	Forward	ACG TTG GAT GAG GGC CAA AAC TGA CTA GAG
		Reverse	ACG TTG GAT GGA GCC ACT TAT CCT AGA GAG
		Extension	CAA CTG ACT AGA GGA ATT GTA
MC4R	rs17782313	Forward	ACG TTG GAT GCT TAA ATG TCA CCT TCC CCC
		Reverse	ACG TTG GAT GAG AAG TTT AAA GCA GGA GAG
		Extension	GGA CGC TTT TCT TGT CAT TTC CAT C
MTCH2	rs10838738	Forward	ACG TTG GAT GTG CTT TTA CTG AGA GTT GAC
		Reverse	ACG TTG GAT GAA AAG TAG ACG GCG AGA CAG
		Extension	GTT ACA TAA TTA CCT CAT GCA C
FTO	rs7206010	Forward	ACG TTG GAT GGC TCT TCT GCA GAG GAA ATG
		Reverse	ACG TTG GAT GCA CAC AGT CTG GTG AAA TGC
		Extension	TTC GGC AGA GGA AAT GAG ACT G
SDCCAG8	rs10926984	Forward	ACG TTG GAT GGG GTC TAT TAC TGG ACT GTG
		Reverse	ACG TTG GAT GGA CTT GGT CTG CCA GAT TTC
		Extension	TGC TAA TAC TAT ACT GTC TTG ATT G
SEC16B, RASAL2	rs10913469	Forward	ACG TTG GAT GCT CTG CAA GGT TTT GCC TTC
		Reverse	ACG TTG GAT GAT TAG CTT AAG CGT GGG AGG
		Extension	GAA AGG TTT TGC CTT CAT ATT ATA AAA

Table H-4: Primer sequences (forward, reverse, extension) used for PCR and extension reaction in the WW and LOGIC study. Together with other polymorphisms these SNPs were genotyped in a 33plex.

Locus	SNP #	Primer	Sequence
TNKS-MSRA	rs13278851	Forward	ACG TTG GAT GCT GGA TGT TAA GGC CTC AGC
		Reverse	ACG TTG GAT GGA CCA AGC AGA CGT AAT GTG
		Extension	GAA GCC CGC TAT GAC
INSIG2	rs11684454	Forward	ACG TTG GAT GCT CCT GGG ATT AGA GGT GTG
		Reverse	ACG TTG GAT GGC ATC CTC AAG AAG ACA AAG
		Extension	GAT GAG AGG TGT GAG CCA C
PCSK1	rs12186664	Forward	ACG TTG GAT GGG AAA TGT TCA GAG ACT GGC
		Reverse	ACG TTG GAT GTG TCC AGG AAG TTG ATT TGC
		Extension	GCT ACA CAA CAT GTG TTT CT
SDCCAG8	rs12145833	Forward	ACG TTG GAT GGG AAA AAA GCA GCA GCC TTG
		Reverse	ACG TTG GAT GCA GTC TCC ACA TTC TTT CCC
		Extension	CTT TGA GGG CAA AAG GGA GCC AC
MAF	rs1424233	Forward	ACG TTG GAT GAG ATT CCA CTG CAT GTT GAG
		Reverse	ACG TTG GAT GGT AAC TCA AGA TAG GGA CAG
		Extension	GCC AAT GCA TGT TGA GCT CAA ACC
FTO	rs9935401	Forward	ACG TTG GAT GTC TGT CTT AGT CAC ACT CAG
		Reverse	ACG TTG GAT GGA ACT GCC ACT CAT TCA ACC
		Extension	GGG GCG TCA CAC TCA GTA TCC TTA
MTNR1B	rs10830963	Forward	ACG TTG GAT GGG CAG AAT ATT CCC ATC AGG
		Reverse	ACG TTG GAT GCC CCC AGT GAT GCT AAG AAT
		Extension	GGC AAG GCA GTT ACT GGT TCT GGA TAG
MC4R	rs502933	Forward	ACG TTG GAT GGG TTA CTT AGT TAC GAA GCC
		Reverse	ACG TTG GAT GTG TGT GTG ATG GAC AAA AGC
		Extension	CGT ATT TAC GAA GCC AAT ACC AAC CTA T

Table H-5: Primer sequences (forward, reverse, extension) used for PCR and extension reaction in the WW and LOGIC study. Together with other polymorphisms these SNPs were genotyped in a 23plex.

Locus	SNP #	Primer	Sequence
GNB3	rs5443	Forward	ACG TTG GAT GTC TCC CAC GAG AGC ATC ATC
		Reverse	ACG TTG GAT GTC GTA GCC AGC GAA TAG TAG
		Extension	CTG CGG CAT CAC GTC
UCP1	rs45539933	Forward	ACG TTG GAT GCG ACG TCC AGT GTT ATT AGG
		Reverse	ACG TTG GAT GTA GAG TTT CAT CCG CCC TTC
		Extension	TCC TGG GAA CAA TCA CC
KCTD15	rs11084753	Forward	ACG TTG GAT GTG TGA GTC ACC GCA CTT GG
		Reverse	ACG TTG GAT GGA AGC GCT AAT ACA TGC TAC
		Extension	TTG GCC ACA CAA TGT TTT
ADRB2	rs12654778	Forward	ACG TTG GAT GGC ACA TAC AGG CAC AAA TAC
		Reverse	ACG TTG GAT GGG TGT GTC TCA GTG TCT ATG
		Extension	TCC ACC CTG GCA GAC ATG CT
TMEM18	rs7561317	Forward	ACG TTG GAT GGG AGG ATC TTT GGG AAC TTG
		Reverse	ACG TTG GAT GTG CTA GCA CTG GCT TAG AAG
		Extension	GTT TGG AAC TTG TAG GCA GA
SFRS10, ETV5, DGKG	rs7647305	Forward	ACG TTG GAT GGG CCT GTT TTG CAT GTT TGT
		Reverse	ACG TTG GAT GCT TTG TGA AAA CTC ATA GAG
		Extension	CAT ACA AGA AAA TAC ACA AAT CA
HTR2C	rs6318	Forward	ACG TTG GAT GGT TAC TAT AGC TGC TAC TGG
		Reverse	ACG TTG GAT GTC AGT GTG CAC CTA ATT GGC
		Extension	CCT CAT GGG CTC ACA GAA ATA TCA
PRL	rs4145443	Forward	ACG TTG GAT GAA AGT ATC TCA TTA CGA GG
		Reverse	ACG TTG GAT GAA TGC CAG ATA CAT GCT GAG
		Extension	GAT CTC ATT ACG AGG AAT GTA AGT
MC4R	rs1673482	Forward	ACG TTG GAT GCC ATG AAG GGA TGT TGA ATT
		Reverse	ACG TTG GAT GGA GAT ACA TCA CAG CAA CAG
		Extension	TCT ATT GAG ATC ATT ATA TGG TTT TT

Table H-6: Primer sequences (forward, reverse, extension) used for PCR and extension reaction in the MONICA/KORA study. Together with other polymorphisms not analyzed in this work these SNPs were genotyped in a 25plex.

Locus	SNP #	Primer	Sequence
NEGR1	rs10789336	Forward	ACG TTG GAT GCA AAT GGA GAT ATG GAA GAT G
		Reverse	ACG TTG GAT GAC TCT GGC ATA GGT GGA ATC
		Extension	AGG TCC AAA TTG GTA GTA TA
TMEM18	rs6548238	Forward	ACG TTG GAT GAA TAG GCC CCA GCA TAA GTC
		Reverse	ACG TTG GAT GAA AGA GAC AGG AGA AGG GAG
		Extension	GAC ACA GCA TAA GTC ACC CGA
MTCH2	rs10838738	Forward	ACG TTG GAT GTG CTT TTA CTG AGA GTT GAC
		Reverse	ACG TTG GAT GAA AAG TAG ACG GCG AGA CAG
		Extension	CTT GAC ATA ATT ACC TCA TGC AC
FTO	rs9935401	Forward	ACG TTG GAT GTC TGT CTT AGT CAC ACT CAG
		Reverse	ACG TTG GAT GGA ACT GCC ACT CAT TCA ACC
		Extension	CCG TCA CAC TCA GTA TCC TTA
MC4R	rs17700144	Forward	ACG TTG GAT GGA GCC ACT TAT CCT AGA GAG
		Reverse	ACG TTG GAT GAG GGC CAA AAC TGA CTA GAG
		Extension	ACG TTG CTT ACA TAG GAA
SH2B1	rs7498665	Forward	ACG TTG GAT GTG TTT CCG GAG TGT CCA AGG
		Reverse	ACG TTG GAT GCG CAT CCC CAT TGA AGA GG
		Extension	GCG GCG AGG GGA TGA ACT GTC CCT G
KCTD15	rs11084753	Forward	ACG TTG GAT GGC GCT AAT ACA TGC TAC AAC
		Reverse	ACG TTG GAT GTC GGA TTA CAG GTG TGA GTC
		Extension	CTA CAA CAT GGG CAA ACT TC
GNPDA2	rs10938397	Forward	ACG TTG GAT GCG ATA ATA ATG CTA AGA AC
		Reverse	ACG TTG GAT GCA TTA GTA TTG TAC ACA CAC C
		Extension	TGC TAA GAA CAT TCT TGA AAA C

Appendix I: Abstract Weight Watchers (WW) (Jebb S et al.)

Talk at International Congress on Obesity, 2010, Stockholm, Sweden (Abstracts in Obesity Reviews, Volume 11, Issue Supplement S1)

Referral to a commercial weight management programme enhances weight loss achieved in primary care

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Introduction: The scale of weight management services needed in primary care is daunting. This study compared the effectiveness of 12 month referral to a commercial weight loss programme with 12 month standard care by health professionals in three countries: Australia, Germany and the UK.

Methods: Participants (N=772; 87% female; median age = 47 years; median start BMI = 31.3 kg/m²) were randomly allocated to receive 12 months standard care (SC) in general practice or free vouchers for 12 months attendance at Weight Watchers (WW). Participants' weight was recorded at measurement appointments at 0, 2, 4, 6, 9 and 12 months.

Results: 59% WW and 50% SC completed the 12 month assessment. Analysis using BOCF showed mean weight loss at 12 months was significantly greater for Weight Watchers (WW) than standard care (SC) (-4.02 kg SEM 0.31 vs -1.59 kg SEM 0.19; p<0.001). 36% WW and 16% SC lost ≥5% initial weight. 19% WW and 6% SC lost ≥10%. Among completers, mean weight loss was significantly greater in WW than SC (-6.87kg SEM 0.43 vs -3.17 kg SEM 0.34; p<0.001). 61% WW and 32% SC lost ≥5%. 33% WW and 12% SC lost ≥10%.

Conclusion: Referral to Weight Watchers over 12 months significantly enhanced weight loss achieved through standard care. Meaningful weight loss (≥5%) was achieved by 36% of all WW patients referred and 61% those who completed the 12 month assessment. These findings support the use of Weight Watchers as an option for large scale provision of weight management services in primary care.

