DIETARY FACTORS, OBESITY AND SERUM LIPOPROTEIN PROFILE: A nutritional epidemiological study in young adult twins

Leonie-Helen Bogl

ACADEMIC DISSERTATION

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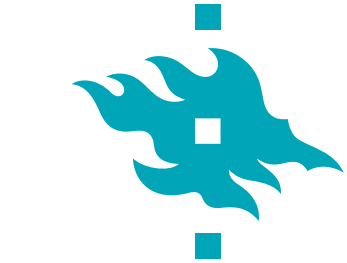

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”To eat is a necessity, but to eat intelligently is an art.” - La Rochefoucauld.

To my family

Contents

Abstract **8**
Tiivistelmä **10**
List of original publications **12**
Abbreviations **13**
1 Introduction **15**
2 Review of the literature **18**
2.1 The value of twin studies in health research 18
2.1.1 Twinning 18
2.1.2 Twin study designs 20
2.1.3 Heritability of diet and physical activity 21
2.1.4 Heritability of obesity and serum lipids 22
2.2 Non-dietary factors associated with obesity and serum lipids 24
2.2.1 Common genetic variants 24
2.2.2 Sex differences 24
2.2.3 Aging 25
2.2.4 Physical activity 26
2.2.5 Smoking 27
2.2.6 Other non-dietary factors 28
2.3 Dietary factors associated with obesity and serum lipids 29
2.3.1 Eating behaviors 29
2.3.2 Meal patterns: snacking and meal skipping 31
2.3.3 Portion size and energy density 33
2.3.4 Dietary macronutrients 36
2.3.5 Food groups 39
2.3.6 Dietary patterns 40
2.4 Assessment of habitual diet and physical activity 43
2.4.1 Dietary and physical activity assessment methods 43
2.4.2 Misreporting of self-reported food intake and physical activity behavior 46
3 Aims of the Study **48**
4 Materials and Methods **49**
4.1 Study populations 49
4.1.1 FinnTwin16 and FinnTwin12 cohorts 50
4.1.2 Obesity-discordant monozygotic twin pairs 51
4.1.3 Ethical considerations 52

4.2 Measures 52
4.2.1 Measures of obesity and body fat 53
4.2.2 Assessment of diet, physical activity and smoking 53
4.2.3 Biochemical analysis of serum lipid profiles 57
4.2.4 Zygosity 58
4.3 Statistical methods 58
6.3.1 Individual-level analyses 59
6.3.2 Multivariate nutrient density model 59
6.3.3 Within-pair analyses 60
5 Results 61
5.1 Co-twin comparison of eating and physical activity habits (I) 61
5.2 Diet and physical activity in obesity-discordant monozygotic twin pairs (II) 68
5.3 Associations between macronutrient intake and serum lipoprotein profile (III) 71
5.4 Associations between obesity and lipoprotein profile (IV) 73
5.5 Associations between dietary patterns and lipoprotein profile (V) 76
5.6 Associations between serum docosahexaenoic acid and lipoprotein profile (V) 80
6 Discussion 81
6.1 Summary of the main findings 81
6.2 Comparison with previous studies 82
6.2.1 Co-twin comparisons of eating and physical activity habits 82
6.2.2 Energy underreporting in obesity-discordant monozygotic twin pairs 85
6.2.3 Associations between dietary factors and serum lipoprotein profile 87
6.2.4 Association between obesity and serum lipoprotein profile 90
6.3 Methodological considerations 92
6.3.1 Cross-sectional design 92
6.3.2 Confounding 92
6.3.3 Self-reported anthropometric measures 93
6.3.4 Diet and physical activity assessment methods 93
6.3.5 Generalizability 94
6.3.6 Genetic and epigenetic discordance in MZ twins 95
6.3.7 Other methodological considerations 96
7 Conclusions and future directions 97
Acknowledgements 99
References 102
Appendices 126
Original publications 134

Abstract

Background: Energy-dense food and low physical activity have been blamed for the dramatic rise in the prevalence of obesity and related metabolic disorders. However, few dietary and physical activity factors have been consistently associated with obesity in observational studies. Self-reported behaviors are prone to misreporting, which may partly explain the inconsistency of previous results. Nutritional biomarkers provide an objective approach to measure habitual intake, but valid biomarkers are not available for all types of dietary exposures.

Aims: The aims of this dissertation were to 1) validate self-reported dietary intake and physical activity by using objective co-twin comparison assessments (I) and the doubly labeled water (DLW) technique (II); 2) determine whether eating and physical activity behaviors are associated with body mass index (BMI) and waist circumference (WC) (I, II); 3) examine whether acquired obesity (IV) and dietary factors (III, V) are associated with serum lipoprotein profiles.

Materials and methods: The following participants were recruited from the FinnTwin12 and FinnTwin16 studies, two population-based studies on young adult Finnish twins: 1) 713 monozygotic (MZ) and 698 dizygotic (DZ) twin pairs of the same sex who filled in food-frequency questionnaires (FFQ) and co-twin comparison questions (I); 2) 14 MZ twin pairs discordant (BMI difference > 3kg/m^2) and 10 pairs concordant for obesity who provided 3-day food and activity diaries, eating behavior questionnaires, co-twin comparison questions and total energy expenditure (TEE) measurements determined by DLW (II); 3) 15 obesity-discordant and 9 concordant MZ twin pairs who completed 3-day food diaries, physical activity questionnaires and measurements of subcutaneous adipose tissue, visceral adipose tissue and liver fat by magnetic resonance imaging and spectroscopy and of serum lipid determinations by ultracentrifugation, gradient gel electrophoresis and enzymatic techniques (III, IV); 4) 663 twin individuals who provided FFQs and nuclear magnetic resonance spectroscopy-derived measurements of serum lipoproteins and serum docosahexaenoic acid (DHA) as an objective biomarker of DHA intake (V).

Results: In both zygosity groups, the co-twins for whom both twin pair members concordantly answered that the identified twin eats more (overall), or eats more fatty foods, or eats more sweet and fatty delicacies, or eats faster, or selects food less according to healthiness or makes less active choices in daily life had significantly higher BMIs and WCs than their twin siblings. Eating more (overall), eating more sweet and fatty delicacies and exercising less remained significant predictors of intrapair differences in BMI and WC independent of each other as evaluated by multivariate regression analysis. Co-twin comparison questions on snacking, fatty foods, sweet and fatty delicacies and healthy foods corresponded well with self-reported food intake in the FFQs. Twin pairs who differed in the overall amount of food they consumed had the largest intrapair differences in BMI (MZ: 1.9 ± 0.1, DZ: 2.9 ± 0.2 kg/m^2) and WC (MZ: 5.5 ± 0.6, DZ: 7.5 ± 0.7 cm). However, there were no differences in self-reported intake between these pairs (I). Analysis of obesity-discordant MZ twin pairs confirmed these results, as most pairs agreed that the heavier twins ate more (overall), snacked more and exercised less than the leaner co-twins. Eating behaviors such as

eating too much, striving to be thin and body dissatisfaction were more frequently reported by obese co-twins than their lean counterparts. Total energy intake did not differ between the obese and lean co-twins and few differences were found in the 3-day food diary data. Underreporting of actual energy intake was significant for the obese (24.7% of TEE) but not for the lean co-twins (8.4% of TEE) (II). Obesity-discordant pairs differed significantly in their serum lipoprotein profiles, and the acquired accumulation of liver fat was particularly associated with increased concentrations of atherogenic lipids, including low-density lipoprotein (LDL-C) and Apolipoprotein B (ApoB), while physical activity was related to reduced concentrations of atherogenic lipids (IV).

Omega-3 polyunsaturated fatty acid intake, as evaluated by self-reporting and serum measurements of DHA, was significantly related to a shift in the serum high-density lipoprotein (HDL) subclass distribution toward larger HDL particle size (III, V). In addition, serum DHA was positively associated with serum LDL particle diameter and negatively associated with triglyceride (TG) concentrations, medium and large very-low density lipoprotein (VLDL) particle concentrations and VLDL particle diameter. A high-fat, high-sucrose, low fiber dietary pattern (labeled “junk food”) was positively associated with TG concentrations, a shift in the subclass distribution of VLDL toward larger particles and LDL toward smaller particles, and an increased concentration of small HDL particles in the serum (V). The associations were independent of adiposity and other lifestyle factors, and most were independent of the potential confounding effects of genotype and early environmental factors shared by twins.

Conclusions: By using mutual responses of twin pairs, this study provides compelling evidence that acquired eating and physical patterns are important determinants of obesity, but they may be overlooked in population studies that use self-reported data due considerable misreporting of actual energy intakes and exercise behavior by obese subjects. Habitual physical activity and fish intake were related to a favorable serum lipoprotein profile, whereas a high-fat, high-sucrose, low-fiber dietary pattern and accumulation of liver fat associated with an unfavorable serum lipoprotein profile. These results emphasize a healthy lifestyle, in particular reduced portion sizes of energy-dense, nutrient poor foods and regular physical activity as the cornerstone of preventing obesity and lipid disturbances in young adults.

Tiivistelmä

Tausta: Energiatiheää ravintoa ja vähäistä liikuntaa on pidetty lihavuuden ja siihen liittyvien aineenvaihduntahäiriöiden huomattavan lisääntymisen syinä. Havainnointitutkimuksissa lihavuuteen on kuitenkin johdonmukaisesti liitetty vain joitakin ruokavalioon ja liikuntaan liittyviä tekijöitä. Kun henkilö ilmoittaa itse käyttäytymisestään, raportit sisältävät herkästi virheitä, mikä saattaa ainakin osittain selittää aiempien tulosten epäjohdonmukaisuutta. Ravitsemukselliset biomerkkiaineet edustavat objektiivista lähestymistapaa tavanomaisen ravinnonsaannin mittaamiseen, mutta valideja biomerkkiaineita ei ole käytettävissä kaikille ravintoaltistuksille.

Tavoitteet: Tämän väitöskirjan tavoitteina oli 1) validoida itseraportoitua ruoankäyttöä ja liikuntaa objektiivisten kaksossisarukselle tehtyjen objektiivisten vertailukysymysten (I) ja kaksoismerkityn veden avulla (II); 2) määrittää, ovatko syömis- ja liikuntakäyttäytyminen yhteydessä painoindeksiin (BMI) ja vyötärön ympärysmittaan (1, II); ja 3) tutkia, liittyvätkö hankittu lihavuus (IV) ja ruokavaliotekijät (III, V) seerumin lipoproteiiniprofiiliin.

Materiaalit ja menetelmät: Tutkimukseen otettiin mukaan seuraavat osallistujat FinnTwin12- ja FinnTwin16-tutkimuksista (kaksi aikuisten nuorten suomalaisten kaksosten populaatiopohjaista tutkimusta): 1) 713 monotsygoottista (eli geneettisesti identtisiä) ja 698 samaa sukupuolta olevaa ditsygoottista kaksosparia (eli ns. epäidenttisiä kaksosia), jotka vastasivat ruokailutiheyteen liittyviin kyselyihin (FFQ) ja kaksossisarukseen liittyviin vertailukysymyksiin (I); 2) 14 monotsygoottista kaksosparia, jotka erosivat lihavuuden suhteen (BMI-ero > 3kg/m^2), ja 10 samanpainoista paria; parit pitivät kolmen päivän ruoka- ja liikuntapäiväkirjoja, vastasivat syömistapakyselyihin ja kaksossisaruksen vertailukysymyksiin sekä osallistuivat kokonaisenergiankulutuksen mittauksiin, jotka tehtiin kaksoisleimatulla vedellä (II); 3) 15 lihavuuden suhteen eroavaa ja 9 samanpainoista monotsygoottista kaksosparia, jotka pitivät kolmen päivän ruokapäiväkirjaa, vastasivat liikuntakyselyihin ja osallistuivat ihonalaisen rasvakudoksen, vatsaontelon sisäisen rasvakudoksen ja maksan rasvan (magneettikuvaus ja magneettispektroskopialla sekä seerumin lipidien mittaukseen (ultrasentrifugointi, gradienttigeelielektroforeesi ja entsymaattiset tekniikat) (III, IV); 4) 663 aikuista kaksosta, jotka vastasivat ruokafrekvenssikyselyihin ja osallistuivat ydinmagneettisella resonanssilla ja spektroskopialla tehtäviin seerumin lipoproteiinien ja seerumin dokosaheksaeenihapon (DHA) mittauksiin, joista jälkimmäinen toimi DHA:n saannin objektiivisena biomerkkiaineena.