1. Conflict of Interest

None

2. Funding

Research relating to this abstract was funded by Weight Watchers

Appendix J: Characteristic Caucasian Weight Watchers (WW) population

Table J-1: Characteristics of the whole Caucasian study population (completer)

	Visit A (0 months)		Visit B (2 months)		Visit C (4 months)		Visit D (6 months)		Visit E (9 months)		Visit F (12 months)	
Parameter	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**
Age [years]	577	48.89 (12.70)	-	-	-	-	-	-	-	-	-	-
Height [m]	577	1.66 (0.08)	-	-	-	-	-	-	-	-	-	-
Systolic blood pressure [mmHg]	577	125.07 (16.30)	562	121.54 (15.41)	498	122.43 (15.75)	464	122.13 (15.58)	409	122.54 (15.68)	391	122.91 (15.85)
Diastolic blood pressure [mmHg]	577	78.70 (9.33)	562	77.17 (9.49)	498	77.29 (9.72)	464	77.16 (9.54)	408	76.75 (9.62)	391	76.84 (9.81)
Heart rate [mmHg]	559	71.51 (10.18)	549	70.85 (9.59)	490	71.96 (10.33)	447	70.83 (9.66)	396	71.64 (10.53)	369	70.27 (10.59)
BMI [kg/m²]	577	31.41 (2.60)	562	30.52 (2.70)	498	30.00 (2.94)	464	29.76 (3.09)	410	29.64 (3.21)	394	29.59 (3.28)
Weight [kg]	577	87.05 (11.53)	562	84.57 (11.64)	498	83.21 (12.02)	464	82.54 (12.57)	410	82.39 (12.59)	394	82.03 (12.46)
Waist circumference [cm]	571	100.09 (9.36)	557	96.92 (9.69)	496	95.71 (9.96)	458	94.87 (10.17)	402	94.84 (10.87)	384	94.46 (10.81)
Fat mass [kg]	517	33.31 (7.17)	510	31.34 (7.34)	446	29.84 (7.82)	420	29.45 (7.92)	365	29.18 (8.10)	357	29.23 (8.04)
Plasma glucose [mmol/l]	572	5.03 (0.83)	551	4.97 (0.78)	-	-	450	4.96 (0.74)	-	-	380	5.03 (0.83)
HbA1c [%]	570	5.64 (0.54)	-	-	-	-	263	5.54 (0.43)	-	-	209	5.50 (0.46)
Triglycerides [mmol/l]	573	1.45 (0.83)	551	1.39 (0.87)	-	-	450	1.39 (0.91)	-	-	379	1.36 (0.79)
Total cholesterol [mmol/l]	573	5.36 (1.01)	551	5.14 (0.99)	-	-	450	5.28 (1.01)	-	-	381	5.43 (1.03)
HDL cholesterol [mmol/l]	551	1.46 (0.37)	546	1.39 (0.33)	-	-	449	1.49 (0.42)	-	-	379	1.55 (0.39)
LDL cholesterol [mmol/l]	550	3.26 (0.88)	544	3.15 (0.85)	-	-	447	3.22 (0.90)	-	-	378	3.31 (0.88)

Means (s.d.) of anthropometric and biochemical parameters are shown at different time points (visit A to visit F); the time point-specific (visit A, B, D, F) median (IQR) for the not normally distributed triglycerides (mmol/l) is 1.28 (0.88), 1.20 (0.79), 1.17 (0.78), and 1.19 (0.76), respectively

Table J-2: Characteristics of the Caucasian study population

	Visit A (0 months)		Visit B (2 months)		Visit C (4 months)		Visit D (6 months)		Visit E (9 months)		Visit F (12 months)	
Parameter	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**
Systolic blood pressure [mmHg]	577	125.07 (16.30)	577	121.61 (15.27)	577	122.58 (15.88)	577	122.28 (15.57)	577	122.88 (15.59)	577	123.40 (16.20)
Diastolic blood pressure [mmHg]	577	78.70 (9.33)	577	77.24 (9.46)	577	77.48 (9.64)	577	77.33 (9.42)	577	77.25 (9.42)	577	77.43 (9.75)
Heart rate [mmHg]	559	71.51 (10.18)	571	70.81 (9.66)	571	72.19 (10.23)	571	71.05 (9.65)	570	72.16 (10.42)	569	71.12 (10.46)
Weight [kg]	577	87.05 (11.53)	577	84.62 (11.69)	577	83.63 (11.95)	577	83.21 (12.21)	577	83.49 (12.23)	577	83.59 (12.33)
Waist circumference [cm]	571	100.09 (9.36)	576	97.08 (9.67)	575	96.48 (10.08)	576	95.91 (10.12)	575	96.34 (10.57)	575	96.40 (10.72)
Fat mass [kg]	517	33.31 (7.17)	521	31.40 (7.29)	514	30.37 (7.74)	516	30.19 (7.84)	510	30.34 (8.02)	519	30.57 (7.92)
Plasma glucose [mmol/l]	572	5.03 (0.83)	575	4.97 (0.77)	-	-	573	4.96 (0.73)	-	-	572	4.98 (0.78)
HbA1c [%]	570	5.64 (0.54)	-	-	-	-	571	5.59 (0.52)	-	-	570	5.58 (0.53)
Triglycerides [mmol/l]	573	1.45 (0.83)	575	1.39 (0.86)	-	-	574	1.39 (0.89)	-	-	573	1.39 (0.83)
Total cholesterol [mmol/l]	573	5.36 (1.01)	575	5.14 (0.99)	-	-	574	5.30 (1.02)	-	-	573	5.41 (1.02)
HDL cholesterol [mmol/l]	551	1.46 (0.37)	567	1.40 (0.34)	-	-	567	1.48 (0.41)	-	-	562	1.52 (0.38)
LDL cholesterol [mmol/l]	550	3.26 (0.88)	566	3.15 (0.85)	-	-	566	3.24 (0.90)	-	-	561	3.30 (0.88)

Missing values are replaced by baseline values (BCF). Means (s.d.) of anthropometric and biochemical parameters are shown at different time points (visit A to visit F); the time point-specific (visit A, B, D, F) median (IQR) for the not normally distributed triglycerides (mmol/l) is 1.28 (0.88), 1.20 (0.80), 1.20 (0.82), and 1.20 (0.80), respectively

Table J-3: Changes of anthropometric parameters.

	BCF		Completers		WW BCF		WW completers		GP BCF		GP completers	
Parameter	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**	**N**	**mean (s.d.) median (IQR)**
After 2 months (visit B)												
Delta weight [kg]	577	-2.42 (2.86) -2.10 (3.70)	562	-2.49 (2.87) -2.20 (3.60)	285	-3.04 (3.07) -2.90 (3.70)	275	-3.15 (3.07) -3.00 (3.50)	292	-1.83 (2.50) -1.50 (3.10)	287	-1.86 (2.51) -1.60 (3.10)
Delta waist circumference [cm]	571	-3.04 (4.72) -2.00 (5.00)	552	-3.14 (4.77) -2.00 (5.50)	284	-3.44 (4.96) -2.00 (6.00)	272	-3.59 (5.01) -2.00 (6.00)	287	-2.64 (4.45) -2.00 (5.00)	280	-2.71 (4.49) -2.00 (5.00)
Delta fat mass [kg]	506	-1.98 (3.05) -1.70 (3.60)	495	-2.05 (3.08) -1.70 (3.60)	252	-2.49 (3.89) -2.40 (3.90)	244	-2.61 (3.43) -2.60 (3.60)	254	-1.47 (2.58) -1.25 (3.20)	251	-1.51 (2.60) -1.30 (3.20)
After 6 months (visit D)												
Delta weight [kg]	577	-3.83 (4.83) -2.80 (6.50)	464	-4.77 (4.96) -4.15 (5.85)	285	-5.09 (5.38) -4.50 (7.30)	238	-6.10 (5.34) -5.70 (5.90)	292	-2.61 (3.86) -1.45 (4.80)	226	-3.37 (4.08) -2.80 (5.20)
Delta waist circumference [cm]	571	-4.22 (5.66) -3.00 (7.50)	453	-5.32 (5.87) -5.00 (8.00)	284	-5.24 (6.08) -4.00 (9.00)	234	-6.36 (6.14) -5.00 (8.00)	287	-3.21 (5.02) -2.00 (6.50)	219	-4.20 (5.37) -3.00 (7.00)
Delta fat mass [kg]	504	-3.25 (4.48) -2.10 (5.80)	408	-4.06 (4.66) -3.60 (6.00)	253	-4.51 (5.10) -3.70 (7.60)	214	-5.42 (5.11) -4.85 (6.70)	251	-1.98 (3.31) -0.70 (4.10)	194	-2.56 (3.56) -2.30 (4.80)
After 12 months (visit F)												
Delta weight [kg]	577	-3.45 (5.50) -1.00 (5.90)	394	-5.06 (6.02) -3.80 (7.10)	285	-4.62 (6.36) -2.60 (7.80)	195	-6.76 (6.69) -5.70 (8.30)	292	-2.31 (4.22) -0.50 (4.20)	199	-3.39 (4.74) -2.30 (5.50)
Delta waist circumference [cm]	571	-3.73 (6.31) -1.00 (6.50)	380	-5.61 (7.03) -5.00 (9.00)	284	-4.40 (6.89) -1.60 (8.00)	187	-6.68 (7.55) -6.00 (10.00)	287	-3.08 (5.62) 0.00 (6.00)	193	-4.57 (6.34) -4.00 (8.50)
Delta fat mass [kg]	509	-2.74 (4.67) -0.60 (5.00)	347	-4.10 (5.20) -3.00 (6.80)	257	-3.70 (5.38) -1.30 (6.80)	178	-5.51 (5.72) -5.05 (7.70)	252	-1.75 (3.55) 0.00 (3.00)	169	-2.62 (4.11) -2.10 (4.80)

Means (s.d.) as well as medians (IQR) are shown for the Caucasian BCF dataset as well as for Caucasian completers. Furthermore, results are shown for both intervention groups (WW and GP). Delta = value visit B or D or F – value visit A

Appendix K: Characteristic Caucasian LOGIC population

Characteristics of the Caucasian LOGIC study cohort are shown in **table K-1** to **K-2**. Weight and BMI-SDS are available for all children after four weeks. Biochemical parameters are available after four weeks or after six weeks dependent on child`s duration of stay (visit 2).

Table K-1: Characteristics of the Caucasian study population

	Visit 1 (0 weeks)		Visit 2 (4 weeks)		Visit 2 (6 weeks)	
Parameter	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**	**N**	**mean (s.d.)**
Age [years]	312	13.78 (2.22)	-	-	-	-
Height [m]	312	162.97 (10.97)	-	-	-	-
Weight [kg]	312	90.08 (22.91)	299	82.06 (20.73)	189	82.15 (19.52)
BMI-SDS	312	2.74 (0.55)	299	2.40 (0.61)	189	2.36 (0.63)
Plasma glucose [mmol/l]	309	3.93 (0.45)	102	4.01 (0.43)	179	4.01 (0.48)
Plasma insulin [mU/l]	308	11.30 (6.07)	106	10.95 (4.78)	182	10.67 (6.01)
Triglycerides [mg/dl]	309	63.71 (25.04)	105	74.74 (35.84)	184	68.86 (26.67)
Total cholesterol [mg/dl]	308	157.01 (31.05)	105	139.44 (25.17)	184	134.05 (27.31)
HDL cholesterol [mg/dl]	307	50.76 (12.71)	105	51.23 (11.57)	183	49.72 (13.21)
LDL cholesterol [mg/dl]	309	103.43 (33.27)	100	83.03 (24.53)	184	78.49 (24.88)

Means (s.d.) of anthropometric and biochemical parameters are shown at different time points (visit 1 and 2); the time point-specific median (IQR) for the not normally distributed plasma insulin (mU/l) is 10.00 (6.28), 9.52 (5.44), 9.61 (6.98) and for triglycerides (mg/dl) is 60.00 (29.00), 66.00 (36.00), 67.00 (35.00)

Table K-2: Changes of anthropometric parameters.

Parameter	**N**	**mean (s.d.) median (IQR)**
After 4 weeks		
Delta weight [kg]	299	-8.24 (2.82) -7.80 (3.50)
Delta BMI-SDS	299	-0.36 (0.10) -0.35 (0.13)
After 6 weeks		
Delta weight [kg]	189	-10.90 (3.63) -10.40 (4.40)
Delta BMI-SDS	189	-0.47 (0.13) -0.46 (0.19)

Means (s.d.) as well as medians (IQR) are shown after four and six weeks for weight and BMI-SDS. Delta = value visit 2 – value visit 1 (visit 2 is after four or six weeks depending on child´s duration of stay)

Appendix L: Genotyping results for the Caucasian population of both studies

Table L-1: Genotype information of SNPs

Locus	SNP	Chr.	Minor allele	MAF	Minor allele	N (Cau.)	HWE (ChiSq)	HWE (Fisher)	Genotyping Success Rate	MAF	Minor allele	N (Cau.)	HWE (ChiSq)	HWE (Fisher)	Genotyping Success Rate	MAF
	HapMap				WW Study						LOGIC Study					
LEPR	rs1805134	1	-	-	C	574	0.429	0.458	99.48	21.60	C	311	0.340	0.392	99.36	21.06
NEGR1	rs2568958	1	G	36	G	574	0.239	0.240	99.48	36.50	G	313	0.923	0.904	100	34.66
	rs2815752		G	36	**C**	574	0.239	0.239	99.48	36.50	**C**	313	0.923	0.902	100	34.66
SDCCAG8	rs10926984	1	G	11	G	567	0.111	0.141	98.27	13.58	G	311	0.527	0.658	99.36	14.31
	rs12145833		G	13	G	564	**0.049**	**0.049**	97.75	13.65	G	310	0.521	0.649	99.04	14.35
	rs2783963		T	12	T	572	0.197	0.286	99.13	13.64	T	312	0.704	1.000	99.68	13.62
SEC16B, RASAL2	rs10913469	1	C	25	C	568	0.274	0.286	98.44	19.63	C	308	0.834	0.843	98.40	17.53
INSIG2	rs11684454	2	A	28	A	563	0.968	1.000	97.57	32.86	A	308	0.289	0.273	98.40	30.19
TMEM18	rs7561317	2	A	15	A	546	0.461	0.434	94.63	15.84	A	313	0.579	0.539	100	16.61
ADIPOQ	rs17300539	3	A	7	A	568	0.948	1.000	98.44	7.39	A	311	0.518	0.515	99.36	9.81
PPARG	rs1801282	3	G	10	G	563	0.789	1.000	97.57	13.14	G	313	0.614	0.802	100	12.62
SFRS10, ETV5, DGKG	rs7647305	3	T	20	T	555	0.854	1.000	96.19	21.53	T	312	0.244	0.355	99.68	18.91
UCP1	rs45539933	4	-	-	T	557	0.125	0.260	96.53	6.10	T	312	0.239	0.626	99.68	6.25
PCSK1	rs12186664	5	T	28	T	564	0.412	0.410	97.75	29.17	T	310	0.397	0.441	99.04	32.26
ADRB2	rs12654778	5	A	34	A	557	0.215	0.235	96.53	36.89	A	312	0.574	0.561	99.68	41.83
PRL	rs4145443	6	C	42	C	556	0.237	0.253	96.36	43.08	C	310	0.148	0.173	99.04	42.42
IL6	rs1554606	7	G	46	Genotyping failure						**T**	311	0.515	0.576	99.36	48.07
TNKS-MSRA	rs13278851	8	A	11	A	562	0.983	1.000	97.40	11.12	A	311	0.677	1.000	99.36	9.16
	rs17150703		A	11	A	570	0.623	0.661	98.79	10.96	A	312	0.722	1.000	99.68	8.97
	rs516175		A	11	**T**	567	0.419	0.439	98.27	12.52	**T**	312	0.709	0.753	99.68	10.42
TRHR	rs7832552	8	T	33	T	574	0.128	0.142	99.48	31.01	T	309	0.270	0.318	98.72	27.18
ADRA2A	rs1800544	10	-	-	G	568	0.940	1.000	98.44	26.32	G	312	0.741	0.890	99.68	27.24
PFKP	rs17132175	10	C	13	C	564	0.221	0.201	97.75	9.04	C	313	0.498	0.752	100	9.90
PTER	rs10508503	10	T	9	T	560	**0.049**	0.062	97.05	7.68	T	304	0.232	0.619	97.12	6.41
BDNF	rs16917237	11	T	22	T	554	0.071	0.075	96.01	21.30	T	311	0.176	0.168	99.36	21.22
MTCH2	rs10838738	11	G	36	G	567	0.901	0.924	98.27	32.89	G	312	0.353	0.385	99.68	33.65
MTNR1B	rs10830963	11	G	30	Genotyping failure						G	311	0.504	0.487	99.36	28.14
UCP2	rs659366	11	T	37	Genotyping failure						T	312	0.386	0.406	99.68	38.14
GNB3	rs5443	12	T	39	T	557	0.625	0.635	96.53	32.94	T	312	0.496	0.529	99.68	33.17
PLIN	rs894160	15	T	32	**A**	564	0.669	0.763	97.75	30.41	**A**	312	0.968	1.000	99.68	30.93
FTO	rs6499640	16	G	36	G	564	0.702	0.713	97.75	35.46	G	311	0.524	0.531	99.36	34.24
	rs7206010		A	36	A	568	0.857	0.927	98.44	36.09	A	311	0.637	0.615	99.36	34.08
	rs9935401		A	45	A	556	0.849	0.860	96.36	42.18	**A/G**	310	0.088	0.087	99.04	50.00
	rs9939609		A	46	A	563	0.841	0.860	97.57	42.81	**T**	311	0.079	0.091	99.36	49.84
MAF	rs1424233	16	C	44	**A**	562	0.933	0.934	97.40	49.82	**G**	311	0.061	0.073	99.36	49.68
SH2B1	rs7498665	16	G	38	Genotyping failure						G	313	0.130	0.138	100	43.93
MC4R	rs1673482	18	G	39	G	553	0.936	0.923	95.84	33.91	G	308	0.161	0.189	98.40	39.12
	rs17700144		A	25	A	568	0.843	0.804	98.44	21.48	A	311	**0.047**	**0.048**	99.36	28.30
	rs17782313		C	26	C	568	0.940	1.000	98.44	23.86	C	311	**0.033**	**0.033**	99.36	30.55
	rs502933		A	34	A	531	0.528	0.568	92.03	35.88	A	310	0.141	0.159	99.04	39.68
NPC1	rs1805081	18	C	47	**G**	560	0.466	0.488	97.05	41.88	**G**	311	0.120	0.119	99.36	39.07
KCTD15	rs11084753	19	A	31	A	555	0.528	0.559	96.19	31.98	A	311	**0.044**	0.058	99.36	32.48
	rs29941		A	32	**T**	573	0.858	0.919	99.31	31.24	**T**	312	0.594	0.695	99.68	32.05
HTR2C	rs6318*	X	C	17	C	482	0.466	0.590	96.98	15.15	C	188	0.942	1	99.47	14.36

Complementary minor alleles to the reference (HapMap) are bold/grey; in LOGIC for rs9935401 and rs9939609 (*FTO*) and for rs1554606 (*IL6*) the "other" allele is the minor allele also highlighted in bold/grey; violated p-values of HWE (<0.05) are bold/grey; *HWE only measured in women because SNP is on the X-chromosome; ChiSq=Chi-square test; Fisher=Fisher´s exact test; MAF=minor allele frequency in percent; Cau.=Caucasian; HWE=Hardy-Weinberg equilibrium

Appendix M: Details about genotyped polymorphisms

Table M-1: Position on the chromosome (chr.), alleles, region, amino acid exchange, and proxy SNPs in the literature are shown for the different SNPs

Locus	SNP	Chr. Position genome build 37.1	Alleles	Comment	in LD with following SNP described in literature (alleles, chr. Position, comment)
LEPR	rs1805134	66067109	C/T	allele change T > C, Ser343Ser	
NEGR1	rs2568958	72765116	A/G	near *NEGR1*	
	rs2815752	72812440	C/T	near *NEGR1*	
SDCCAG8	rs10926984	243462153	G/T	intron region	
	rs12145833	243483754	G/T	intron region	
	rs2783963	243501583	C/T	intron region	
SEC16B, RASAL2	rs10913469	177913519	C/T	intron region (*SEC16B*)	
INSIG2	rs11684454	118763068	A/G	intron region (*CCDC93*, coiled-coil domain containing 93)	rs7566605 (C/G; 118836025; near *INSIG2*)
TMEM18	rs7561317	644953	A/G	near *TMEM18*	
ADIPOQ	rs17300539	186559460	A/G	near *ADIPOQ*	
PPARG	rs1801282	12393125	C/G	allele change C > T, Pro12Ala	
SFRS10, ETV5, DGKG	rs7647305	185834290	C/T	near *SFRS10*, *ETV5*, *DGKG*	
UCP1	rs45539933	141489068	C/T	allele change G > A, Ala64Thr	
ADRB2	rs12654778	148205741	A/G	near *ADRB2*	rs1042713 (A/G; 148206440; allele change A > G, Arg16Gly)
PCSK1	rs12186664	95630225	A/T	near *PCSK1*	rs6234 (C/G; 95728974; allele change C > G, Gln665Glu) rs6235 (C/G; 95728898; allele change G > C, Ser690Thr)
PRL	rs4145443	22068174	A/C	near *PRL*	rs4712652 (A/G; 22078615; near *PRL*)
IL6	rs1554606	22768707	G/T	intron region	rs1800795 (C/G; 22766645; near *IL6*)
TNKS-MSRA	rs13278851	9750872	A/G	near *TNKS-MSRA*	
	rs17150703	9745798	A/G	near *TNKS-MSRA*	
	rs516175	9769573	C/T	near *TNKS-MSRA*	
TRHR	rs7832552	110115676	C/T	intron region	
ADRA2A	rs1800544	112836503	C/G	near *ADRA2A*	
PFKP	rs17132175	3150814	C/G	intron region	rs6602024 (A/G; 3155237; intron region)
PTER	rs10508503	16299951	C/T	near *PTER*	
BDNF	rs16917237	27702383	G/T	intron region	rs6265 (A/G; 27679916; allele change G > A, Val66Met)
MTCH2	rs10838738	47663049	A/G	intron region	
MTNR1B	rs10830963	92708710	C/G	intron region	
UCP2	rs659366	73694754	C/T	near *UCP2*	rs660339 (A/C/G/T; 73689104; allele change C > A, Ala55Asp; C > G, Ala55Gly; C > T, Ala55Val)
GNB3	rs5443	6954875	C/T	allele change C > T, Ser275Ser	
PLIN	rs894160	90211823	A/G	intron region	
FTO	rs6499640	53769677	A/G	intron region	
	rs7206010	53755177	A/G	intron region	rs6499640
	rs9935401	53816838	A/G	intron region	rs9939609
	rs9939609	53820527	A/T	intron region	
MAF	rs1424233	79682751	A/G	near *MAF*	
SH2B1	rs7498665	28883241	A/G	allele change A > G, Thr484Ala	
MC4R	rs1673482	57890212	G/T	near *MC4R*	rs477181 (G/T; 57896038; near *MC4R*), rs502933
	rs17700144	57811982	A/G	near *MC4R*	rs12967135 (A/G; 57849023; near *MC4R*) in LD with rs17782313
	rs17782313	57851097	C/T	near *MC4R*	
	rs502933	57896474	A/C	near *MC4R*	
NPC1	rs1805081	21140432	A/G	allele change A > G, His215Arg	
KCTD15	rs11084753	34322137	A/G	near *KCTD15*	
	rs29941	34309532	C/T	near *KCTD15*	
HTR2C	rs6318	113965735	C/G	allele change G > C, Cys23Ser	

LD=linkage disequilibrium

Appendix N: Genotype frequencies in Weight Watchers (WW) and LOGIC

Table N-1: Numbers of persons for the specific genotypes are shown for the WW and the LOGIC study (whole study populations)