Tulokset: Sekä mono- että ditsygoottiryhmissä niillä kaksossisaruksilla, joiden kohdalla kaksosparin molemmat kaksossisarukset vastasivat yhdenmukaisesti, että kyseinen kaksonen joko syö enemmän (yhteensä), syö rasvaisempia ruokia, syö rasvaisia herkkuja, syö nopeammin, ajattelee ruokaa valitessaan ruuan terveellisyyttä vähemmän kuin toinen kaksonen tai tekee arkielämässä vähemmän liikunnallisesti aktiivisia valintoja, oli merkitsevästi suuremmat painoindeksit ja vyötärönympärykset kuin heidän kaksossisaruksillaan. Enemmän syöminen (yhteensä), rasvaiset herkut ja vähäisempi liikunta säilyivät parinvälisten erojen merkitsevinä ennustajina monitekijäisessä regressioanalyysissä toisistaan riippumatta. Kaksossisarusten vertailukyselyt napostelusta, rasvaisista ruuista,

rasvaisista herkuista ja terveellisistä ruuista vastasivat hyvin ruokafrekvenssikyselyissä itseraportoitua ravinnonsaantia. Niillä kaksospareilla, joilla ruuan nautittu kokonaismäärä oli erilainen, oli suurin parinsisäinen ero painoindeksissä (monotsygootit: 1,9 ± 0,1, ditsygootit: 2,9 ± 0,2 kg/m2) ja vyötärönympäryksessä (monotsygootit: 5,5 ± 0,6, ditsygootit: 7,5 ± 0,7 cm). Näiden parien välillä ei kuitenkaan ollut eroja itseraportoidussa ravinnonsaannissa (I). Monotsygoottisten, lihavuuden suhteen eroavien kaksosten analysointi varmisti nämä tulokset, sillä useimmat kaksossisarukset kertoivat, että painavampi kaksonen syö enemmän (yhteensä), napostelee useammin ja liikkuu vähemmän kuin laihempi kaksosista. Lihavat kaksossisarukset raportoivat laihoja useammin liiallista syömistä, halua laihtua ja tyytymättömyyttä omaa kehoa kohtaan. Energiansaannin kokonaismäärä ei poikennut näiden lihavien ja laihojen kaksossisarusten välillä, ja kolmen päivän ruoka- ja liikuntapäiväkirjojen välillä havaittiin vain vähän eroja. Energiansaannin aliraportointi on merkitsevää lihavilla (24,7 % kokonaisenergiansaannista), mutta ei laihoilla (8,4 % kokonaisenergiansaannista) kaksossisaruksilla (II). Lihavuuden suhteen eroavilla pareilla oli merkitsevästi erilaiset lipoproteiiniprofiilit, ja erityisesti maksaan kertynyt rasva liittyi aterogeenisten lipidien (LDL-C, ApoB) lisääntyneisiin pitoisuuksiin ja liikunta niiden pienentyneisiin pitoisuuksiin (IV).

Itseraportoitujen tietojen ja DHA-seerumimittausten arvioinnin perusteella monityydyttymättömän omega-3-rasvahapon saanti liittyi merkitsevästi HDL-alaluokan jakautumiseen suuremman HDL-partikkelikoon suuntaan (III, V). Seerumin DHA-pitoisuus liittyi lisäksi positiivisesti LDL-partikkelin läpimittaan ja negatiivisesti triglyseridipitoisuuteen ja keskikokoisten ja isojen VLDL- ja VLDL-partikkelien läpimittaan. Runsasrasvainen, runsaasti sakkaroosia sisältävä, vähäkuituinen ruokavalio ("roskaruoka") liittyi korkeampiin triglyseridipitoisuuksiin, VLDL:n alaluokkajakautuman siirtymiseen suurempien partikkelien suuntaan ja LDL:n siirtymiseen pienempien partikkeleiden suuntaan sekä pienten HDL-partikkelien suurentuneeseen pitoisuuteen (V). Nämä yhteydet olivat lihavuudesta ja muista elämäntapatekijöistä riippumattomia, ja useimmat olivat myös kaksosten yhteisen genotyypin ja varhaisten ympäristötekijöiden mahdollisista sekoittavista vaikutuksista riippumattomia.

Päätelmät: Tämä tutkimus antaa kiistatonta näyttöä siitä, että omaksutut syömis- ja liikuntatottumukset ovat tärkeitä lihavuutta selittäviä tekijöitä, mutta näitä eroja ei ehkä huomata populaatiotutkimuksissa itseraportoituja tietoja käytettäessä, mikä johtuu lihavien tutkittavien virheellisestä itseraportoinnista. Liikunta ja kalan nauttiminen liittyivät edulliseen seerumin lipoproteiiniprofiiliin, ja runsasrasvainen, runsaasti sakkaroosia sisältävä, vähäkuituinen ruokavalio ja rasvan kertyminen maksaan liittyivät epäsuotuisaan profiiliin. Nämä tulokset korostavat terveellisen ruokavalion, erityisesti vähäisen energiatiheiden, niukasti suojaravintoaineita sisältävien ruokien käytön ja säännöllisen liikunnan merkitystä lihavuuden ja lipidihäiriöiden ehkäisemisessä nuorilla aikuisilla.

List of original publications

This dissertation is based on the following original communications, which are referred to in the text by the Roman numerals I-V. In addition, some unpublished results are presented. The original publications are reprinted with the permission of the copyright holders.

I **Bogl LH**, Pietiläinen KH, Rissanen A, Kaprio J. Improving the accuracy of self-reports on diet and physical exercise: the co-twin control method. *Twin Res Hum Genet* 2009; **12**: 531–40.

II Pietiläinen KH, Korkeila M, **Bogl LH**, Westerterp KR, Yki-Järvinen H, Kaprio J, Rissanen A. Inaccuracies in food and physical activity diaries of obese subjects: complementary evidence from doubly labeled water and co-twin assessments. *Int J Obes (Lond)* 2010; **34**: 437–45.

III **Bogl LH**, Maranghi M, Rissanen A, Kaprio J, Taskinen M-R, Pietiläinen KH. Dietary omega-3 polyunsaturated fatty acid intake is related to a protective high-density lipoprotein subspecies profile independent of genetic effects: a monozygotic twin pair study. *Atherosclerosis* 2011; **219**: 880–6.

IV Kaye SM, Maranghi M, **Bogl LH**, Kaprio J, Hakkarainen A, Lundbom J, Lundbom N, Rissanen A, Taskinen MR, Pietiläinen KH. Acquired liver fat is a key determinant of serum lipid alterations in healthy monozygotic twins. *Obesity (Silver Spring)* 2012; **21**: 1815-22

V **Bogl LH**, Pietiläinen KH, Rissanen A, Kangas AJ, Soininen P, Rose RJ, Ala-Korpela M, Kaprio J. Association between habitual dietary intake and lipoprotein subclass profile in healthy young adults. *Nutr Metab Cardiovasc Dis* 2013; **23**: 1071-8.

Abbreviations

ANCOVA	Analysis of covariance
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
BMI	Body mass index
BMR	Basal metabolic rate
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CVD	Cardiovascular diseases
DHA	Docosahexaenoic acid
DLW	Doubly labeled water
DXA	Dual-energy X-ray absorptiometry
DZ	Dizygotic
EPA	Eicosapentaenoic acid
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food-frequency questionnaire
FTO	Fat mass and obesity-associated (gene)
GEE	Gradient gel electrophoresis
GWA	Genome-wide association study
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
IDL-C	Intermediate-density lipoprotein
Kcal	Kilocalories
KJ	Kilojoule
LDL	Low-density lipoprotein
MET	Metabolic equivalent
MHO	Metabolically healthy obese
MJ	Megajoule
MUFAs	Monounsaturated fatty acids
MZ	Monozygotic
n-3	Omega-3
NHANES	National Health and Nutrition Examination Survey
NMR	Nuclear magnetic resonance
PCA	Principal component analysis
PUFAs	Polyunsaturated fatty acids
RS	Restraint Scale
SD	Standard deviation
SE	Standard error

SES	Socioeconomic status
SFAs	Saturated fatty acids
SNPs	Single-nucleotide polymorphisms
SSAGA	Semi-Structured Assessment of Genetics of Alcoholism
SSB	Sugar-sweetened beverages
TEE	Total energy expenditure
TFEQ	Three-Factor Eating Questionnaire
TG	Triglycerides
VLDL	Very-low density lipoprotein
WC	Waist circumference

1 Introduction

Obesity represents a growing public health challenge which has reached epidemic proportions. Globally, 9.8% of men and 13.8% of women are obese, which is almost twice the 1980 prevalence of 4.8% and 7.9%, respectively. However, there are large geographical differences in the distribution. For example, in North America, Australia, Latin America and Europe high rates of obesity occur whereas obesity rates are lower in South Asia and Central Africa (Finucane et al., 2011). The dramatic rise in the prevalence of obesity is particularly worrying. Among Finnish adults, the prevalence of obesity has increased from 11.3% to 20.7% in men and 17.9% to 24.1% in women between the 20-year period from 1978–1980 to 2000–2001 (Lahti-Koski et al., 2010), but has remained relatively stable thereafter (Mannistö et al., 2012). Obesity leads to numerous health consequences, including reduced quality of life, sleep apnea, diseases of the joints and bones, certain forms of cancer and a decrease in life expectancy (Bray, 2004). Further, obesity is a strong risk factor for type 2 diabetes mellitus and cardiovascular disease (CVD) and is typically accompanied by a cluster of metabolic abnormalities, including atherogenic lipid profile, hypertension, impaired glucose metabolism, and systemic inflammation (Bray, 2004). However, there is considerable metabolic heterogeneity among equally obese subjects. Some patients show a relatively healthy metabolic risk profile despite being obese, whereas others seem to be especially predisposed to develop metabolic complications. The subset of individuals who do not develop the expected obesity-associated metabolic complications despite their excess body fat have been termed the “metabolically healthy obese” (MHO) (Karelis et al., 2004; Primeau et al., 2011).

The proximal cause of obesity is a prolonged energy imbalance between energy intake and expenditure that results from multiple behavioral and physiological factors, which are themselves influenced by genetic and environmental factors and their complex interactions. The proposition that large portion sizes promote overconsumption of food and lead to obesity is intuitive and logical, though few dietary factors have been robustly associated with obesity or weight gain in large epidemiologic studies, and energy intake is generally not associated with obesity in observational research. For reviews on this topic, see Fogelholm et al., 2012; Summerbell et al., 2009; Togo et al., 2001. Methodological difficulties in accurately measuring habitual dietary intake could partly explain the lack of associations observed in many previous studies. Underreporting of energy intake by self-reported dietary methods is well-documented, and is more common among obese than lean subjects (Goris et al., 2000; Lafay et al., 1997; Schoeller et al., 1990), which is particularly troublesome when searching for dietary determinants of obesity. One potential solution is the use of nutritional biomarkers, which are objective and lack the bias created by self-reported dietary intake errors. Doubly labeled water (DLW) is the gold standard for measuring free-living energy expenditure and validate energy intakes from self-reports (Westerterp et al., 1995). Other examples of biomarkers are fatty acids measured in serum, plasma and adipose tissue, particularly those fatty acids that are not endogenously synthesized in large amounts, such as long-chain omega-3 (n-3) polyunsaturated fatty acids (PUFAs) and trans fatty acids (Baylin and Campos, 2006;

Willet et al., 2013). However, valid nutrient biomarkers are not available for all dietary exposures of interest.

CVD are the leading cause of death worldwide (WHO, 2011) and refer to a group of diseases of the heart and blood vessels. CVD due to atherosclerosis include ischemic heart disease or coronary heart disease (CHD) (e.g. heart attack), cerebrovascular disease (e.g. stroke) and peripheral arterial disease, which is a disease of the blood vessels that supply the arms and legs. As lipoproteins carry cholesterol and triglycerides (TG) in the bloodstream, they are the direct mediators of the atherosclerotic process. Elevated low-density lipoprotein cholesterol (LDL-C) is a major risk factor for CVD, and lowering LDL-C concentrations by statins reduces the incidence of CHD and decreases CHD-mortality rates (Downs et al., 1998; Shepherd et al., 1995). Excess LDL-C in the vessel wall initiates the atherosclerotic process because LDL particles become oxidized and ingested by macrophages, which initially develop into foam cells and then eventually become plaque. High-density-lipoprotein cholesterol (HDL-C) counterbalances this deleterious effect by inhibiting the oxidation of LDL-C and by the removal of cholesterol from peripheral cells back to the liver, a process called reverse cholesterol transport. In addition, HDL-C exerts antioxidant, anti-inflammatory, vasodilatory and antithrombotic effects (Assmann and Gotto, 2004).

Lipoproteins are not a single entity but a heterogeneous group of particles that differ in size, composition and atherogenic properties. Conventional lipid risk factors do not perfectly predict CHD in patients. Thus, lipoprotein subclasses may have the potential to improve risk prediction (Krauss, 2010; Lamarche et al., 1997; Mueller et al., 2008). A distinct subclass pattern has been identified by nondenaturating gradient gel electrophoresis (GEE) and is characterized by predominantly small, dense LDL particles which have been associated with a two to threefold increased risk of coronary artery disease (Austin et al., 1988; Campos et al., 1992). Higher levels of HDL2b particles have been inversely associated with CHD risk factors and higher levels HDL3b particles have been positively associated with CHD risk factors including adiposity, resting heart rate and physical activity (Williams et al., 1992, 1995). Using nuclear magnetic resonance (NMR) spectroscopy to quantify lipoprotein subclasses, elevated concentrations of small HDL and LDL, decreased concentrations of large HDL and also LDL and increased concentrations of large VLDL have been linked to obesity (Magkos et al., 2008), metabolic syndrome (Rivellese et al., 2008), subclinical atherosclerosis (Würtz et al., 2012) and type 2 diabetes (Mora et al., 2010). Moreover, HDL and LDL particle size have been shown to be significantly increased in families with exceptional longevity (Barzilai et al., 2003).

Dietary guidelines have focused on reducing intakes of saturated fatty acids (SFAs), primarily as a means of lowering LDL-C concentrations. The effect of macronutrients on the lipoprotein profile needs to be seen in the context of the replacement macronutrient in the diet. According to a large meta-analysis of 60 controlled trials, the replacement of dietary SFAs by carbohydrates does not improve the ratio of total to HDL-C, but the ratio is improved when SFAs are replaced by unsaturated fat, especially PUFAs (Mensik et al., 2003). Replacement of carbohydrates by protein primarily from beef also high in monounsaturated fatty acids (MUFAs) has been shown to improve multiple features of atherogenic dyslipidemia, including ApoB and small LDL in the context of a low SFA intake (Mangravite et al., 2011).

Alcohol intake increases HDL-C, ApoA1 and TG concentrations (Rimm et al., 1999), and has been related to lipoprotein particle sizes towards larger particles (Mukamal, et al., 2007; Muth et al., 2010). The long-chain n-3 PUFAs are well known for their TG lowering effects (Harris, 1997; Roche and Gibney, 2000). Physical activity raises HDL-C (Leon and Sanchez, 2001), decreases concentrations of small LDL, increases mean LDL particle size and decreases concentrations of TG and also large VLDL particles independent of weight loss (Krauss et al., 2002).

Besides lifestyle, genetic factors contribute to the variation in obesity (Elks et al., 2012), lipid profile (Bayoumi et al., 2007) and dietary intake (Hasselbalch et al., 2008). Thus, genetic factors could act as a confounder in the association between diet and metabolic health, i.e. two traits could be influenced by the same genetic factors rather than one trait causing the other (van Dongen et al., 2012). The only study design in humans that accurately distinguishes between associations that reflect causality from those that reflect the confounding effects of genes is the study involving MZ twins. Twins are, by definition, matched for many unmeasured factors, including cultural background, prenatal exposure, parental characteristics and childhood rearing environment, and in addition, MZ twins also share the same genotype. If MZ twin pairs differ, it provides a unique opportunity to find a probable causal pathway between the exposure (such as diet) and the outcome (such as obesity and dyslipidemia). This assumption of plausible causality is based on the fact that genes and shared environmental experiences cannot explain differences between the two members of an MZ pair, because these factors are methodologically controlled (McGue et al., 2010; Vitaro et al., 2009).

This thesis explores the associations between habitual diet, obesity and serum lipoprotein profile by using the unconventional approach of using monozygotic (MZ) and dizygotic (DZ) twins: a) as mutual respondents to increase the accuracy of self-reported dietary intake and b) to control for the potential confounding effects of early environmental factors, and in the case of MZ twins, DNA sequence. The following literature review gives an introduction to twin studies in health research and discusses the determinants of obesity and lipoprotein profile. In addition, dietary and physical activity assessment methods are described and their advantages and limitations are discussed.

2 Review of the literature

2.1 The value of twin studies in health research

2.1.1 Twinning

Zygosity refers to whether the twins arise from one zygote (one egg fertilized by one sperm) or from two zygotes (two separate eggs each fertilized by two different sperms). Approximately two thirds of twins are DZ and one third is MZ. The arrangement of placentas and membranes in twin pregnancies depends on the twin type and the stage of gestation at which the fertilized egg splits. All DZ twins are dichorionic and diamniotic, i.e. they have separate placentas and membranes, although the placentas can sometimes be fused. MZ twin pregnancies are commonly divided into 3 subtypes based on placentation and membrane layers (**Figure 1**).

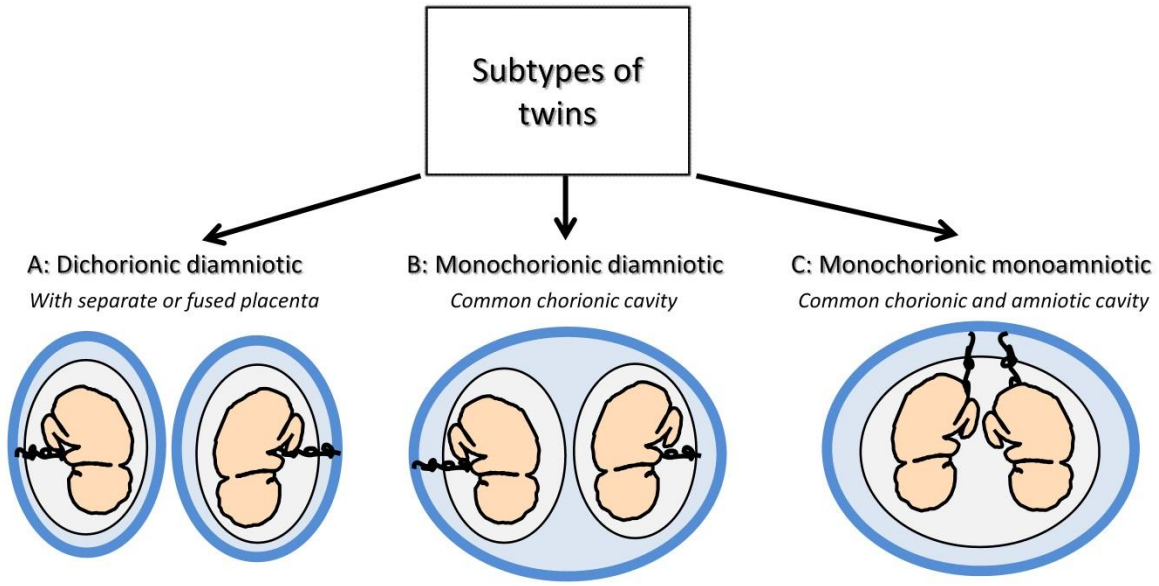

Figure 1. Subtypes of twins.

In about 25-30% of MZ twins, the zygote divides between the first 3 days after conception yielding twins with separate placentas and amniotic sacs (dichorionic diamniotic twins). In the great majority of MZ twins (>70%), the division occurs between day 4 and 7 after conception resulting in a shared placenta with two amniotic sacs (monochorionic diamniotic twins). Only in about 1% of MZ twin gestations, the splitting of the zygote happens so late (propably between day 7 and 14) that both fetuses share the same placenta and are surrounded by the same amniotic sac (monochorionic monoamniotic). When the separation of the zygote occurs even later, the division is incomplete giving rise to conjoined twins (Hall, 2003).

The twinning rate, defined as the number of twin maternities per 1000 maternities including still birth and also live births, varies greatly among countries and over time. Twinning rates are lowest in South Asia, South-East Asia and Latin America (less than 6-9 per 1000), intermediate in Europe and North America (9-20 per 1000) and highest in Central Africa (above 18 per 1000) (Little, 1988, Smits and Monden, 2011). After a long-term decline in twinning and multiple birth rates since the 1920s, rates have gradually started to increase during the last two or three decades in most developed countries, including the USA, Japan, South Korea and many European countries (Chauhan et al., 2010; Hur and Song, 2009; Imaizumi and Nonaka, 1998). As the rate of MZ twinning has been fairly constant worldwide, with about 3.5-4 per 1000 maternities, differences in twinning rates are mostly due to variation in DZ twinning (Hall, 2003).

The recent increase in the use of assisted reproductive technologies is related to increased occurrence of MZ and DZ twin births (Derom et al., 1995). Maternal reproductive age is a major factor influencing DZ twinning (Bulmer, 1970). The underlying mechanisms of DZ twinning is multiple ovulation, which is related to increased concentrations of follicle-stimulating hormone in the mother (Lambalk et al., 1998). Amounts of follicle-stimulating hormones are raised in taller, heavier and older mothers and concentrations of these hormones are related to seasonality, ethnic origin and increased parity. Family studies have also implicated a genetic disposition for DZ twinning as mothers of DZ twins and their relatives have an increased probability of giving birth to DZ twins (Hoekstra et al., 2008; Lewis et al., 1996). Recent genome-wide linkage scans suggested new candidate loci that may harbor genetic variants that contribute to variation in familial DZ twinning, and highlight the complex and heterogeneous nature of the inheritance that underlies DZ twinning (Painter et al., 2010; Palmer et al., 2006).