Locus	SNP	Homozygous major allele		Heterozygous		Homozygous minor allele		Homozygous major allele		Heterozygous		Homozygous minor allele	
		WW Study						LOGIC Study					
		Genotype	N	Genotype	N	Genotype	N	Genotype	N	Genotype	N	Genotype	N
LEPR	rs1805134	TT	406	CT	209	CC	34	TT	219	CT	127	CC	11
NEGR1	rs2568958	AA	278	AG	281	GG	90	AA	152	AG	164	GG	42
	rs2815752	TT	278	CT	281	CC	90	TT	152	CT	165	CC	42
SDCCAG8	rs10926984	TT	483	GT	151	GG	**6**	TT	264	GT	86	GG	**7**
	rs12145833	TT	479	GT	153	GG	**5**	TT	263	GT	86	GG	**7**
	rs2783963	CC	479	CT	160	TT	**8**	CC	268	CT	81	TT	**7**
SEC16B, RASAL2	rs10913469	TT	415	CT	198	CC	28	TT	243	CT	101	CC	10
INSIG2	rs11684454	GG	284	GA	282	AA	70	GG	175	GA	143	AA	36
TMEM18	rs7561317	GG	444	GA	158	AA	17	GG	254	GA	94	AA	10
ADIPOQ	rs17300539	GG	554	AG	84	AA	**3**	GG	296	AG	57	AA	**4**
PPARG	rs1801282	CC	490	GC	139	GG	**9**	CC	278	GC	77	GG	**4**
SFRS10, ETV5, DGKG	rs7647305	CC	386	CT	214	TT	30	CC	227	CT	120	TT	10
UCP1	rs45539933	CC	551	TC	81	TT	**1**	CC	311	TC	46	-	-
ADRB2	rs12654778	GG	251	GA	307	AA	75	GG	122	GA	171	AA	64
PCSK1	rs12186664	AA	319	TA	261	TT	57	AA	164	TA	160	TT	32
PRL	rs4145443	AA	198	CA	297	CC	136	AA	117	CA	166	CC	72
IL6	rs1554606	-	-	-	-	-	-	GG	103	GT	181	TT	72
TNKS-MSRA	rs13278851	GG	501	GA	123	AA	11	GG	294	GA	61	AA	**2**
	rs17150703	GG	512	GA	121	AA	10	GG	295	GA	60	AA	**2**
	rs516175	CC	491	CT	134	TT	15	CC	286	CT	64	TT	**8**
TRHR	rs7832552	CC	311	CT	266	TT	72	CC	182	CT	148	TT	24
ADRA2A	rs1800544	CC	326	GC	260	GG	54	CC	189	GC	143	GG	26
PFKP	rs17132175	GG	533	CG	99	CC	**7**	GG	296	CG	61	CC	**2**
PTER	rs10508503	CC	546	TC	88	-	-	CC	304	TC	46	-	-
BDNF	rs16917237	GG	386	GT	218	TT	24	GG	232	GT	103	TT	22
MTCH2	rs10838738	AA	289	GA	277	GG	73	AA	162	GA	153	GG	43
MTNR1B	rs10830963	-	-	-	-	-	-	CC	187	GC	139	GG	31
UCP2	rs659366	-	-	-	-	-	-	CC	141	TC	161	TT	56
GNB3	rs5443	CC	279	CT	273	TT	81	CC	159	CT	153	TT	45
PLIN	rs894160	GG	303	GA	273	AA	63	GG	174	GA	151	AA	33
FTO	rs6499640	AA	251	AG	285	GG	102	AA	158	AG	153	GG	46
	rs7206010	GG	245	GA	296	AA	100	GG	158	GA	153	AA	46
	rs9935401	GG	221	AG	301	AA	106	AA	96	AG	165	GG	95
	rs9939609	TT	219	AT	311	AA	108	AA	97	AT	165	TT	95
MAF	rs1424233	AA	166	GA	317	GG	152	AA	109	GA	158	GG	90
SH2B1	rs7498665	-	-	-	-	-	-	AA	123	GA	166	GG	70
MC4R	rs1673482	TT	273	GT	274	GG	82	TT	139	GT	156	GG	58
	rs17700144	GG	401	GA	208	AA	32	GG	194	GA	128	AA	35
	rs17782313	TT	371	TC	227	CC	43	TT	183	TC	134	CC	40
	rs502933	CC	244	CA	277	AA	82	CA	158	CC	138	AA	60
NPC1	rs1805081	AA	224	AG	312	GG	98	AA	154	AG	148	GG	55
KCTD15	rs11084753	GG	292	GA	260	AA	79	GG	153	GA	173	AA	30
	rs29941	CC	298	TC	276	TT	74	CC	160	TC	164	TT	34
HTR2C	rs6318*	GG	399	GC	143	CC	**9**	GG	149	GC	58	CC	**5**

<10 subjects are highlighted in bold/grey; *only analyzed in women/girls

Appendix O: Results Kruskal-Wallis test – Weight Watchers (WW) study

Table O-1: P-values from the Kruskal-Wallis test are given for the WW study population

Locus	SNP	Delta weight (6 months)	Delta weight BCF (6 months)	Delta weight (12 months)	Delta weight BCF (12 months)	Delta fat mass (6 months)	Delta fat mass BCF (6 months)	Delta fat mass (12 months)	Delta fat mass BCF (12 months)	Delta waist (6 months)	Delta waist BCF (6 months)	Delta waist (12months)	Delta waist BCF (12 months)
		p-value Kruskall-Wallis test											
LEPR	rs1805134	0.772	0.437	0.967	0.997	0.874	0.874	0.995	0.939	0.676	0.714	0.232	0.253
NEGR1	rs2568958	0.128	0.065	**0.014**	0.162	0.209	0.478	0.168	0.618	0.076	0.154	**0.025**	0.227
	rs2815752	0.128	0.065	**0.014**	0.162	0.209	0.478	0.168	0.618	0.076	0.154	**0.025**	0.227
SDCCAG8	rs10926984	0.294	0.326	**0.019**	**0.030**	0.058	0.113	**0.054**	**0.025**	0.907	0.923	0.364	0.624
	rs12145833	0.503	0.429	0.083	0.111	0.061	0.119	0.055	**0.024**	0.860	0.891	0.755	0.840
	rs2783963	0.162	0.165	**0.020**	**0.014**	**0.034**	0.072	0.055	**0.017**	0.963	0.996	0.347	0.358
SEC16B, RASAL2	rs10913469	0.409	0.961	0.541	0.876	0.496	0.313	0.996	0.844	0.574	0.611	0.557	0.584
INSIG2	rs11684454	0.300	0.326	0.302	0.815	0.473	0.449	0.174	0.585	0.247	0.297	0.236	0.360
TMEM18	rs7561317	0.531	0.221	0.124	0.114	0.858	0.317	0.422	0.181	0.252	**0.048**	0.190	0.057
ADIPOQ	rs17300539	0.174	0.310	0.248	0.449	0.083	0.157	0.192	0.671	0.280	0.644	**0.053**	0.273
PPARG	rs1801282	0.889	0.963	0.095	0.091	0.685	0.564	0.354	0.327	0.328	0.562	0.314	0.247
SFRS10, ETV5, DGKG	rs7647305	**0.034**	**0.021**	0.065	**0.051**	0.140	0.169	0.229	0.400	0.744	0.457	0.801	0.466
UCP1	rs45539933	0.724	0.397	0.700	0.947	0.404	0.076	0.284	0.263	0.884	0.653	0.593	0.919
ADRB2	rs12654778	0.993	0.813	0.240	0.139	0.995	0.965	0.078	0.170	0.830	0.514	0.378	0.134
PCSK1	rs12186664	0.177	0.124	0.659	0.955	0.135	0.159	0.591	0.996	0.228	0.366	0.172	0.852
PRL	rs4145443	0.634	0.582	0.876	0.312	0.372	0.210	0.138	**0.003**	0.435	0.808	0.995	0.467
TNKS-MSRA	rs13278851	0.528	0.355	0.928	0.653	**0.038**	**0.033**	0.675	0.957	0.087	0.092	0.743	0.596
	rs17150703	0.577	0.322	0.989	0.934	**0.036**	**0.027**	0.582	0.741	0.242	0.228	0.760	0.863
	rs516175	0.120	0.090	0.872	0.798	**0.0003**	**0.0004**	0.111	0.248	0.132	0.115	0.801	0.578
TRHR	rs7832552	0.539	0.134	0.920	0.768	0.422	0.168	0.834	0.690	0.752	0.213	0.120	0.654
ADRA2A	rs1800544	0.825	0.970	0.343	0.445	0.880	0.813	0.692	0.542	0.801	0.847	0.991	0.413
PFKP	rs17132175	0.864	0.835	0.829	0.920	0.773	0.839	0.691	0.697	0.439	0.725	0.528	0.742
PTER	rs10508503	**0.033**	0.152	0.200	0.294	0.714	0.909	0.470	0.962	0.303	0.694	0.852	0.841
BDNF	rs16917237	0.123	**0.050**	0.493	0.294	0.596	0.458	0.479	0.234	0.489	0.288	0.983	0.786
MTCH2	rs10838738	0.792	0.397	0.270	0.393	0.252	0.124	0.318	0.329	0.308	0.736	0.860	0.970
GNB3	rs5443	0.498	0.262	0.617	0.779	0.946	0.464	0.766	0.835	0.882	0.321	0.949	0.293
PLIN	rs894160	0.510	0.412	0.841	0.869	0.224	0.455	0.548	0.853	0.822	0.956	0.844	0.968
FTO	rs6499640	0.422	0.318	0.134	0.685	0.203	0.145	0.096	0.468	0.809	0.647	0.685	0.710
	rs7206010	0.473	0.424	0.128	0.703	0.283	0.257	0.082	0.444	0.642	0.662	0.917	0.864
	rs9935401	0.797	0.172	0.825	0.372	0.750	0.237	0.148	**0.050**	0.749	0.182	0.257	0.121
	rs9939609	0.933	0.384	0.832	0.439	0.975	0.510	0.227	**0.049**	0.940	0.376	0.464	0.197
MAF	rs1424233	0.208	0.368	0.346	0.457	0.143	0.290	**0.030**	0.122	0.705	0.633	0.603	0.766
MC4R	rs1673482	**0.020**	**0.008**	**0.035**	**0.002**	0.307	0.134	0.063	**0.005**	0.408	0.144	0.936	0.338
	rs17700144	**0.019**	**0.005**	0.093	**0.010**	0.109	0.059	0.343	**0.054**	0.290	0.172	0.837	0.417
	rs17782313	0.254	**0.032**	0.403	**0.015**	0.655	0.273	0.812	0.146	0.622	0.248	0.793	0.638
	rs502933	**0.033**	**0.020**	0.083	**0.003**	0.439	0.253	0.248	**0.025**	0.644	0.350	0.994	0.415
NPC1	rs1805081	0.899	0.863	0.683	0.487	0.730	0.929	0.692	0.990	0.354	0.612	0.969	0.923
KCTD15	rs11084753	0.287	0.304	0.169	0.296	0.589	0.405	0.640	0.471	0.628	0.397	0.384	0.702
	rs29941	0.944	0.769	0.926	0.685	0.970	0.789	0.879	0.115	0.106	**0.037**	0.935	0.686
HTR2C	rs6318*	0.224	0.755	0.531	0.940	0.920	0.995	0.192	0.732	0.523	0.675	0.117	0.261

Delta weight, fat mass, and waist circumference were analyzed after six and twelve months in both datasets (completer and BCF); p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Appendix P: Results from logistic regression – delta weight in Weight Watchers (WW) study

Table P-1: Results from logistic regression concerning delta weight after two, six and twelve months