The causes for the occurrence of MZ twins are largely unknown. There are no naturally occurring animal models for MZ twinning, apart from armadillos, which produce identical quadruplets or octuplets in normal regular reproduction and not in exceptional cases (Blickstein and Keith, 2007). In a large population-based registry study, Lichtenstein et al. (1998) reported that mothers of MZ twins have an increased probability of giving birth to MZ offsprings, and the genes increasing the liability for the division of the embryo are likely to be expressed in the mother as no paternal effect on MZ twinning was found. Familial aggregation of MZ twins has also been reported in a few pedigrees (Hamamy et al., 2004; Machin, 2009). One possible biological mechanism of MZ twinning involves the discordance among cells of the inner cell mass leading to two points of regrowth due to genetic or epigenetic mechanisms (Machin, 1996; Shur, 2009). *In vitro* fertilization methods increase the incidence of MZ twins between two to five times, which could be due to injury of the zona pellucida thereby cutting the early zygote (Hall, 2003).

Multiple pregnancies are high-risk pregnancies characterized by an increased incidence of both fetal and maternal complications. Mothers of multiples suffer from pregnancy related nausea and vomiting and have a higher risk for gestational hypertension and preeclampsia than mothers of singletons. Intrauterine growth restriction, preterm delivery (<37 weeks of gestation) and low birth weight are more common in twin pregnancies than in singleton pregnancies and are therefore important factors that contribute to excess perinatal mortality and morbidity in twin gestations (Rao et al., 2004). Knowledge of chorionicity is crucial,

because monochorionic placentation is a risk factor for low birth weight and perinatal death (Dubé et al., 2002). In addition, in about 15% of monochorionic gestations, the shared placenta can result in twin-to-twin transfusion syndrome resulting in an asymmetrical growth and a perinatal mortality rate of over 90% if untreated (Duncan et al., 1997).

2.1.2 Twin study designs

Over the last decades, the comparison of resemblance of MZ and DZ twins has been used increasingly to estimate heritabilities of diseases or quantitative traits in the so-called classical twin study. The results have contributed to the understanding that variation in almost any human condition is influenced to some extent by genetic factors. The estimation of heritability depends on the mathematical partitioning of observed phenotypic variation into unobserved genetic and environmental components. The variance components can be obtained by fitting quantitative genetic models with twin data on observed resemblances (phenotypic covariation) in MZ and DZ twin pairs (Neale et al., 2003). As MZ twins result from a single fertilized egg, they are (nearly) genetically identical, whereas DZ twins are derived from two different zygotes and share, like siblings 50% of their segregating genes on average. By definition, both types of twins share 100% of their common environment and 0% of their unshared environment.

Heritability is formally defined as the proportion of the total variance in a population for a particular measurement taken at a particular time or age that is attributable to the variation in additive genetic or total genetic values, which are respectively termed narrow-sense heritability and broad-sense heritability. It measures the fraction of variation between individuals in a population that is due to their genotype (Visscher et al., 2008). The classical twin study has also been extended to estimate whether multiple traits are influenced by the same genetic or environmental factors (multivariate twin model) or whether a trait is influenced by the same genetic and environmental factors over time (longitudinal twin model). Further, twin studies can be used to estimate gene-environmental interactions without measured genotype data by testing whether the heritability of a trait varies across different levels of environmental exposures. The presence of cultural transmission, assortative mating and genotype x environment covariance can be estimated using an extended twin-family design (Boomsma et al., 2002).

For heritable traits, the MZ co-twin control design offers a unique opportunity for isolating the role of environmental factors in the development of a certain phenotype or disease. Gesell (1942) was one of the first that used the co-twin control design to study behavior resemblances and differences in identical twin children. This method is similar to a case-control study but with the additional advantages that cases and controls are perfectly matched for age, sex and genetic background, and a wide range of early environmental factors shared between the pair of twins. Thus, differences between both members of a twin pair cannot be attributable to differences in DNA sequence or to the shared environment. The particular strength of the co-twin control design is the ability to distinguish between associations that may reflect causality from those that reflect confounding effects of genes or the shared environment, i.e. two traits could be associated because of the same genes or same shared environmental factors (McGue et al., 2010; Vitaro et al., 2009).

The longitudinal phenotypic data available in many twin registries worldwide together with collected biological materials and new technologies opens up new opportunities for twin studies in the future (Boomsma et al., 2002; van Dongen et al., 2012). The classical twin method can be used to assess the extent to which twins resemble each other at the level of molecular processes, such as gene expression levels, metabolite concentrations or epigenetic mechanisms. The roles of disease-causing mutations, copy-number variation, epigenetic variation, gene expression alterations in the source of phenotypic variation and discordance of MZ twins will continue to be investigated and may facilitate the understanding of genes and molecular pathways that underlie complex traits and disease pathogenesis. The rising interest in rare genetic variants may bring back interest in linkage studies, in which discordant DZ twins can increase statistical power. In contrast to nontwin siblings, DZ twin pairs are of the same age and have similar pre- and postnatal environmental exposures, all of which are important factors in reducing the within-pair variance (van Dongen et al., 2012).

2.1.3 Heritability of diet and physical activity

A number of twin studies have shown that in addition to environmental factors, genetic factors contribute to the variation in physical activity and food intake between individuals. The contribution of genetic factors to food preferences and choices is already documented in childhood, with substantial individual differences in children's liking and disliking of specific foods (Wardle and Cooke, 2008). Studies of twin children and adults also indicate that genetic factors contribute to the number and timing of meals (de Castro, 1993), dietary patterns (Teucher et al., 2007), eating behaviors (Tholin et al., 2005), macronutrient composition (de Castro, 1993; Dubois et al., 2013; Hasselbalch et al., 2008), and food choices (Faith et al., 2008; Teucher et al., 2007).

Twin studies have also evaluated the heritability of *ad libitum* food intake and taste preferences in laboratory tests. Faith et al. (1999) reported a heritability of 24-33% for *ad libitum* caloric intake in a controlled laboratory buffet meal. Moreover, genetic variation in perception of sour (Törnwall et al., 2012), sweet (Keskitalo et al., 2007) and bitter (Hansen et al., 2006) tastes might contribute to differences in food preferences and choices. The heritability of intakes of calories, nutrients and foods was also examined in 66 MZ and 51 DZ adult twins reared apart. About 30% of the variance in self-reported dietary intake assessed by using a food-frequency questionnaire (FFQ) was explained by genetic factors. The addition of 101 individuals, who were mostly spouses, provided an opportunity to test the influence of family environment on current dietary habits. There were moderate correlations between twins and their spouses. However, the lengths of the marriage was unrelated to spouse resemblance in diet, indicating that spouses dietary habits do not converge over time (Hur et al., 1998).

Twin and family studies have also examined the contributions of genetic and unique environmental factors to participation in sports (Boomsma et al., 1989; Stubbe et al., 2005) and daily physical activity levels (Choh et al., 2009; Mustelin et al., 2012). Notably, the largest study to date (more than 37 000 twin pairs from 7 countries), found genetic factors explained 27-70% of the variance of self-reported exercise participation depending on the country with the remaining variance explained by unique environmental factors. The effects

of the common environment on variations in exercise participation were largely absent, except in Norwegian males. Genes contributing to exercise participation could act through mechanisms influencing personality, such as self-motivation or self-discipline, responsiveness to training programs in terms of physical fitness or ability to lose weight or reward pathways of the nervous system (Stubbe et al., 2005).

It is plausible that the same genes that act to increase obesity or other metabolic disorders also promote increased intake of calories or energy-dense foods. Alternatively, genes that are protective against obesity could predispose the subjects to engage in more physical activity or affect the intake of healthy food or fewer calories. Only a subset of twin and family studies tested this possibility, and reported positive genetic correlations between bread and butter intake and body mass index (BMI) among 7 y old girls (Faith et al., 2008), and between total caloric intake and serum TG concentrations in adults (McCaffery et al., 2001) whereas negative genetic correlations were found between fruit and vegetable intake and BMI (Martin et al., 2011) and between sport and leisure physical activity and percent body fat in adulthood (Mustelin et al., 2011).

Genome-wide linkage studies have identified chromosomal regions that may harbor loci that contribute to dietary and exercise behavior phenotypes, including: energy and macronutrient intake (Choquette et al., 2008), eating behavior (Steinle et al., 2002), physical activity and inactivity (Simonen et al., 2003). Candidate gene association studies have typically focused on genes related to neurotransmitter pathways and involved in satiety and energy balance. Although some positive associations have been reported, replication in consecutive studies has been inconsistent (Rankinen and Bouchard, 2006). To date, there have been only a few genome-wide association (GWA) studies on dietary intake and physical activity. The first and so far only GWA study for exercise behavior in 1772 unrelated Dutch and 978 unrelated American adults reported an association between genetic variants in the *SGIP1*, *CYP19A1* and *LEPR* genes and voluntary exercise behavior independent of BMI (De Moor et al., 2009). Recently, GWA studies have identified variants in genes involved in nutrient metabolism and obesity that are associated with macronutrient consumption (Tanaka et al., 2013), alcohol consumption (Baik et al., 2011; Schumann et al., 2011) and caffeine intake (Cornelis et al., 2011).

2.1.4 Heritability of obesity and serum lipids

Genetic epidemiology is important for understanding the magnitude of the genetic and environmental contributions to obesity and serum lipid concentrations. Numerous twin and family studies have examined the heritability of obesity-related traits and BMI is by far the most studied phenotype. Recently, Elks et al. (2012) summarized data of 88 twin and 27 family studies and reported a major contribution of genetic variation to interindividual differences in BMI. Heritability estimates derived from twin studies generally fall in the range of 47- 90% and those from family studies in the range of 24-81%. The relative importance of genetic and environmental factors to BMI appears to vary with age. Based on the findings from this large systematic review, genetic factors have larger effects in childhood than in adulthood. In a meta-analysis of 9 twin studies in children and adolescence, Silventoinen et al. (2010) found that the effect of genetic factors was lowest and that of the common

environment was highest in mid-childhood, though the common environmental influence disappeared at age 13. The high heritability of BMI reported in studies of twins reared together has been confirmed in studies of twins reared apart (Allison et al., 1996; Stunkard et al., 1990). The rate of change in BMI has also a substantial heritability of more than 60%, but the genetic variants that influence BMI level are possibly different from those that influence change in BMI over time (Hjelmborg et al., 2008; Ortega-Alonso et al., 2012). Heritability estimates from direct measures of whole-body and regional body fat assessed by dual-energy X-ray absorptiometry (DXA) are generally similar to the estimates obtained for BMI (Hsu et al., 2005; Malis et al., 2005). Several twin studies suggest that the heritability for obesity can be modified by environmental factors, and physical activity in particular has repeatedly been shown to modify the action of genes that predispose to obesity (McCaffery et al., 2009; Mustelin et al., 2009; Silventoinen et al., 2009).

Several twin and family studies have shown that genetic factors influence concentrations of conventional lipid risk factors, i.e. total cholesterol, LDL-C and HDL-C, but heritability estimates vary widely. For example, the heritability estimate for total cholesterol concentration ranges from as low as 30% to as high as 73% (Bayoumi et al., 2007; Beekman et al., 2002; Elder et al., 2009; Goode et al., 2007). Heritability estimates are context-dependent and population-specific, which could in part explain the wide range in estimates reported. Heller et al. (1993) compared genetic and environmental influences on serum lipids in 146 twins reared apart and 156 twins reared together. They examined both older and younger twins and males and females separately and found consistently higher correlations for total cholesterol concentrations among twins reared together than twins reared apart. The finding shows the importance of a shared rearing environment in determining total cholesterol concentrations but not in determining concentrations of other serum lipids or apolipoproteins in later life (Heller et al., 1993).

Only a few studies have investigated genetic influences on serum lipoprotein subclasses and particle size. Pietiläinen et al. (2009) reported moderate heritabilities between 46-63% for HDL particle size and HDL subspecies in 52 MZ and 89 DZ young adult twin pairs. Kaess et al. (2008) analyzed NMR spectroscopy data from 1275 coronary artery disease patients from the Regensburg Myocardial Infarction Family Study and reported significant heritability estimates of 23-67% for HDL and LDL subclass profiles. An investigation of the Finnish Twin Cohort has yielded similar or even higher heritability estimates in the range of 48-76% for serum lipids and lipoprotein concentrations in 221 MZ and 340 DZ twin pairs aged 22-25 years (Kettunen et al., 2012). There is evidence from twin and family studies that the same genes influence several of the components of the metabolic syndrome, and that the genes for serum lipid concentrations and adiposity traits also partly overlap (Pietiläinen et al., 2009; Povel et al., 2011). The genetic variants responsible for the genetic pleiotropy remain to be identified.

2.2 Non-dietary factors associated with obesity and serum lipids

2.2.1 Common genetic variants

As described in the previous chapter, the evidence for genetic influences on obesity and lipid phenotypes is substantial. Over the past few years, the availability of GWA studies has led to the discovery of a large number of genetic susceptibility loci associated with common obesity and lipid traits. However, these common variants only explain a small or modest proportion of the overall genetic variance. The strongest signal for BMI at the fat mass and obesity associated (FTO) locus explains 0.34% of the total variance. Altogether, the identified loci explain 1.5% of the variance and 2-4% of the genetic variance in BMI based on a heritability of 40-70% for BMI (Speliotes et al., 2010). There is still uncertainty about the function of the genetic loci, such as FTO, and how it is related to increased adiposity. Although human studies suggest that FTO may act through exerting effects on appetite or satiety but not energy expenditure (Haupt et al., 2009; Speakman et al., 2008; Wardle et al., 2009), animal studies suggest the opposite. Fischer et al. (Fischer et al., 2009) recently developed a mouse model in which the homologous FTO gene was inactivated (FTO-/-) and showed that these mice were protected from obesity. The FTO-/- mice showed no significant differences in food intake relative to wild-type mice but they did have an elevated metabolic rate. For plasma lipid concentrations, common single-nucleotide polymorphisms (SNPs) explain 10-12% of the total variance and 23-30% of the genetic variability (Asselbergs et al., 2012; Teslovich et al., 2010). Thus, much of the genetic variance is currently unexplained, which has led to the concept of "missing heritability" (Manolio et al., 2009). Much of the speculation about missing heritability from GWAs has focused on the possible contribution of rare variants with large effect sizes. In the coming years, advances in next-generation sequencing are expected to enable the quantification of the contribution made by rare alleles. Other researchers using the entire GWA data rather than only SNPs data that reach genome-wide significance suggest that the accumulated effect of many hundred loci with weak effects may be sufficient to explain a vast majority of the genetic variance (Stahl et al., 2012). Yang et al. (2011a) recently developed a software tool called genome-wide complex trait analysis (GCTA). This tool estimates the variance explained by all the SNPs on a chromosome or on the whole genome for a complex trait rather than testing the association of any individual SNP to the trait. For example, GWAs for height have identified genetic variants that cumulatively explain only about 10% of the phenotypic variation, whereas Yang et al. estimated that 45% of the variance for height can be explained by common SNPs (Yang et al., 2011b). Moreover, missing heritability may be largely explained by gene-environment interactions, rather than undiscovered variants (Kaprio, 2012; Zuk et al., 2012).

2.2.2 Sex differences

Men have more atherogenic serum lipid and lipoprotein profiles than women. Compared with men of a similar age, premenopausal women have lower concentrations of LDL-C, TGs and higher concentrations of HDL-C (Hazzard, 1985) in their serum. The predominance of the

small and dense LDL particles (LDL subclass pattern B) is more prevalent among men than women (Campos et al., 1992). Men have smaller NMR-determined LDL and HDL particles and larger VLDL particles and these differences persist after adjusting for differences in plasma lipid concentrations (Freedman et al., 2004). These differences are likely related to differences in the activity of enzymes involved in lipoprotein metabolism. For example, hepatic lipase (HL) hydrolyzes TG and phospholipids in lipoproteins, and increased HL activity is associated with small, dense LDL particles and with reduced HDL2 cholesterol levels. HL concentration has been shown to be almost twice as high in men as in women. The differences in HL activity became smaller but remained significant after adjusting for intra-abdominal fat, which suggests that sex steroid hormones may also contribute to the higher HL activity seen in men compared with premenopausal women (Carr et al., 2001). Differences in these risk factors between sexes, particularly differences in HDL-C concentration and smoking, could partly explain why CHD is more prevalent among men than women (Jousilahti et al., 1999; Tunstall-Pedoe et al., 1994). In a study by Jousilahti et al. (1999) consisting of 14 786 Finnish men and women aged 25 to 64 years at baseline, the CHD incidence in men was about three-fold that of women and mortality was five-fold that of women.

It is well documented that there are sex differences in body fat distribution, largely because of differences in sex hormones between men and women. Men and estrogen-deficient postmenopausal women tend to accumulate more abdominal and visceral fat and therefore have an increased cardiometabolic risk relative to premenopausal women (Tchernof and Després, 2013). Given that women are shorter, weigh less and have less fat-free mass, it is not surprising that in absolute terms women have lower energy expenditure than men. However, whether the sex differences in basal metabolic rate or daily energy expenditure remain after adjustment for body composition differences is subject to debate (Carpenter et al., 1998; Klausen et al., 1997).

2.2.3 Aging

The aging process causes several changes in body composition in men and women that may lead to physical disability and increased metabolic risk (Zamboni et al., 2005). In particular, reductions in fat-free mass and increases in body fat mass, predominantly in the abdominal region are hallmarks of human aging (Hughes et al., 2002). Fat-free mass typically increases until it reaches a peak in the third to fifth decade of life and declines thereafter. Body fat mass generally increases throughout the lifespan with a peak between the fifth and seventh decades of life and then it remains relatively stable or even decreases slightly (Barlett et al., 1991; Borrud et al., 2010; Chumlea et al., 2002; Hasselbalch et al., 2008). Body composition often changes without simultaneous changes in body weight (Zamboni et al., 2003). Total potassium, an index of fat-free mass, has been shown to decline at the ages of 30 and 31 for women and men, respectively and it also differs according to ethnicity and sex. African American women and Hispanic men have the most rapid decline (He et al., 2003). In a study that used magnetic resonance spectroscopy of liver and muscle to compare age cohorts, the elderly subjects had more liver fat and intramyocellular lipids than younger adults (Cree et al., 2004). A 2 year follow-up study in 26 weight-stable elderly African American women showed that skeletal muscle mass at the leg and bone masses decreased and visceral adipose

tissue and intermuscular adipose tissue increased over the follow-up period. These changes occurred without changes in total adipose tissue or food intake (Song et al., 2004). Hughes et al. (2004) comprehensively assessed 10 year changes that had occurred in body composition at 11 anthropometric sites in elderly persons who were aged 46–78 years at baseline. Subcutaneous fat declined whereas total fat mass increased suggesting that weight stability may mask sarcopenia with advancing age. The best predictors of body fat mass change were waist and hip girths, whereas the predictive value for skinfold measures was relatively poor. The decline in thigh girth was diminished by increasing or maintaining physical activity in both men and women (Hughes et al., 2004). The risk of CHD increases markedly with advancing age and total cholesterol and LDL-C concentrations increase gradually until the age of around 70 (Anderson et al., 1987; Ferrara et al., 1997). The reason for the steady increase may be a decreased breakdown of cholesterol to bile acids or increased intestinal cholesterol absorption (Lammert and Wang, 2005; Parini et al., 1999). Although studies suggest that HDL-C concentrations change little during the adult life span (Heitmann, 1991; Wallace and Colsher, 1992), the aging process is associated with unfavorable changes in HDL structure, function and activity, including an increased susceptibility of HDL to lipid peroxidation, a decrease of HDL antioxidant activity and a reduction of cholesterol efflux (Berrougui et al., 2007; Jaouad et al., 2006). Menopausal transition is associated with unfavorable changes in body composition and lipid profile independent of age, such as higher LDL-C, particularly denser LDL (Derby et al., 2009; Matthews et al., 1989).

2.2.4 Physical activity

Most cross-sectional and longitudinal studies have shown an inverse association between self-reported physical activity and measures of obesity or weight gain, though some have failed to show a significant association. For a review on this topic see Hill and Wyatt (2005). It is not fully elucidated whether obesity is a cause or a consequence of physical inactivity. Some studies suggest that engagement in regular physical activity is related to less weight gain over time (Schmitz et al., 2000; Waller et al., 2008), other studies report that obesity may lead to physical inactivity (Golubic et al., 2013; Petersen et al., 2004) and yet others suggest that physical activity is both causative and secondary to the development of obesity (Pietiläinen et al., 2008; Tucker et al., 2013). It has been difficult to distinguish the independent effects of caloric restriction from those of exercise training on weight loss. Those few well-controlled studies that matched energy deficits by either caloric restriction or by increased physical activity have shown that physical activity reduces insulin resistance and total and ectopic fat to a greater extent than caloric restriction alone (Donnelly et al., 2003; Irving et al., 2008). Exercise training influences concentrations of serum lipids and lipoproteins favorably, and HDL-C has been studied most extensively. Aerobic exercise without changes in diet raises HDL-C concentrations to a modest degree, on average by 4.3% (Leon and Sanchez, 2001). Randomized studies suggest that high-volume and high-intensity exercise is required to induce a modest but significant change in HDL-C concentrations. However, in many intervention studies the change in HDL-C concentrations occurred concurrently with weight change and thus the independent effect of exercise per se has been difficult to differentiate (Trejo-Gutierrez and Fletcher, 2007). The exercise induced effect on HDL-C concentrations has been reported to vary by Apolipoprotein E and Cholesteryl ester transfer protein (CETP) genotype (Thompson et al., 2004; Wilund et al., 2002). In a study by Krauss et al. (2002), 111

randomly assigned sedentary overweight men and women with dyslipidemia participated for 6 months in the study control group or for about 8 months in one of three exercise groups that varied in amount and intensity. Importantly, the results showed that high intensity exercise can improve the overall lipoprotein profile even in the absence of weight loss. The high amount high-intensity exercise group had clearly the most beneficial changes, that manifested in decreased concentrations of small LDL and increased LDL particle size (without any changes in plasma LDL-C concentrations), increases in HDL-C concentrations (particularly large HDL particles) and decreased concentrations of TG and large VLDL particles. The low and moderate exercise groups had more beneficial lipoprotein profiles than the sedentary control group. Recently, Kujala et al. (2013) also reported that long-term leisure-time physical activity, quantified by the metabolic equivalent (MET), is associated with a shift in the serum lipoprotein subclass distribution toward lower VLDL and higher large and very large HDL particle concentrations independent of BMI. In addition, they extended their analysis to the whole metabolome, which showed that isoleucine, α1-acid glycoprotein, and glucose were lower in the physically active than in the inactive individuals, whereas the serum fatty acid composition shifted toward a less saturated profile.