Locus	SNP	Delta weight (2 months)		Delta weight (6 months)		Delta weight BCF (6 months)		Delta weight (12 months)		Delta weight BCF (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	0.910	0.484	1.083	0.601	1.167	0.249	0.971	0.858	1.040	0.770
NEGR1	rs2568958	0.842	0.138	1.004	0.978	0.785	**0.035**	0.739	**0.036**	0.903	0.376
	rs2815752	0.842	0.138	1.004	0.978	0.785	**0.035**	0.739	**0.036**	0.903	0.376
SDCCAG8	rs10926984	1.097	0.597	1.044	0.823	1.038	0.830	1.509	0.058	1.281	0.162
	rs12145833	1.037	0.839	1.027	0.892	1.016	0.928	1.456	0.085	1.196	0.314
	rs2783963	1.186	0.314	1.203	0.330	1.132	0.460	1.501	0.057	1.337	0.088
SEC16B, RASAL2	rs10913469	0.955	0.746	1.070	0.659	0.978	0.872	0.866	0.402	0.901	0.458
INSIG2	rs11684454	1.127	0.323	0.882	0.353	1.056	0.650	0.985	0.917	0.889	0.334
TMEM18	rs7561317	1.233	0.186	0.868	0.409	0.826	0.225	0.714	0.076	0.824	0.221
ADIPOQ	rs17300539	0.838	0.430	1.203	0.469	1.204	0.401	1.089	0.755	1.077	0.738
PPARG	rs1801282	0.774	0.145	0.992	0.967	1.019	0.912	0.940	0.764	0.743	0.091
SFRS10, ETV5, DGKG	rs7647305	1.114	0.438	1.333	0.066	1.395	**0.017**	1.460	**0.032**	1.265	0.092
UCP1	rs45539933	0.835	0.455	0.864	0.559	0.713	0.152	0.666	0.148	0.947	0.816
ADRB2	rs12654778	1.340	**0.018**	1.101	0.471	1.063	0.612	1.156	0.314	1.000	0.999
PCSK1	rs12186664	0.919	0.500	0.822	0.151	0.840	0.157	1.020	0.892	1.057	0.653
PRL	rs4145443	0.963	0.735	1.052	0.683	1.013	0.910	1.008	0.954	1.165	0.174
TNKS-MSRA	rs13278851	0.844	0.334	0.822	0.322	0.901	0.549	0.912	0.663	1.069	0.705
	rs17150703	0.925	0.661	0.847	0.408	0.935	0.705	0.907	0.647	1.056	0.760
	rs516175	0.954	0.773	0.887	0.515	0.960	0.804	0.971	0.884	1.015	0.930
TRHR	rs7832552	0.967	0.779	0.927	0.558	0.797	**0.054**	1.017	0.907	0.893	0.342
ADRA2A	rs1800544	1.115	0.388	0.842	0.220	0.959	0.737	0.752	0.072	1.026	0.839
PFKP	rs17132175	0.919	0.668	1.041	0.852	1.063	0.753	0.830	0.444	1.100	0.627
PTER	rs10508503	1.998	**0.004**	1.748	**0.033**	1.479	0.094	1.531	0.133	1.178	0.486
BDNF	rs16917237	0.884	0.392	1.032	0.842	0.859	0.285	1.058	0.745	0.851	0.262
MTCH2	rs10838738	0.998	0.986	0.972	0.828	1.107	0.389	1.186	0.232	1.073	0.558
GNB3	rs5443	0.858	0.198	0.971	0.824	0.963	0.749	0.798	0.129	0.982	0.876
PLIN	rs894160	1.017	0.892	0.917	0.519	0.819	0.102	0.897	0.466	0.991	0.939
FTO	rs6499640	0.803	0.057	0.868	0.259	1.074	0.529	0.851	0.250	0.946	0.626
	rs7206010	0.801	0.056	0.865	0.252	1.038	0.744	0.866	0.311	0.917	0.451
	rs9935401	1.088	0.470	0.952	0.704	0.874	0.242	1.053	0.719	0.842	0.140
	rs9939609	1.105	0.391	0.984	0.902	0.893	0.322	1.114	0.449	0.878	0.264
MAF	rs1424233	0.988	0.919	0.912	0.467	0.981	0.861	0.836	0.195	1.027	0.818
MC4R	rs1673482	0.947	0.642	0.784	0.064	0.730	**0.008**	0.882	0.375	0.688	**0.002**
	rs17700144	0.872	0.318	0.767	0.075	0.729	**0.022**	0.819	0.212	0.680	**0.006**
	rs17782313	1.026	0.844	0.877	0.350	0.816	0.116	0.946	0.719	0.684	**0.004**
	rs502933	0.969	0.794	0.780	0.066	0.734	**0.011**	0.902	0.478	0.712	**0.006**
NPC1	rs1805081	1.072	0.558	0.981	0.882	0.943	0.616	0.839	0.218	0.939	0.592
KCTD15	rs11084753	0.883	0.292	0.838	0.173	0.903	0.383	0.772	0.067	0.874	0.251
	rs29941	0.851	0.175	0.915	0.496	1.020	0.863	0.976	0.860	0.960	0.730
HTR2C	rs6318*	1.158	0.406	1.689	**0.009**	1.205	0.288	1.122	0.602	0.933	0.693

Both datasets (completers and BCF) were analyzed for delta weight after six and twelve months; odds ratios (ORs) and p-values for lower weight loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age and sex was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Appendix Q: Results from logistic regression – percent weight loss in Weight Watchers (WW) study

Table Q-1: Results from logistic regression concerning percent weight loss after six and twelve months

Locus	SNP	5% delta weight (6 months)		5% delta weight (12 months)		10% delta weight (6 months)		10% delta weight (12 months)	
		OR	**p-value**	**OR**	**p-value**	**OR**	**p-value**	**OR**	**p-value**
LEPR	rs1805134	1.085	0.593	0.963	0.821	1.078	0.723	1.208	0.364
NEGR1	rs2568958	0.919	0.509	0.733	**0.031**	0.785	0.159	0.690	**0.028**
	rs2815752	0.919	0.509	0.733	**0.031**	0.785	0.159	0.690	**0.028**
SDCCAG8	rs10926984	1.150	0.474	1.429	0.104	1.455	0.196	1.691	0.066
	rs12145833	1.091	0.660	1.374	0.150	1.349	0.295	1.470	0.166
	rs2783963	1.307	0.160	1.411	0.110	1.550	0.129	1.546	0.114
SEC16B, RASAL2	rs10913469	1.039	0.804	0.959	0.808	1.134	0.561	0.918	0.668
INSIG2	rs11684454	0.883	0.361	0.966	0.811	0.864	0.417	1.114	0.535
TMEM18	rs7561317	0.807	0.215	0.698	0.058	0.661	0.055	0.640	**0.032**
ADIPOQ	rs17300539	1.154	0.575	0.920	0.762	0.987	0.971	0.907	0.758
PPARG	rs1801282	1.075	0.713	1.194	0.396	1.090	0.749	0.824	0.410
SFRS10, ETV5, DGKG	rs7647305	1.398	**0.033**	1.563	**0.013**	1.279	0.267	1.647	**0.029**
UCP1	rs45539933	0.871	0.583	0.558	**0.039**	0.677	0.204	0.808	0.495
ADRB2	rs12654778	1.050	0.716	1.172	0.272	0.917	0.626	1.014	0.933
PCSK1	rs12186664	0.921	0.549	1.112	0.476	1.060	0.753	1.006	0.975
PRL	rs4145443	1.043	0.735	1.085	0.548	1.079	0.652	1.028	0.863
TNKS-MSRA	rs13278851	0.815	0.304	0.970	0.886	0.917	0.740	1.001	0.997
	rs17150703	0.856	0.438	0.918	0.690	0.921	0.756	0.932	0.779
	rs516175	0.905	0.589	1.004	0.985	0.834	0.449	1.043	0.862
TRHR	rs7832552	0.960	0.752	1.088	0.559	1.054	0.766	1.259	0.191
ADRA2A	rs1800544	0.956	0.750	0.837	0.259	0.845	0.363	0.824	0.290
PFKP	rs17132175	1.190	0.421	0.865	0.549	1.839	0.091	1.599	0.152
PTER	rs10508503	1.718	**0.039**	1.455	0.194	1.404	0.372	1.227	0.557
BDNF	rs16917237	1.116	0.490	1.003	0.988	0.820	0.343	0.784	0.226
MTCH2	rs10838738	1.012	0.928	1.169	0.278	1.356	0.100	1.344	0.093
GNB3	rs5443	0.862	0.269	0.832	0.217	0.876	0.457	0.960	0.814
PLIN	rs894160	0.920	0.537	0.904	0.499	0.743	0.093	0.896	0.528
FTO	rs6499640	0.856	0.219	0.776	0.073	0.801	0.187	0.884	0.455
	rs7206010	0.887	0.343	0.798	0.114	0.841	0.311	0.945	0.737
	rs9935401	0.971	0.821	1.099	0.509	0.974	0.882	0.941	0.718
	rs9939609	0.995	0.966	1.141	0.357	0.982	0.916	0.971	0.864
MAF	rs1424233	0.926	0.542	0.844	0.220	0.960	0.809	0.941	0.711
MC4R	rs1673482	0.778	0.056	0.843	0.233	0.621	**0.006**	0.706	**0.037**
	rs17700144	0.720	**0.029**	0.753	0.077	0.682	**0.043**	0.823	0.291
	rs17782313	0.851	0.253	0.856	0.311	0.808	0.247	0.890	0.517
	rs502933	0.779	0.065	0.861	0.309	0.664	**0.020**	0.718	**0.054**
NPC1	rs1805081	0.915	0.497	0.899	0.455	0.864	0.405	0.886	0.472
KCTD15	rs11084753	0.812	0.109	0.727	**0.025**	0.909	0.578	0.911	0.570
	rs29941	0.904	0.439	0.877	0.355	0.931	0.686	1.044	0.800
HTR2C	rs6318*	1.513	**0.037**	1.167	0.485	1.249	0.416	1.051	0.848

Only completer dataset was analyzed; odds ratios (ORs) and p-values for lower percent weight loss are shown; variables were dichotomized according to their ≤ and > 5 or 10 percent weight loss; an additive genetic model was assumed; adjustment for age, and sex was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Appendix R: Results from linear regression – Weight Watchers (WW) study

Table R-1: Results from linear regression concerning delta weight, fat mass, and waist circumference after two, six and twelve months