2.2.5 Smoking

Nicotine affects hormones such as norepinephrine, dopamine and serotonin that are released by the central nervous system that influence brain chemicals that could suppress appetite and increase metabolic rate (Jo et al., 2002), which could explain why smokers have a lower body weight than non-smokers and why smoking cessation is frequently followed by weight gain (Filozof et al., 2004). There is widespread belief that cigarette smoking helps to control body weight and this could even be a reason why adolescents, especially females, start smoking (Potter et al., 2004). Moreover, smoking is strongly linked to weight concerns and a lifetime history of recurrent intentional weight-loss episodes (Luostarinen et al., 2013; Saarni et al., 2007). However, the relationship between smoking and obesity is far more complex, and among smokers as a group, the greater the number of cigarettes smoked, the higher is the body weight (John et al., 2005; Rásky et al., 1996), probably because smoking is clustered with other risk behaviors, such as low physical activity, low fruit and vegetable intake and high alcohol intake (Chiolero et al., 2006). Other harmful metabolic consequences of smoking include central fat accumulation (Bamia et al., 2004), glucose intolerance (Houston et al., 2006) and an increased risk of metabolic syndrome and type 2 diabetes (Willi et al., 2007). Smokers are also characterized by a more atherogenic lipid and lipoprotein subclass profile. Smokers have higher concentrations of serum TGs, total cholesterol and LDL-C, which is mainly due to increased concentrations of small and medium LDL particles. They also have lower concentrations of HDL-C, which is largely due to lower concentrations of large HDL particles (Beauchamp et al., 2010; Craig et al., 1989; Freeman et al., 1993). In a double-blind randomized controlled trial that lasted for 1 year, Gepner et al. (2011) examined the effect of smoking cessation on serum lipoproteins. Despite the weight gain upon smoking cessation, subjects who quit smoking had increased concentrations of HDL-C, large and total HDL particles compared to those who continued smoking.

2.2.6 Other non-dietary factors

The etiology of obesity and dyslipidemia is complex and multifactorial, and thus, several other factors have been suggested to contribute or associate with obesity and abnormal lipid levels. Large ethnic differences exist, especially among women. Nationally representative examination surveys in the US show that African Americans have the highest prevalence of obesity, i.e. more than 80 % were overweight or obese, and more than 50 % were obese among women aged 40 years or older (Wang and Beydoun, 2007). African American ethnicity is also associated with lower LDL particle size after adjustment for CHD risk factors, statin use and estrogen use (in women), physical activity and alcohol intake (Kullo et al., 2007).

Socioeconomic status (SES) is inversely associated with CVD morbidity and mortality in developed countries (Kaplan and Keil, 1993). As obesity and serum lipids represent important CVD risk factors, they could party explain this finding. Sobal and Stunkard (1989) published an extensive review on the association between SES and obesity. The main findings include a strong inverse association among women in developed economies, with a higher likelihood of obesity among women in lower socioeconomic groups, whereas results for men and children in developed societies were inconsistent. However, in developing economies, there was a strong direct association, with a higher likelihood of obesity among men and women in higher socioeconomic groups. More recently, McLaren (2007) updated Sobal and Stunkard´s review and included indicators of SES. In countries with high levels of socioeconomic development, the majority of studies show a negative association between SES and obesity, especially for the SES indicators of education and occupation. In contrast, the lower the development of a country is, the higher is the proportion of positive associations between SES and obesity, especially for the indicators of income and material possessions. Interestingly, population studies have found that associations between SES and serum lipids follow the same trend. For example, in China, the country with the world's fastest developing economy, people belonging to lower SES strata had more favorable serum lipid profiles compared with those in higher SES strata (Yu et al., 2002). In contrast, in Sweden, low SES was associated with an unfavorable lipid profile, especially low HDL-C concentrations (Wamala et al., 1997). The associations between SES and CVD risk factors are likely the result of multiple behavioral and psychosocial risk factors that differ between people in different social strata.

In addition, social and psychological factors influence eating and physical activity habits and physical characteristics such as body weight. Transition into marriage appears to be associated with weight gain, whereas transition out of marriage is associated with weight loss (Dinour et al., 2012). Chronic stress leads to increased secretion of glucocorticoids, which, in animal studies, increases the intake of palatable foods (Dallman et al., 2005). The association between depression and obesity is bidirectional, overweight and obesity increase the risk of developing depression and depression increases the risk of developing overweight and obesity (Luppino et al., 2010).

There is a growing body of work that indicates that the *in utero* environment and factors early in life influence the risk of being overweight or obese later in life. These factors include, for example, gestational diabetes mellitus, maternal smoking, rapid infant growth and not being breastfed (Monasta et al., 2010). Animal studies have suggested that epigenetic influences,

such as those induced by maternal obesity or prenatal exposure to nicotine, can alter gene expression and further affect the risk for obesity (Gao et al., 2005; Shankar et al., 2008). Other putative factors that could potentially contribute to the obesity epidemic include sleep deprivation, certain pharmaceuticals, endocrine disruptors, demographic changes, reduction in the variability of the ambient temperature, increase in maternal age and assortative mating (Keith et al., 2006).

2.3 Dietary factors associated with obesity and serum lipids

2.3.1 Eating behaviors

Energy intake is a consequence of behaviors (e.g. food choices) that are themselves genetic or environmental in origin. Two major cognitive behavioral theories have dominated the eating behavior research over the past decades, namely externality and dietary restraint. Schachter´s externality theory (1968) suggests that obese subjects are more reactive to external food cues such as smell of food or a situation cue such as passing a restaurant and less sensitive to internal cues of satiety and hunger. In an environment of easily accessible, abundant and highly palatable food such cues would stimulate appetite and overeating in some individuals. Studies in animals demonstrate that non-food cues that have become signals for food can stimulate appetite and feeding independent of physiological hunger. Weingarten (1983) used Pavlovian conditioning to teach rats an association between an external cue and food. He presented an external stimulus, a buzzer and a light that were activated together with a meal delivery into a food cup, and an intermittent pure tone unpaired with meals. Later, presentation of the conditioned cue elicited feeding in the animals even though the rats were tested while still in a satiated state. The results show that cues that have become signals for food can subsequently initiate a meal.

In humans, exposure to the sensory properties of food and even the thought of eating increases reported hunger (Ferriday and Brunstrom, 2008) and initiates cephalic phase responses, including insulin release and changes in salivation, heart rate, skin conductance, blood pressure and gastric activity (Nederkoorn et al., 2000). Cephalic phase responses can be seen as adjustments of the body in anticipation of an impending meal, and these events are believed to optimize the digestion, absorption and use of ingested nutrients (Mattes, 1997). Overweight and obese individuals seem particularly sensitive to the effects of food cues, as demonstrated by their significantly slower decline in salivation and larger desire to eat cued and non-cued foods as compared to their leaner counterparts (Epstein et al., 1996; Ferriday and Brunstrom, 2011; Tetley et al., 2009).

Herman and Mack (1975) developed the Restraint Scale (RS) as a measure of the degree of cognitive restriction of food intake in order to control body weight. The restraint eating theory proposed that chronic dieting may induce counter-regulatory processes that suppress signals of satiety and hunger resulting in disinhibition and binge eating episodes. Their hypothesis draws from the theory espoused by Nisbett (1972), which suggests that obese people are actually underweight with regard to their biological set-point, and consequently they are in state of chronic hunger because they continuously diet in order to maintain a reduced weight.

In the study that first demonstrated this phenomenon, participants consumed zero, one, or two tasty high caloric milk shakes (the preload) and were then given ice cream. The preload reduced subsequent ice cream consumption in normal eaters, but restrained eaters paradoxically ate more ice cream when they had violated their diets with a milk-shake preload than when they had not. It was further shown that induced negative emotions, such as those experienced by watching an unpleasant film, induced overeating, particularly among those that tried to restrict their food intake (Wardle and Beales, 1987). The validity of the RS was later questioned since it contains items that reflect not only restrained but also disinhibited eating and weight fluctuation. Thus, it may be biased towards a selection of restrained eaters who have a high tendency to overeat (Heatherton et al., 1988; van Strien, 1999).

The Three-Factor Eating Questionnaire (TFEQ), that was developed by Stunkard and Messick (1985), addressed the confounding of restraint and disinhibition in the RS factors by including three factors of eating behavior. The first factor, cognitive restraint refers to concern over weight control and strategies to achieve this (e.g. avoiding fattening foods or eating small portions). The second factor, disinhibition reflects the tendency towards over-eating and eating opportunistically in an obesogenic environment (e.g. overeating when others do so or in response to the palatability of food). Finally, hunger measures the extent to which hunger feelings are perceived and to what extent they evoke feelings for food intake (Bryant et al., 2008; Stunkard and Messick, 1985). In contrast to the original RS, the restrained subscale of the TFEQ assesses the cognitive tendency and behavioral strategies towards restricting food intake that are linked with successful dieting (Heatherton et al., 1988). The factor most consistently related to a higher BMI and weight gain is disinhibition. Disinhibition associates with a lower diet quality, higher preference for fatty foods, increased energy intake, eating disorders including binge eating disorder, lower success at weight-loss and the adoption of unhealthy behaviors such as smoking as a weight control strategy (Bryant et al., 2008). The other TFEQ factors may modulate the association of disinhibition and obesity. Cross-sectional studies have shown that subjects with low restraint and high disinhibition have the highest BMIs and largest waist circumferences (WC), whereas those with low restrained and low disinhibition have the lowest BMIs and smallest WCs (Dykes et al., 2004; Williamson et al., 1995). This is supported by prospective studies, which report that the positive association between disinhibition and weight gain was attenuated by dietary restraint (Hays and Roberts, 2008; Savage et al., 2009).

A more recent theory of eating regulation, the Goal Conflict Model of Eating, postulates that the eating behavior of restrained eaters is dominated by a conflict between two incompatible goals, namely the goal of eating enjoyment and the goal of weight control (Stroebe et al., 2013). According to the model, exposure to palatable food temporarily activates the eating enjoyment goal while it inhibits the weight control goal, which leads to diet violation and unhealthy eating. Several other dimensions of eating behavior, including food responsiveness, food enjoyment, satiety responsiveness, eating in the absence of hunger, reinforcing value of food, impulsivity/self-control have been examined in relation to energy intake, BMI and weight gain over time, mainly in children but also adults. A recent review on these behaviors concluded that most available data show positive cross-sectional associations between these behaviors and BMI, but fewer studies found associations between energy intake or food choices and BMI (French et al., 2012).

Food intake is regulated by a complex system, including both homeostatic and hedonic pathways. Two of the hormones that play an important role in the regulation of energy balance through their actions on the hypothalamus are leptin, secreted by the adipose tissue and ghrelin, secreted by the stomach. Leptin is mainly involved in long-term regulation of energy balance, as it is released into the circulatory system by the energy stores, whereas ghrelin is a fast-acting hormone, whose circulatory levels are affected by meal intakes. In obese subjects the circulating levels of leptin are increased, and levels of ghrelin are decreased (Klok et al., 2007).

Studies using neuroimaging have given insight into hedonic systems in the brain that influence food intake. These images show that obese subjects have altered neuronal responses to food intake and food cues in brain regions known to be involved in reward, emotion and memory, homeostatic regulation of food intake, sensory processing, motor processing, cognitive control and attention (Carnell et al., 2012).

2.3.2 Meal patterns: snacking and meal skipping

Studies that examined trends in snacking behaviors among children and adults have suggested that snacking prevalence (i.e. occasions of snacking) may have contributed to the obesity epidemic (Jahns et al., 2001; Zizza et al., 2001). Jahns et al. (2001) reported national representative data of more than 21 000 US individuals aged from 2 to 18 years. The prevalence of snacking has increased in all age groups. For example, in adolescents aged from 12 to 18 years, the prevalence increased from 76% in 1977 to 88% in 1996. Although the size of a snack and the energy content per snack remained relatively constant between 1977 and 1996, the number of snacking occasions increased significantly among children and adolescents, which lead to an increased contribution of snacks to total energy intake. Piernas and Popkin (2010) analyzed 4 nationally representative surveys of food intake in the US population, which included more than 44 500 adults aged 19 years and older. The prevalence of snackers increased for all adults from 71% in 1977 to 97% in 2003–2006. In all adults, snacking occasions increased by about one snack per day over this time period and the contribution of snacks to total energy intake increased from 18 to 24%. The energy density of snacks also increased from 357 kilocalories (kcal) to 579 kcal per snack over the studied time period. The definition of a snack in both studies was based on the respondent´s classification of an eating occasion as a snack or other meal.