Locus	SNP	Delta weight (2 months)		Delta weight (6 months)		Delta weight (12 months)		Delta fat mass (2 months)		Delta fat mass (6 months)		Delta fat mass (12 months)		Delta waist (2 months)		Delta waist (6 months)		Delta waist (12 months)	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	-0.058	0.759	0.214	0.564	0.108	0.829	-0.036	0.867	0.243	0.513	0.154	0.736	0.043	0.894	0.180	0.688	0.706	0.235
NEGR1	rs2568958	-0.284	0.079	-0.498	0.108	-1.053	**0.013**	-0.280	0.128	-0.157	0.612	-0.490	0.207	-0.390	0.159	-0.374	0.322	-0.968	0.055
	rs2815752	-0.284	0.079	-0.498	0.108	-1.053	**0.013**	-0.280	0.128	-0.157	0.612	-0.490	0.207	-0.390	0.159	-0.374	0.322	-0.967	0.055
SDCCAG8	rs10926984	0.224	0.365	0.676	0.154	1.433	**0.025**	0.179	0.529	0.461	0.333	0.588	0.319	0.263	0.534	-0.011	0.985	0.983	0.196
	rs12145833	0.105	0.673	0.545	0.256	1.127	0.080	0.105	0.710	0.338	0.479	0.480	0.413	0.236	0.578	-0.153	0.793	0.565	0.460
	rs2783963	0.368	0.122	0.801	0.080	1.374	**0.029**	0.302	0.264	0.611	0.185	0.554	0.338	0.272	0.503	0.076	0.890	0.986	0.185
SEC16B, RASAL2	rs10913469	-0.020	0.922	-0.068	0.857	-0.486	0.342	0.092	0.680	0.467	0.199	-0.149	0.746	-0.188	0.583	-0.573	0.211	-0.734	0.228
INSIG2	rs11684454	0.090	0.596	0.092	0.781	0.010	0.982	0.214	0.265	0.421	0.203	0.395	0.319	0.370	0.203	0.417	0.301	-0.158	0.757
TMEM18	rs7561317	-0.033	0.882	-0.533	0.201	-0.662	0.235	-0.294	0.246	-0.400	0.336	-0.467	0.359	0.094	0.807	-1.149	**0.022**	-1.014	0.127
ADIPOQ	rs17300539	-0.322	0.303	-0.239	0.700	-0.298	0.716	-0.175	0.628	0.257	0.688	-0.206	0.789	-0.440	0.408	-0.997	0.183	-1.748	0.072
PPARG	rs1801282	-0.077	0.754	-0.032	0.947	-0.543	0.381	0.077	0.782	-0.083	0.861	-0.627	0.268	-0.501	0.228	-0.743	0.199	-0.684	0.348
SFRS10, ETV5, DGKG	rs7647305	0.266	0.173	0.513	0.173	0.905	0.081	0.035	0.872	0.363	0.323	0.637	0.171	0.123	0.712	0.206	0.655	0.109	0.860
UCP1	rs45539933	-0.182	0.590	-0.257	0.672	-0.208	0.800	-0.569	0.130	-0.604	0.312	-0.838	0.262	0.670	0.247	0.287	0.698	0.838	0.391
ADRB2	rs12654778	0.104	0.544	-0.070	0.829	0.042	0.922	-0.005	0.980	-0.164	0.618	0.473	0.225	0.388	0.189	0.212	0.593	0.061	0.906
PCSK1	rs12186664	-0.163	0.351	-0.183	0.582	-0.011	0.981	-0.099	0.619	-0.022	0.948	0.162	0.690	0.018	0.951	0.162	0.689	0.319	0.541
PRL	rs4145443	0.065	0.681	0.006	0.983	0.182	0.654	0.320	0.074	0.183	0.543	0.420	0.254	0.258	0.339	-0.364	0.323	0.170	0.725
TNKS-MSRA	rs13278851	-0.342	0.165	-0.390	0.415	-0.158	0.803	-0.392	0.161	-0.773	0.100	-0.527	0.355	0.449	0.285	-0.202	0.729	-0.043	0.954
	rs17150703	-0.286	0.254	-0.329	0.497	-0.263	0.681	-0.390	0.171	-0.769	0.104	-0.615	0.286	0.438	0.304	-0.458	0.433	-0.493	0.514
	rs516175	-0.156	0.498	-0.282	0.529	-0.275	0.649	-0.357	0.174	-0.850	**0.054**	-0.757	0.161	0.416	0.288	-0.035	0.948	-0.325	0.649
TRHR	rs7832552	0.064	0.703	-0.258	0.410	0.147	0.731	0.316	0.092	-0.153	0.623	0.290	0.462	0.285	0.317	-0.147	0.695	0.606	0.229
ADRA2A	rs1800544	0.013	0.941	-0.178	0.602	-0.671	0.152	-0.108	0.587	-0.120	0.722	-0.308	0.467	0.268	0.377	-0.045	0.915	0.069	0.902
PFKP	rs17132175	0.046	0.869	0.416	0.425	0.364	0.616	0.110	0.722	0.295	0.571	0.593	0.360	-0.023	0.961	-0.084	0.899	0.159	0.856
PTER	rs10508503	0.663	**0.045**	1.144	0.064	0.875	0.294	-0.327	0.384	0.257	0.673	0.403	0.593	0.897	0.115	0.749	0.316	-0.104	0.916
BDNF	rs16917237	-0.288	0.157	-0.028	0.943	-0.663	0.201	-0.360	0.119	-0.273	0.474	-0.588	0.205	-0.295	0.390	0.321	0.487	-0.151	0.804
MTCH2	rs10838738	0.060	0.722	0.244	0.443	0.741	0.082	-0.210	0.270	0.340	0.280	0.674	0.083	-0.301	0.293	0.093	0.809	-0.070	0.890
GNB3	rs5443	-0.151	0.363	-0.228	0.483	-0.334	0.449	-0.079	0.676	-0.087	0.788	-0.254	0.521	-0.060	0.832	0.269	0.496	0.051	0.923
PLIN	rs894160	0.086	0.618	-0.287	0.378	-0.141	0.752	0.136	0.483	-0.356	0.268	-0.328	0.410	0.040	0.891	0.058	0.883	-0.340	0.521
FTO	rs6499640	-0.170	0.291	-0.330	0.283	-0.519	0.217	-0.070	0.703	-0.027	0.928	-0.342	0.373	-0.060	0.824	0.297	0.419	-0.455	0.360
	rs7206010	-0.154	0.342	-0.294	0.341	-0.452	0.285	-0.100	0.584	-0.048	0.875	-0.272	0.480	-0.022	0.937	0.430	0.250	-0.217	0.667
	rs9935401	-0.021	0.896	-0.241	0.450	-0.182	0.671	-0.271	0.144	-0.239	0.447	-0.137	0.723	-0.060	0.827	-0.134	0.732	-0.103	0.840
	rs9939609	0.019	0.907	-0.149	0.636	-0.069	0.872	-0.175	0.344	-0.094	0.761	-0.038	0.921	-0.081	0.773	-0.038	0.922	0.065	0.898
MAF	rs1424233	-0.200	0.212	-0.492	0.110	-0.613	0.134	-0.172	0.342	-0.546	0.074	-0.914	**0.014**	0.033	0.903	0.069	0.855	-0.522	0.287
MC4R	rs1673482	-0.241	0.145	-0.853	**0.007**	-0.988	**0.020**	-0.077	0.681	-0.359	0.251	-0.811	**0.033**	0.204	0.475	-0.306	0.427	-0.039	0.939
	rs17700144	-0.209	0.278	-0.867	**0.016**	-0.782	0.100	-0.104	0.632	-0.397	0.265	-0.481	0.262	0.075	0.819	-0.472	0.284	0.009	0.987
	rs17782313	-0.024	0.895	-0.438	0.201	-0.477	0.298	0.114	0.580	-0.001	0.998	-0.210	0.612	0.252	0.420	-0.192	0.646	0.202	0.711
	rs502933	-0.207	0.228	-0.818	**0.013**	-0.918	**0.035**	-0.033	0.862	-0.219	0.505	-0.590	0.134	0.189	0.514	-0.167	0.678	0.058	0.909
NPC1	rs1805081	-0.075	0.651	-0.206	0.516	-0.445	0.294	-0.055	0.771	-0.064	0.840	-0.287	0.460	-0.102	0.720	0.508	0.187	-0.211	0.676
KCTD15	rs11084753	0.040	0.810	-0.293	0.350	-0.610	0.146	0.337	0.073	-0.181	0.558	-0.386	0.305	0.318	0.261	-0.181	0.634	-0.636	0.204
	rs29941	0.060	0.720	0.079	0.804	-0.051	0.903	0.142	0.452	0.047	0.879	-0.143	0.706	0.486	0.087	0.545	0.153	-0.209	0.677
HTR2C	rs6318*	0.126	0.606	0.457	0.330	0.135	0.836	-0.126	0.635	0.042	0.928	-0.243	0.689	-0.158	0.715	0.280	0.631	0.516	0.502

Beta estimates (kg for weight and fat mass; cm for waist circumference) and p-values are shown; an additive genetic model was assumed; adjustment for age and sex was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Appendix S: Results from logistic regression – delta fat mass in Weight Watchers (WW) study

Table S-1: Results from logistic regression concerning delta fat mass after two, six and twelve months

Locus	SNP	Delta fat mass (2 months)		Delta fat mass (6 months)		Delta fat mass BCF (6 months)		Delta fat mass (12 months)		Delta fat mass BCF (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	1.030	0.834	1.090	0.599	1.096	0.521	1.022	0.904	0.956	0.751
NEGR1	rs2568958	0.771	**0.035**	0.880	0.345	0.891	0.341	0.759	0.071	0.931	0.560
	rs2815752	0.771	**0.035**	0.880	0.345	0.891	0.341	0.759	0.071	0.931	0.560
SDCCAG8	rs10926984	1.204	0.324	1.237	0.311	1.318	0.142	0.935	0.771	1.141	0.483
	rs12145833	1.151	0.453	1.202	0.380	1.330	0.128	0.896	0.633	1.106	0.588
	rs2783963	1.176	0.368	1.360	0.133	1.403	0.061	0.926	0.735	1.176	0.367
SEC16B, RASAL2	rs10913469	1.104	0.505	1.204	0.248	1.240	0.144	1.060	0.745	0.994	0.970
INSIG2	rs11684454	1.228	0.107	1.180	0.255	1.059	0.651	1.209	0.221	0.986	0.911
TMEM18	rs7561317	1.212	0.254	1.133	0.496	0.800	0.187	0.884	0.538	0.790	0.163
ADIPOQ	rs17300539	0.946	0.818	1.349	0.290	1.542	0.076	1.165	0.612	1.120	0.636
PPARG	rs1801282	0.976	0.896	0.977	0.910	0.952	0.788	0.921	0.709	0.721	0.079
SFRS10, ETV5, DGKG	rs7647305	1.046	0.754	1.381	**0.047**	1.252	0.119	1.301	0.151	1.157	0.316
UCP1	rs45539933	0.737	0.225	0.744	0.260	0.466	**0.003**	0.448	**0.010**	0.807	0.390
ADRB2	rs12654778	1.083	0.537	1.108	0.474	1.074	0.579	1.441	**0.019**	1.094	0.490
PCSK1	rs12186664	0.881	0.334	1.024	0.867	0.943	0.658	1.050	0.758	0.926	0.564
PRL	rs4145443	1.019	0.872	0.978	0.865	1.059	0.625	1.073	0.625	1.329	**0.018**
TNKS-MSRA	rs13278851	0.782	0.188	0.698	0.086	0.778	0.177	1.032	0.887	0.993	0.969
	rs17150703	0.776	0.182	0.683	0.073	0.767	0.161	0.995	0.981	0.993	0.971
	rs516175	0.815	0.243	0.686	0.056	0.836	0.305	0.942	0.776	0.968	0.850
TRHR	rs7832552	1.188	0.169	0.967	0.806	0.708	**0.007**	1.030	0.847	0.879	0.307
ADRA2A	rs1800544	0.858	0.248	1.104	0.504	1.043	0.749	0.869	0.394	1.126	0.368
PFKP	rs17132175	1.292	0.215	1.182	0.465	1.141	0.526	1.086	0.742	1.090	0.675
PTER	rs10508503	1.048	0.850	1.145	0.613	0.894	0.647	1.026	0.931	0.971	0.905
BDNF	rs16917237	0.996	0.981	1.074	0.669	0.790	0.124	1.114	0.549	0.742	**0.052**
MTCH2	rs10838738	0.996	0.975	0.987	0.927	1.051	0.692	1.049	0.752	1.091	0.491
GNB3	rs5443	1.026	0.834	1.116	0.435	1.249	0.074	0.836	0.250	1.078	0.546
PLIN	rs894160	1.234	0.101	0.897	0.441	0.943	0.647	0.855	0.312	1.050	0.700
FTO	rs6499640	0.913	0.449	0.923	0.551	1.084	0.499	0.888	0.425	1.026	0.830
	rs7206010	0.899	0.380	0.928	0.578	1.061	0.623	0.910	0.531	1.020	0.873
	rs9935401	0.847	0.179	0.877	0.337	0.794	0.060	1.014	0.927	0.784	**0.049**
	rs9939609	0.918	0.488	0.965	0.792	0.865	0.230	1.072	0.643	0.817	0.098
MAF	rs1424233	0.870	0.245	0.857	0.251	0.841	0.149	0.744	**0.044**	0.815	0.090
MC4R	rs1673482	0.939	0.609	0.887	0.384	0.854	0.203	0.806	0.150	0.675	**0.002**
	rs17700144	0.869	0.332	0.707	**0.029**	0.677	**0.008**	0.876	0.428	0.689	**0.011**
	rs17782313	1.023	0.867	0.865	0.329	0.778	0.068	1.019	0.907	0.694	**0.009**
	rs502933	0.995	0.967	0.914	0.523	0.867	0.259	0.843	0.263	0.699	**0.005**
NPC1	rs1805081	1.072	0.581	0.996	0.975	1.094	0.474	0.901	0.491	1.035	0.785
KCTD15	rs11084753	0.966	0.779	0.839	0.196	0.856	0.206	0.868	0.336	0.941	0.620
	rs29941	0.921	0.509	0.940	0.650	0.956	0.717	0.950	0.726	1.024	0.851
HTR2C	rs6318*	1.168	0.396	1.325	0.172	1.125	0.518	0.842	0.464	0.851	0.385

Both datasets (completers and BCF) were analyzed for delta fat mass after six and twelve months; odds ratios (ORs) and p-values for lower fat mass loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age and sex was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Appendix T: Results from logistic regression – delta waist circumference in Weight Watchers (WW) study

Table T-1: Results from logistic regression concerning delta waist circumference after two, six and twelve months