The role of meal frequency and snacking in obesity is controversial. Some studies report a positive cross-sectional association between the number of eating occasions and BMI, whereas others found the opposite. For instance, Howarth et al. (2007) analyzed the relationship between eating patterns and BMI in 1792 younger and 893 older adults. Eating frequency in both age groups was positively associated with energy intake. In a Swedish study of more than 5000 subjects, Bertéus Forslund and colleagues (2005) reported that obese middle-aged individuals snacked more frequently compared to the Swedish reference population and that women were more frequent snackers than men. Sweet and fatty food groups were associated with snacking and contributed significantly to energy intake, irrespective of physical activity. In contrast, Ma et al. (2003) investigated the association between eating patterns and obesity in about 500 adult participants. A greater number of

eating episodes was associated with a lower risk of obesity independent of age, sex, physical activity and total energy intake. A large study that involved 5811 individuals conducted by Keast et al. (2010), found that weight status and abdominal obesity were inversely associated with snacking and the percentage of energy from snacks in adolescents 12–18 years of age. A longitudinal study by Phillips and others (2004) found no significant association between energy-dense snack foods and body weight or percent body fat change in 196 adolescents over a 10 year follow up period. The exception to this was a significant association between sugar-sweetened beverages (SSB) and BMI.

Several studies have reported that the most popular snacks are foods high in fat and sugar, such as cookies, candies, chocolate, bars, savory snacks and SSB (Kerr et al., 2009; Phillips et al., 2004; Piernas and Popkin, 2010). Thus, it is possible that snacking will contribute to weight gain and obesity, when the excess in energy is not compensated for in later eating occasions. Chapelot (2011) developed an experimental approach to test whether the energy of snacks consumed in the satiety state were compensated for in subsequent meals. In their first study, 11 young men were deprived of time cues from lunchtime until they requested dinner. The consumption of a 1 megajoule (MJ) afternoon snack under conditions of satiety had no effect on the requested dinner time or the energy intake at dinner (Marmonier et al., 1999). In a similar study design, 8 time-blinded men were subjected to a 1MJ snack high in carbohydrates, fat or protein. The snack high in protein induced a longer satiety than other types of snacks due to the lower insulin secretion attributed to it, however energy and macronutrient composition of the dinner were the same across all conditions (Marmonier et al., 2000, 2002). Taken together, observational studies on the associations between meal frequency and obesity are inconsistent, which may be due to different definitions for snacking, difficulty in differentiating meals from snacks, different covariate adjustments or dietary assessment methods that are prone to misreporting. Experimental evidence suggests that the high energy density of snack foods is not compensated for in later eating episodes, which may lead to overeating and could potentially lead to weight gain.

Several other aspects of meal patterns, such as eating rate (Andrade et al., 2008), temporal distribution of meals (Berg et al., 2009), and the number of meals eaten away from home (Bes-Rastrollo et al., 2010) have been associated with obesity or weight gain. In particular, research on breakfast consumption has gained a lot of attention. Breakfast eating by US children and adults appears to have declined over the past decades (Haines et al., 1996; Siega-Riz et al., 1998). Breakfast consumption has been related to a better diet quality (Dubois et al., 2009; Kant et al., 2008), lower risk of obesity in cross-sectional studies in various age groups (Azadbakht et al., 2013; Kant et al., 2008; Keski-Rahkonen et al., 2003), and less weight gain in prospective studies of children (Barton et al., 2005), adolescents (Affenito et al., 2005) and adults (van der Heijden et al., 2007). Recent data from the population-based Northern Finland Birth Cohort suggests that a five-meal-a-day pattern including regular breakfast consumption is associated with a reduced risk of obesity and attenuates the BMI-increasing effects of obesity-susceptibility loci in adolescents (Jääskeläinen et al., 2012, 2013).

The type of breakfast and amount of energy consumed at breakfast may also play a role in body weight regulation. For example, Cho et al. (2003) examined data from the National Health and Nutrition Examination Survey (NHANES) III and found that subjects who

regularly ate ready-to-eat cereals, cooked cereals or quick breads had significantly lower BMIs than those who ate meat and eggs for breakfast or those who skipped breakfast. Purslow et al. (2008) looked at the association between the percentage of total daily energy intake consumed at breakfast and weight change in 6764 middle-aged men and women from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. Although all participants gained weight over the course of a follow-up period of about 3.5 years, an increased percentage of daily energy consumed at breakfast was associated with a relatively lower weight gain.

The omission of breakfast is likely to result in overeating later in the day among the obese as a consequence of the production of appetite-stimulating hormones. Ghrelin levels increase with fasting which enhances appetite and stimulates food intake (Cummings et al., 2001). The Intergene study on 3610 randomly selected men and women aged 25-74 in Sweden showed that the meal time pattern of obese subjects is shifted towards later in the day. Specifically, being obese was significantly associated with omitting breakfast, omitting lunch, eating at night and larger self-reported portion sizes of main meals (Berg et al., 2009). Furthermore, it is possible that those who skip breakfast are more likely to snack throughout the day. For example, Savige et al. (2007) showed that adolescents who skip meals are more likely to snack on the run, e.g. on the way to or from school.

2.3.3 Portion size and energy density

Portion size

The increase in the prevalence of obesity has occurred simultaneously with the increase in portion sizes of foods and beverages both inside and outside the home (Nielsen and Popkin, 2004), which suggests that larger portion sizes may play a role in the obesity epidemic. Rolls and colleagues (2000) conducted a series of well-controlled laboratory studies to determine experimentally whether portion size influences food intake and the subsequent energy intake in a single meal. In one of their earlier studies, 16 three-year-old and 16 five-year-old children participated in three sessions conduced at their usual lunchtime during which they were served different portion sizes of macaroni and cheese at each occasion. The older preschool children consumed more of the lunch when served the large portion than when served the small portion, whereas food intake of the three-year olds was not affected by the portion size served. The same authors found that adults eat more food and energy when served the larger macaroni and cheese lunch or a sandwich, which was similar to what they found for the older children. In particular, subjects consumed 30% more energy when offered the largest portion than when they were offered the smallest portion of the lunch and adult females consumed 12% more energy and adult males 23% more energy when presented with the larger 12-inch sandwich than when they were offered the smaller 8-inch sandwich. Despite the differences in food portions, ratings of hunger and fullness were not different after eating the larger or smaller portions (Rolls et al., 2002, 2004). These studies suggest that very young children quit eating in response to physiological cues such as satiety rather than responding to food cues such as portion size, whereas external factors have more influence on food intake than internal cues in older children and adults.

From these single meal studies it is not possible to know whether the effects of portion size is sustained over several days, which might subsequently lead to increased food intake and body weight. Those studies that examined the effects of large portion sizes on food intake over multiple days strongly suggest that subjects continue to overeat when portion sizes are increased and there is little evidence that they would compensate for the excess in energy intake (Kelly et al., 2009; Rolls et al., 2007). In the longest of these experimental studies, a 50% increase in portion sizes over 11 days led to a mean increase in daily energy intake of 423 kcal per day, which was not influenced by body weight status. The effect of portion size on increased intake was seen at all meals and for all foods except for fruit and vegetables (Rolls et al., 2007). In another study under a more naturalistic setting, the food intake of customers who purchased a pasta entreé from a public caferia-style restaurant on a university campus was recorded on 10 days over a period of 5 months. The size of the entreé was increased from a standard to a large portion size without changing the price on half of the days. Those customers who received the larger portion had a 43% increased energy intake than customers who got the standard portion size. Responses to the customer survey showed no differences in the ratings of the appropriateness of the portion size or of the amount that was eaten in relation to their usual meal between the two groups of customers (Diliberti et al., 2004). Thus, laboratory-based studies over multiple days and the study among restaurant customers support the evidence that environmental influences such as portion size contribute to the over-consumption of food and energy, which may lead to body weight gain.

Few observational studies have examined the relationship between portion size and weight status. In the Intergene study, obese men and women reported significantly larger portions of main meals and had a 13% increased risk of being obese for each of 9 increments in portion size (Berg et al., 2009). Portion sizes of 10 commonly eaten foods, number of eating occasions per day and number of foods consumed per day were examined as predictors of energy intake after adjustment for body weight among 2 to 5 year old children from the Continuing Survey of Food Intakes by Individuals. Portion size alone accounted for 17% to 19% of the variance in energy intake, whereas body weight predicted only 4% (McConahy et al., 2004). In French children aged 3–11 years, overweight status was positively correlated to portion sizes of croissant-like pastries and other sweetened pastries but inversely associated with portion sizes of liquid dairy products (Lioret et al., 2009).

Energy density

Energy density, i.e. the amount of energy in a given weight of food (Ello-Martin et al., 2007), directly influences energy intake independent of the macronutrient composition of a food. For example, in a crossover study of normal-weight women, energy density was manipulated but portion size and macronutrient composition was kept constant. Subjects were provided all meals for 2 days on 3 different test sessions. The meals were either low 3.5 kilojoule (kJ)/g; 0.8 kcal/g), medium (4.4 kJ/g; 1.1 kcal/g), or high (5.6 kJ/g; 1.3 kcal/g) in energy density. Despite the variation in energy density, the women ate a constant weight of food at each meal over the 2 days under all three conditions so that daily energy intakes varied directly with the energy density of the diets. As a result, 30% more energy was consumed on the high-energy dense meal as compared to the lower energy-dense condition (Flood and Rolls, 2007). The same laboratory also showed that consuming a low-energy-density first course meal in the

form of a soup or salad can lead to significant reductions in subsequent energy intake. For example, in their study of normal-weight subjects, consuming a soup as a preload resulted in a reduced meal (preload + test meal) energy intake of 20% (Flood and Rolls, 2007). As dietary fat has a higher energy density (9 kcal/g) than carbohydrates or protein (4 kcal/g), it significantly influences the energy density of foods. However, other factors such as fiber and water content also determine the energy-density of foods. Thus, it is possible to investigate the separate effects of fat and energy density on energy intake by manipulating either the energy density of meals while keeping the fat content constant, or by manipulating the fat content of meals while keeping the energy density constant. Studies using this approach point toward the energy density of foods, but not the fat content *per se*, in influencing energy intake during meals in both lean and obese subjects (Rolls et al., 1999; Saltzman et al., 1997; Stubbs et al., 1996).

Several clinical trials of weight loss also show that reducing the energy density of the diet is a successful strategy for weight management while controlling hunger (Ello-Martin et al., 2007; de Oliveira et al., 2008; Rolls et al., 2005). In one of the trials, 97 obese women were assigned to either of two groups with two different dietary strategies: one group was advised to reduce fat and the other group to increase the consumption of water-rich foods, particularly fruit and vegetables along with a reduction in fat intake. Both groups were instructed to eat *ad libitum*. After 1 year, both groups had lost weight, the group that was counseled to decrease the energy-density of foods by increasing the intake of water-rich foods lost significantly more weight, consumed a greater weight of food and experienced less hunger (Ello-Martin et al., 2007).