Locus	SNP	Delta waist (2 months)		Delta waist (6 months)		Delta waist BCF (6 months)		Delta waist (12months)		Delta waist BCF (12 months)	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
LEPR	rs1805134	0.914	0.514	1.043	0.784	1.140	0.331	1.049	0.780	1.277	0.075
NEGR1	rs2568958	0.780	**0.037**	0.819	0.126	0.846	0.145	0.745	**0.044**	0.897	0.348
	rs2815752	0.780	**0.037**	0.819	0.126	0.846	0.145	0.745	**0.044**	0.897	0.348
SDCCAG8	rs10926984	1.001	0.997	1.001	0.996	0.853	0.366	1.113	0.621	1.105	0.573
	rs12145833	1.061	0.742	0.953	0.809	0.825	0.278	1.050	0.825	1.070	0.704
	rs2783963	1.092	0.605	0.941	0.751	0.850	0.336	1.170	0.461	1.202	0.281
SEC16B, RASAL2	rs10913469	0.900	0.468	1.037	0.816	0.915	0.532	0.900	0.544	0.862	0.300
INSIG2	rs11684454	1.138	0.292	1.171	0.251	1.138	0.283	1.030	0.841	0.786	**0.050**
TMEM18	rs7561317	1.158	0.362	0.768	0.129	0.686	**0.019**	0.722	0.088	0.759	0.084
ADIPOQ	rs17300539	0.993	0.976	0.911	0.716	1.002	0.994	0.565	**0.049**	0.971	0.893
PPARG	rs1801282	0.753	0.115	0.740	0.133	0.909	0.583	0.674	0.063	0.731	0.076
SFRS10, ETV5, DGKG	rs7647305	1.139	0.353	1.205	0.236	1.054	0.705	1.241	0.224	1.227	0.143
UCP1	rs45539933	1.636	**0.043**	1.059	0.821	0.750	0.229	1.250	0.425	0.663	0.089
ADRB2	rs12654778	1.248	0.076	0.962	0.778	1.160	0.225	0.987	0.930	0.978	0.855
PCSK1	rs12186664	0.975	0.843	0.990	0.944	0.924	0.520	1.264	0.120	0.914	0.467
PRL	rs4145443	1.057	0.627	0.953	0.700	1.030	0.791	0.985	0.916	1.187	0.129
TNKS-MSRA	rs13278851	1.203	0.293	0.815	0.302	1.025	0.887	0.899	0.620	1.102	0.582
	rs17150703	1.190	0.331	0.707	0.090	0.901	0.558	0.769	0.229	0.997	0.986
	rs516175	1.253	0.172	0.840	0.350	0.957	0.790	0.780	0.226	0.976	0.883
TRHR	rs7832552	1.144	0.263	0.887	0.358	0.949	0.655	1.248	0.126	0.979	0.860
ADRA2A	rs1800544	1.033	0.797	0.842	0.227	1.000	0.997	1.021	0.896	1.133	0.325
PFKP	rs17132175	1.100	0.635	0.920	0.711	1.095	0.647	0.993	0.978	1.133	0.531
PTER	rs10508503	1.442	0.122	1.307	0.297	1.199	0.432	0.823	0.489	0.904	0.667
BDNF	rs16917237	0.946	0.705	0.982	0.909	0.956	0.750	0.921	0.639	0.889	0.411
MTCH2	rs10838738	1.067	0.593	1.054	0.692	1.004	0.973	0.995	0.972	0.940	0.605
GNB3	rs5443	0.974	0.827	0.973	0.837	1.069	0.569	0.968	0.827	1.062	0.611
PLIN	rs894160	1.113	0.388	0.926	0.572	0.997	0.979	0.931	0.636	1.042	0.737
FTO	rs6499640	1.010	0.929	1.005	0.967	1.149	0.220	0.994	0.969	0.978	0.848
	rs7206010	1.045	0.704	1.030	0.814	1.159	0.198	1.069	0.642	0.983	0.880
	rs9935401	1.055	0.653	0.941	0.644	0.837	0.126	1.030	0.840	0.929	0.529
	rs9939609	1.028	0.813	0.972	0.827	0.858	0.188	1.026	0.860	0.941	0.603
MAF	rs1424233	1.094	0.439	1.088	0.513	1.053	0.651	0.815	0.148	1.137	0.264
MC4R	rs1673482	0.942	0.621	0.952	0.708	0.794	**0.052**	0.982	0.899	0.783	**0.041**
	rs17700144	0.921	0.554	0.806	0.154	0.755	**0.043**	0.961	0.806	0.779	0.071
	rs17782313	1.008	0.953	0.885	0.394	0.767	**0.043**	1.082	0.611	0.778	0.056
	rs502933	0.976	0.844	1.027	0.842	0.835	0.136	1.003	0.984	0.832	0.133
NPC1	rs1805081	0.980	0.866	1.246	0.097	1.076	0.533	1.032	0.829	1.062	0.613
KCTD15	rs11084753	1.081	0.515	0.914	0.490	1.126	0.310	0.872	0.338	0.952	0.676
	rs29941	1.179	0.171	1.203	0.163	1.275	**0.041**	0.955	0.747	1.036	0.765
HTR2C	rs6318*	1.082	0.658	1.207	0.345	1.083	0.652	1.276	0.273	1.152	0.424

Both datasets (completers and BCF) were analyzed for delta waist circumference after six and twelve months; odds ratios (ORs) and p-values for lower waist circumference loss are shown; variables were dichotomized according to their median (≤ and >); waist circumference loss after twelve months (BCF) were dichotomized as < and ≥; an additive genetic model was assumed; adjustment for age and sex was done; p-values ≤ 0.05 are bold/grey; *) only analyzed in women

Appendix U: Results Kruskal-Wallis test – LOGIC study

Table U-1: P-values from the Kruskal-Wallis test are given for the LOGIC study population

Locus	SNP	Delta weight (4 weeks)	Delta weight (6 weeks)	Delta weight (4 or 6 weeks)	Delta BMI-SDS (4 weeks)	Delta BMI-SDS (6 weeks)	Delta BMI-SDS (4 or 6 weeks)
		p-value Kruskall-Wallis test					
LEPR	rs1805134	0.372	0.750	0.552	0.429	0.447	0.781
NEGR1	rs2568958	0.887	0.614	0.553	**0.042**	0.078	**0.002**
	rs2815752	0.921	0.676	0.597	**0.043**	0.081	**0.002**
SDCCAG8	rs10926984	**0.044**	0.407	**0.027**	0.205	0.693	0.061
	rs12145833	**0.044**	0.409	**0.027**	0.210	0.675	0.060
	rs2783963	**0.039**	0.210	**0.020**	0.253	0.495	0.062
SEC16B, RASAL2	rs10913469	0.804	0.645	0.769	0.807	0.795	0.548
INSIG2	rs11684454	0.135	0.636	0.288	0.586	0.635	0.472
TMEM18	rs7561317	0.839	0.640	0.707	0.136	0.195	0.120
ADIPOQ	rs17300539	0.472	0.526	0.393	0.294	0.177	0.375
PPARG	rs1801282	0.478	0.966	0.281	0.689	0.414	0.623
SFRS10, ETV5, DGKG	rs7647305	0.742	0.152	0.660	0.970	0.943	0.986
UCP1	rs45539933	0.106	0.576	0.206	0.793	0.464	0.718
ADRB2	rs12654778	0.176	0.801	0.155	0.315	0.143	0.340
PCSK1	rs12186664	0.978	0.182	0.999	0.151	0.261	0.140
PRL	rs4145443	0.603	0.684	0.335	0.965	0.898	0.831
IL6	rs1554606	0.179	0.575	0.167	0.143	0.770	0.176
TNKS-MSRA	rs13278851	0.619	0.707	0.374	0.843	0.382	0.334
	rs17150703	0.550	0.725	0.500	0.775	0.280	0.523
	rs516175	0.675	0.444	0.675	0.995	0.505	0.573
TRHR	rs7832552	0.417	0.315	0.638	0.319	0.597	0.218
ADRA2A	rs1800544	0.798	0.058	0.461	0.534	0.262	0.687
PFKP	rs17132175	0.845	0.301	0.898	0.345	**0.037**	0.321
PTER	rs10508503	0.439	0.551	0.298	0.581	0.962	0.681
BDNF	rs16917237	0.823	0.393	0.812	0.396	0.155	0.550
MTCH2	rs10838738	0.076	0.065	**0.035**	0.811	0.699	0.623
MTNR1B	rs10830963	0.292	0.584	0.130	0.247	0.545	0.128
UCP2	rs659366	0.196	0.841	0.582	0.298	0.415	0.714
GNB3	rs5443	0.409	0.408	0.276	0.735	0.956	0.778
PLIN	rs894160	0.231	0.373	0.160	0.651	0.414	0.286
FTO	rs6499640	0.600	**0.042**	0.238	0.340	0.306	0.956
	rs7206010	0.591	0.083	0.214	0.233	0.269	0.919
	rs9935401	0.618	0.451	0.560	0.241	0.109	0.197
	rs9939609	0.630	0.504	0.552	0.215	0.086	0.187
MAF	rs1424233	0.963	0.749	0.865	0.248	0.314	0.061
SH2B1	rs7498665	0.355	0.233	0.721	0.565	0.856	0.360
MC4R	rs1673482	0.171	0.152	0.126	0.947	0.877	0.431
	rs17700144	0.984	0.769	0.892	0.523	0.832	0.925
	rs17782313	0.734	0.688	0.603	0.193	0.471	0.802
	rs502933	0.139	0.172	0.128	0.931	0.887	0.449
NPC1	rs1805081	0.709	0.470	0.456	0.721	0.519	0.447
KCTD15	rs11084753	0.992	0.498	0.908	0.399	0.267	0.500
	rs29941	0.634	0.234	0.499	0.591	0.549	0.571
HTR2C	rs6318*	**0.003**	**0.013**	**0.005**	0.352	0.253	0.250

Delta weight and BMI-SDS were analyzed after four and six weeks as well as after four and six weeks together; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls

Appendix V: Results from logistic regression – LOGIC study

Table V-1: Results from logistic regression concerning delta weight and BMI-SDS after four or six weeks or after four and six weeks together

Locus	SNP	Delta weight (4 weeks)		Delta weight (6 weeks)		Delta weight (4 or 6 weeks)		Delta BMI-SDS (4 weeks)		Delta BMI-SDS (6 weeks)		Delta BMI-SDS (4 or 6 weeks)	
		OR	**p-value**	**OR**	**p-value**	**OR**	**p-value**	**OR**	**p-value**	**OR**	**p-value**	**OR**	**p-value**
LEPR	rs1805134	1.188	0.429	0.823	0.471	1.395	0.171	1.121	0.570	1.000	1.000	1.226	0.348
NEGR1	rs2568958	1.199	0.306	1.091	0.699	1.526	**0.038**	0.773	0.117	0.726	0.127	0.688	**0.040**
	rs2815752	1.205	0.294	1.096	0.684	1.532	**0.036**	0.777	0.123	0.730	0.132	0.686	**0.039**
SDCCAG8	rs10926984	1.373	0.194	1.397	0.324	1.252	0.426	1.024	0.915	1.129	0.694	1.294	0.304
	rs12145833	1.391	0.176	1.425	0.295	1.249	0.431	1.045	0.841	1.160	0.630	1.308	0.285
	rs2783963	1.489	0.112	1.477	0.259	1.218	0.489	1.086	0.714	1.224	0.521	1.211	0.450
SEC16B, RASAL2	rs10913469	0.916	0.697	0.916	0.755	0.968	0.895	1.055	0.795	1.135	0.620	0.798	0.322
INSIG2	rs11684454	0.920	0.647	0.815	0.386	0.792	0.253	0.765	0.111	0.847	0.446	0.790	0.200
TMEM18	rs7561317	0.804	0.343	1.456	0.190	0.833	0.468	0.819	0.340	0.488	**0.010**	0.760	0.232
ADIPOQ	rs17300539	0.810	0.459	1.260	0.481	0.963	0.903	0.792	0.377	0.719	0.292	1.011	0.971
PPARG	rs1801282	1.145	0.607	1.244	0.547	1.244	0.482	0.886	0.613	0.850	0.624	0.801	0.407
SFRS10, ETV5, DGKG	rs7647305	1.219	0.369	1.489	0.162	1.886	**0.011**	1.039	0.849	1.184	0.511	1.109	0.637
UCP1	rs45539933	1.643	0.176	1.065	0.896	1.339	0.481	0.929	0.820	0.689	0.384	0.757	0.446
ADRB2	rs12654778	0.641	**0.011**	0.822	0.371	0.561	**0.004**	1.036	0.818	1.126	0.552	0.861	0.382
PCSK1	rs12186664	1.142	0.473	0.895	0.647	0.660	**0.053**	1.175	0.340	1.139	0.559	1.164	0.416
PRL	rs4145443	1.198	0.293	0.867	0.504	1.075	0.711	1.071	0.657	1.031	0.875	1.051	0.773
IL6	rs1554606	0.968	0.847	1.043	0.844	1.059	0.762	0.903	0.514	1.115	0.581	1.000	0.999
TNKS-MSRA	rs13278851	0.881	0.675	0.744	0.409	0.828	0.571	1.097	0.735	0.646	0.192	0.946	0.853
	rs17150703	0.835	0.555	0.730	0.396	0.772	0.444	1.066	0.816	0.579	0.117	0.993	0.981
	rs516175	0.955	0.861	0.943	0.848	0.834	0.527	1.040	0.867	0.697	0.207	0.886	0.637
TRHR	rs7832552	1.110	0.591	0.845	0.504	0.783	0.268	1.011	0.952	0.788	0.307	0.749	0.143
ADRA2A	rs1800544	1.183	0.380	1.781	**0.031**	1.284	0.243	0.778	0.158	0.849	0.488	0.770	0.177
PFKP	rs17132175	0.903	0.734	0.782	0.514	0.807	0.527	0.751	0.300	0.435	**0.024**	0.833	0.548
PTER	rs10508503	1.467	0.276	1.217	0.682	0.933	0.857	0.862	0.643	0.635	0.312	0.901	0.766
BDNF	rs16917237	1.313	0.180	1.080	0.765	0.972	0.904	1.056	0.767	0.808	0.374	0.813	0.313
MTCH2	rs10838738	0.837	0.308	0.563	**0.011**	0.865	0.458	1.199	0.260	0.985	0.939	1.126	0.499
MTNR1B	rs10830963	1.010	0.958	0.855	0.510	0.877	0.529	1.058	0.737	0.891	0.596	0.954	0.800
UCP2	rs659366	0.639	**0.011**	0.793	0.308	0.828	0.331	0.932	0.653	0.909	0.643	0.868	0.409
GNB3	rs5443	1.035	0.844	1.036	0.874	1.219	0.305	0.987	0.936	0.791	0.256	1.041	0.819
PLIN	rs894160	0.858	0.408	0.855	0.509	0.877	0.528	1.121	0.499	1.131	0.574	1.117	0.553
FTO	rs6499640	0.900	0.542	0.642	**0.043**	0.868	0.468	1.028	0.860	1.258	0.247	1.231	0.235
	rs7206010	0.893	0.514	0.655	**0.052**	0.865	0.459	1.021	0.898	1.266	0.229	1.244	0.211
	rs9935401	1.052	0.759	1.051	0.815	0.988	0.948	0.995	0.975	1.268	0.228	1.179	0.319
	rs9939609	1.031	0.851	1.038	0.861	1.005	0.976	0.985	0.921	1.254	0.245	1.203	0.262
MAF	rs1424233	0.849	0.308	0.984	0.935	1.026	0.887	0.853	0.279	0.924	0.672	0.722	**0.046**
SH2B1	rs7498665	0.931	0.671	1.398	0.115	0.997	0.986	0.905	0.512	0.899	0.579	0.756	0.100
MC4R	rs1673482	0.892	0.502	0.898	0.612	0.913	0.630	1.148	0.366	1.020	0.918	0.861	0.376
	rs17700144	0.883	0.493	0.872	0.552	0.928	0.713	1.130	0.454	1.164	0.465	1.012	0.947
	rs17782313	0.838	0.320	0.776	0.256	0.932	0.720	1.260	0.150	1.241	0.283	1.095	0.607
	rs502933	0.926	0.645	0.907	0.645	0.950	0.786	1.136	0.401	1.035	0.856	0.850	0.334
NPC1	rs1805081	1.136	0.446	1.120	0.614	0.950	0.791	0.869	0.356	0.708	0.098	0.866	0.393
KCTD15	rs11084753	0.972	0.883	1.008	0.974	1.062	0.779	1.364	0.077	1.239	0.347	1.195	0.350
	rs29941	1.055	0.774	1.024	0.924	1.067	0.756	1.318	0.106	1.061	0.792	1.200	0.330
HTR2C	rs6318*	1.563	0.142	1.812	0.133	1.991	**0.052**	0.972	0.919	1.688	0.172	1.260	0.458

Odds ratios (OR) and p-values for lower loss are shown; variables were dichotomized according to their median (≤ and >); an additive genetic model was assumed; adjustment for age and sex was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls

Appendix W: Results from linear regression – delta weight in LOGIC study

Table W-1: Results from linear regression concerning delta weight after four or six weeks or after four and six weeks together

Locus	SNP	Delta weight (log) (4 weeks)		Delta weight (log) (6 weeks)		Delta weight (log) (4 or 6 weeks)	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	0.004	0.897	-0.027	0.458	-0.010	0.743
NEGR1	rs2568958	-0.023	0.340	-0.003	0.908	-0.018	0.455
	rs2815752	-0.022	0.366	-0.002	0.942	-0.017	0.480
SDCCAG8	rs10926984	0.045	0.172	-0.018	0.683	0.047	0.153
	rs12145833	0.046	0.162	-0.017	0.702	0.047	0.147
	rs2783963	0.053	0.113	-0.002	0.969	0.054	0.106
SEC16B, RASAL2	rs10913469	-0.025	0.416	-0.016	0.667	-0.025	0.425
INSIG2	rs11684454	0.008	0.751	-0.022	0.491	0.004	0.868
TMEM18	rs7561317	-0.016	0.601	0.008	0.837	-0.007	0.815
ADIPOQ	rs17300539	0.008	0.829	0.013	0.768	0.034	0.382
PPARG	rs1801282	0.059	0.095	0.042	0.379	0.045	0.210
SFRS10, ETV5, DGKG	rs7647305	0.035	0.246	0.105	**0.004**	0.048	0.110
UCP1	rs45539933	0.083	0.085	0.032	0.603	0.074	0.123
ADRB2	rs12654778	-0.061	**0.008**	-0.032	0.268	-0.058	**0.011**
PCSK1	rs12186664	-0.004	0.865	-0.049	0.128	-0.037	0.145
PRL	rs4145443	0.033	0.150	0.029	0.298	0.028	0.233
IL6	rs1554606	-0.029	0.210	0.027	0.338	-0.020	0.393
TNKS-MSRA	rs13278851	-0.002	0.967	0.007	0.885	-0.006	0.887
	rs17150703	-0.003	0.943	0.004	0.939	-0.009	0.830
	rs516175	0.040	0.246	0.050	0.211	0.033	0.349
TRHR	rs7832552	0.028	0.290	0.002	0.963	0.017	0.512
ADRA2A	rs1800544	0.030	0.252	0.085	**0.012**	0.031	0.240
PFKP	rs17132175	-0.025	0.542	-0.080	0.109	-0.019	0.648
PTER	rs10508503	0.030	0.524	-0.004	0.948	0.014	0.767
BDNF	rs16917237	0.007	0.792	0.024	0.481	-0.006	0.823
MTCH2	rs10838738	-0.022	0.369	-0.036	0.212	-0.016	0.497
MTNR1B	rs10830963	0.006	0.803	-0.020	0.525	-0.007	0.778
UCP2	rs659366	-0.044	0.057	-0.009	0.771	-0.037	0.110
GNB3	rs5443	0.018	0.445	0.020	0.491	0.026	0.281
PLIN	rs894160	-0.021	0.392	-0.037	0.240	-0.011	0.676
FTO	rs6499640	-0.003	0.908	-0.051	0.076	0.004	0.857
	rs7206010	-0.006	0.789	-0.047	0.096	0.001	0.977
	rs9935401	0.010	0.657	0.007	0.816	0.010	0.655
	rs9939609	0.008	0.709	0.010	0.733	0.009	0.686
MAF	rs1424233	-0.009	0.669	0.006	0.833	-0.001	0.981
SH2B1	rs7498665	0.009	0.681	0.028	0.318	0.021	0.352
MC4R	rs1673482	-0.015	0.497	-0.022	0.434	-0.009	0.688
	rs17700144	-0.007	0.764	-0.014	0.639	-0.007	0.763
	rs17782313	-0.021	0.386	-0.026	0.364	-0.016	0.501
	rs502933	-0.012	0.596	-0.021	0.459	-0.006	0.802
NPC1	rs1805081	0.002	0.938	0.026	0.386	-0.003	0.907
KCTD15	rs11084753	-0.035	0.176	-0.005	0.878	-0.039	0.133
	rs29941	-0.008	0.765	0.009	0.790	-0.018	0.468
HTR2C	rs6318*	0.146	**0.0006**	0.139	**0.006**	0.140	**0.0007**

Beta estimates and p-values are shown; an additive genetic model was assumed; adjustment for age and sex was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls; log=logarithmized

Appendix X: Results from linear regression – delta BMI-SDS in LOGIC study

Table X-1: Results from linear regression concerning delta BMI-SDS after four or six weeks or after four and six weeks together

Locus	SNP	Delta BMI-SDS (4 weeks)		Delta BMI-SDS (6 weeks)		Delta BMI-SDS (4 or 6 weeks)	
		beta	**p-value**	**beta**	**p-value**	**beta**	**p-value**
LEPR	rs1805134	0.010	0.335	-0.009	0.583	0.001	0.919
NEGR1	rs2568958	-0.019	**0.025**	-0.021	0.119	-0.023	**0.018**
	rs2815752	-0.018	**0.025**	-0.021	0.119	-0.023	**0.018**
SDCCAG8	rs10926984	0.000	0.970	0.003	0.879	0.003	0.825
	rs12145833	0.001	0.928	0.004	0.858	0.003	0.798
	rs2783963	0.004	0.753	0.012	0.570	0.007	0.582
SEC16B, RASAL2	rs10913469	-0.008	0.473	-0.024	0.151	-0.014	0.251
INSIG2	rs11684454	-0.010	0.236	-0.015	0.288	-0.012	0.221
TMEM18	rs7561317	-0.014	0.179	-0.038	**0.020**	-0.020	0.109
ADIPOQ	rs17300539	-0.010	0.456	-0.009	0.655	-0.003	0.837
PPARG	rs1801282	-0.007	0.561	-0.024	0.265	-0.014	0.321
SFRS10, ETV5, DGKG	rs7647305	0.001	0.923	0.003	0.838	0.006	0.602
UCP1	rs45539933	-0.004	0.795	-0.025	0.349	-0.014	0.456
ADRB2	rs12654778	-0.004	0.565	-0.010	0.422	-0.007	0.457
PCSK1	rs12186664	0.015	0.083	0.009	0.543	0.007	0.508
PRL	rs4145443	0.008	0.316	0.013	0.307	0.007	0.473
IL6	rs1554606	-0.010	0.217	-0.005	0.707	-0.010	0.298
TNKS-MSRA	rs13278851	0.001	0.924	-0.013	0.545	-0.005	0.752
	rs17150703	-0.000	0.986	-0.019	0.388	-0.006	0.733
	rs516175	0.005	0.659	-0.009	0.625	0.001	0.968
TRHR	rs7832552	-0.003	0.708	-0.014	0.354	-0.009	0.398
ADRA2A	rs1800544	-0.009	0.324	-0.016	0.296	-0.008	0.432
PFKP	rs17132175	-0.021	0.121	-0.061	**0.006**	-0.031	**0.053**
PTER	rs10508503	0.003	0.860	0.004	0.881	0.003	0.890
BDNF	rs16917237	-0.002	0.840	-0.009	0.563	-0.008	0.478
MTCH2	rs10838738	-0.001	0.926	-0.001	0.943	0.000	0.988
MTNR1B	rs10830963	0.011	0.216	-0.001	0.967	0.006	0.560
UCP2	rs659366	-0.007	0.364	-0.006	0.671	-0.005	0.599
GNB3	rs5443	-0.001	0.938	-0.008	0.553	0.002	0.810
PLIN	rs894160	0.001	0.910	0.006	0.651	0.006	0.585
FTO	rs6499640	0.004	0.615	0.019	0.132	0.007	0.453
	rs7206010	0.005	0.568	0.020	0.113	0.008	0.416
	rs9935401	0.009	0.220	0.022	0.082	0.013	0.151
	rs9939609	0.010	0.210	0.022	0.074	0.013	0.137
MAF	rs1424233	-0.014	0.065	-0.024	0.050	-0.018	**0.039**
SH2B1	rs7498665	-0.005	0.478	-0.003	0.789	-0.002	0.795
MC4R	rs1673482	0.001	0.866	-0.005	0.679	-0.002	0.824
	rs17700144	0.009	0.258	0.012	0.375	0.009	0.378
	rs17782313	0.014	0.090	0.019	0.143	0.015	0.125
	rs502933	0.001	0.926	-0.005	0.690	-0.002	0.794
NPC1	rs1805081	-0.004	0.612	-0.008	0.550	-0.008	0.401
KCTD15	rs11084753	0.005	0.559	0.011	0.440	0.005	0.618
	rs29941	-0.001	0.915	0.003	0.816	-0.003	0.782
HTR2C	rs6318*	0.019	0.124	0.028	0.149	0.024	0.096

Beta estimates and p-values are shown; an additive genetic model was assumed; adjustment for age and sex was done; the four or six weeks together analysis was adjusted also for duration of stay; p-values ≤ 0.05 are bold/grey; *) only analyzed in girls

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