Ecological analyses suggest an inverse relationship between energy density of foods and their energy cost (dollars per MJ), i.e. more energy-dense diets rich in refined grains, added sugars and added fats are associated with lower food costs and may explain higher obesity rates among lower-income groups (Drewnowski and Darmon, 2005). In cross-sectional and prospective studies, dietary energy density has been positively associated with BMI and weight gain. In the nationally representative NHANES 1988-1994, a high energy density characterized by low fruit and vegetable intake was related to higher BMI in US adults (Kant and Graubard, 2005). In NHANES 1999-2002, dietary energy density was also positively associated with BMI, and in addition, WC, fasting insulin and the metabolic syndrome (Mendoza et al., 2007). A high dietary energy density, which represented a dietary pattern high in saturated fat, trans fat and glycemic index was associated with a greater increase in weight during 8 years follow-up among more than 50 000 women in the Nurses' Health Study (Bes-Rastrollo et al., 2008). The EPIC study (Du et al., 2009) showed that a high energy density reflected a dietary pattern high in fat and sugar and low in fruit and vegetables. In this population-based prospective cohort study of more than 89 000 Europeans, participants were followed for a mean of 6.5 years. Energy density was not associated with body weight change but instead with WC change, whereby an increase in energy density of 1 kcal per gram was associated with a 0.09 cm increase in WC per year. In the Danish Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study, Iqbal et al. (2006) reported that energy density was not related to five-year change in body weight among 862 men and 900 women. The exception was for obese women, for whom energy density was positively associated with subsequent weight gain (Iqbal et al., 2006).

Combined effect of portion size and energy density

Recent experimental studies have examined how energy density and portion size interact to affect energy intake. Kral et al. (2004) demonstrated that there was evidence that when both the energy density and the portion size of a food were increased within a meal, both factors acted independently to affect *ad libitum* energy intake. A total of 39 female participants consumed a main entrée *ad libitum* at lunch once a week for 6 weeks. This study was designed to investigate two energy levels, each of which was served in three different portion sizes making six different energy intakes. There was no interaction between the effects of energy density and portion size on energy intake, which indicated that the two factors led to independent and additive increases in energy intake. Participants consumed 56% more energy (925 kJ) when served the largest portion of the higher energy-dense entrée compared with the smallest portion of the lower energy-dense entrée. Rolls et al. (2006) investigated the combined effect of portion size and energy to influence food intake over multiple meals for 2 days. A crossover designed study was conducted on 24 young women who consumed meals and snacks that varied in energy density and portion size for 2 consecutive days per week over a 4 week period. Reduction in portion size and energy density led to significant and independent decreases in *ad libitum* energy intake over 2 days. In both aforementioned studies, ratings of hunger and fullness did not differ across conditions. The mechanisms underlying this phenomenon are not well understood, but possibly include a combination of cognitive and orosensory factors in addition to physiological controls related to gastric distention and gastric emptying (Kral and Rolls, 2004).

2.3.4 Dietary macronutrients

Meta-analyses have shown that isocaloric increases in dietary SFAs increase serum total cholesterol and LDL-C (Clarke et al., 1997; Mensink et al., 2003). Moreover, *ad libitum* low-fat diets prevent weight gain in normal-weight subjects and consistently associate with weight loss in overweight subjects (Astrup et al., 2002). These findings led to worldwide recommendations to decrease the consumption of fat and increase the consumption of carbohydrates to counteract the obesity epidemic and improve cardiovascular health.

However, there has recently been controversy over the effectiveness of low-fat high-carbohydrate diets. Low-fiber carbohydrates in the diet tend to raise serum TG concentrations and lower serum HDL-C concentrations, which may not be useful in terms of CVD risk prevention (Katan et al., 1997; Reaven, 1986; Mensink et al., 2003). In this context, the effect may depend on the replacement macronutrient in the diet and also on the carbohydrate quality. In a meta-analysis of 60 controlled trials conducted by Mensik et al. (2003), isoenergetic replacement of SFAs by carbohydrates did not improve the serum total:HDL cholesterol and increased fasting triacylglycerol concentrations. Replacing trans fatty acids and SFAs by cis unsaturated fatty acids was most effective in improving blood lipid profiles. This effect was stronger for linolenic acid than for MUFAs such as oleic acid. Using macronutrient substitution models, a pooled analysis of 11 American and European cohort studies that included 344 696 persons (71% women) investigated whether energy from unsaturated fatty acids or carbohydrates should replace energy derived from SFAs to prevent CHD (Jakobsen et al., 2009). For a 5% lower energy intake from SFAs and a concomitant higher intake from PUFAs, there was a significant inverse association with the risk of

coronary events and coronary deaths. For a 5% lower energy intake from SFAs and a compensatory higher intake from carbohydrates, there was a modest significant direct association with coronary events but not coronary deaths, whereas MUFA intake was unrelated to CHD incidence. However, the authors note in the discussion that the effects of substitution of carbohydrates may vary depending on the carbohydrate quality (Jakobsen et al., 2009). Moreover, the investigation of MUFA intake in relation to CHD risk in Western diets is complex, where MUFAs and SFAs are highly correlated in many foods, especially in natural and processed animal products (Ros, 2003; Sundström et al., 2001). A similar study has not been carried out in Mediterranean populations, where MUFAs are largely derived from olive oil.

Ecologic studies that examined the proportion of energy from fat in relation to obesity at the population level have shown positive associations between fat intake, the percentage of the population that is obese and CHD rates across countries (Bray and Popkin, 1998). In the famous Seven Country study, Keys et al. (1986) found a strong relationship between diet, serum cholesterol levels and risk of CHD. Populations with relatively high intakes of saturated fat, especially animal fat and cholesterol, had comparatively high serum cholesterol levels and higher incidence of CHD as compared to populations with low fat intakes. This most basic type of epidemiologic research has many weaknesses, including the inability to control for confounders and failure to relate individual levels to the population level. However, these studies were useful in raising initial hypotheses.

Prospective studies relating dietary fat intake and body fat are inconsistent and likely to be complex. For example, in a relatively small sample of 361 Swedish women aged between 38 to 60 years, high dietary fat intake was significantly associated with 6-year gain in BMI only among those women who were overweight at baseline and those who had at least one obese parent (Heitmann et al., 1995). A much larger sample of around 41 500 women from the Nurses' Health Study revealed a weak positive association between total dietary fat intake and weight gain. Although MUFAs and PUFAs were not associated with weight change, animal fat, SFAs, and trans fatty acids showed a positive association with 8-year weight gain. In contrast to the findings of the Swedish study, there was no evidence that this association would be modified by parental BMI (Field et al., 2007).

Recent attention has turned to the potential beneficial effects of dietary protein on the prevention of weight gain and on weight maintenance. Dietary protein favors the retention or accretion of fat-free mass at the expense of fat mass at a similar physical activity level and stimulates dietary-induced thermogenesis and satiety more than the isoenergetic ingestion of carbohydrates or fat (Paddon-Jones et al., 2008). Intervention studies have suggested that higher protein diets may increase total weight loss, improve body composition and improve blood lipid profiles (Layman et al., 2003). The benefit of a moderately higher-protein diet to limit weight regain after weight loss has also been demonstrated in a few trials. A randomized parallel study design on 113 moderately overweight men and women who lost 5–10% of their body weight during 4 weeks on a very-low-energy diet, found that those who consumed 18% of their energy intake as protein during the 6 months weight maintenance phase regained less weight than did those subjects who consumed only 15% of their energy intake as protein. Importantly, weight regain for the high protein group consisted of only fat-free mass, whereas the 15% protein group gained fat mass as well (Lejeune et al., 2005). In the Diogenes project,

European investigators compared 5 different diets that were all moderate in fat content. They varied the protein and carbohydrate content and glycemic index for weight maintenance among the study participants who had initially successfully lost weight on a calorie-restricted diet. The results showed that both, a modest increase in protein content and a modest reduction in the glycemic index facilitated the maintenance of weight loss. However, compliance in this study was not as good as expected, as the difference in the total energy from protein between high- and low-protein groups was only 5.4%, as compared to the target difference of 12% (Larsen et al., 2010). Epidemiologic evidence that related protein intake to weight gain over time is inconsistent, because positive, inverse or no associations manifest (Halkjær et al., 2011; Halkjaer et al., 2006; Ludwig et al., 1999; Stookey et al., 2005). In a recent large prospective study of several cohorts that belong to the EPIC study, positive associations between protein from animal sources, specifically meat and poultry, but not fish and dairy products, and subsequent long-term weight gains were found (Halkjær et al., 2011).

Currently, there is considerable interest in the ability of n-3 PUFAs to influence cardiovascular health. Meta-analysis of observational studies on fish intake and CHD have shown that fish consumption is inversely associated with CHD mortality and stroke (He et al., 2004a, 2004b). In secondary prevention, a Mediterranean alpha-linolenic acid-rich diet reduced the risk of coronary events and death (de Lorgeril et al., 1994). Furthermore, dietary and supplemental intake of n-3 PUFAs, has been shown to reduce overall mortality, the mortality due to myocardial infarction, and sudden death in patients with CHD in randomized controlled trials (Bucher et al., 2002). However, a daily dose of 1 gram of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) did not prevent death or cardiovascular outcomes in type 2 diabetic subjects who were at increased risk for cardiovascular events (Bosch et al., 2012).

Supplementation with marine-derived long-chain n-3 PUFAs, including EPA and DHA, of about 4 grams per day decreases serum TG concentrations by 25-30% and increases HDL-C concentrations by 1-3%, with accompanying increases in LDL-C concentrations of 5% to 10% (Harris, 1997). These effects occur at relatively high doses of n-3 PUFAs, which are not likely to be attained by diet alone (Harris, 1997; Roche and Gibney, 2000).

The literature on long-chain n-3 PUFA intake in relation to insulin sensitivity and risk of type 2 diabetes is controversial. Intervention studies report either no effect or positive effects of fish oils on insulin resistance in nondiabetic subjects (Giacco et al., 2007; Tsitouras et al., 2008; Waite et al., 2008). High doses of fish oil in diabetic subjects may adversely affect glucose control (Mostad et al., 2006). In the Women´s Health Study, exclusively dietary marine derived n-3 PUFAs were positively associated with the incidence of type 2 diabetes during a follow-up of 12.4 years (Djoussé et al., 2011). Conversely, total fish intake was associated with a lower risk of diabetes among participants of the EPIC-Norfolk cohort study (Patel et al., 2009). A meta-analysis of data of 438 000 individuals in 12 independent prospective cohorts with a mean follow-up time of 11 years did not provide evidence for an association between fish or fish oil intake with incidence of diabetes (Xun and He, 2012).

Higher consumption of fish and higher intakes of EPA and DHA have also been associated with lower body weight in some studies (Cade et al., 2004; Iso et al., 2001). This is further supported by a few human trials suggesting that fish oil containing EPA and DHA may

reduce adiposity, as shown in a study of type 2 diabetic women (Kabir et al., 2007) and reduce postprandial hunger sensations in overweight and obese subjects (Parra et al., 2008). Exploration of the mechanisms shows that marine-derived long-chain PUFAs could potentially alter the expression of several key proteins pertinent to inflammation, lipid metabolism, and energy utilization (Deckelbaum et al., 2006).

2.3.5 Food groups

The obesity epidemic is temporally related to a dramatic rise in fast food and SSB consumption. In the 1970s, fast food accounted for 2% of energy intake, whereas in 1995 it accounted for 10% (Guthrie et al., 2002). The intake of SSB increased from 3.9% of energy intake in late 1970s to 9.2% in 2001 (Nielsen and Popkin, 2004). Fast food is associated with a diet that is high in energy density and low in essential nutrients, and with higher BMIs and overweight status (Bowman and Vinyard, 2004). The Coronary Artery Risk Development in Young Adults Study (Pereira et al., 2005) found that baseline fast-food frequency was associated with changes in bodyweight in both black and white ethnic groups and increases in fast-food frequency over 15 years were associated with increases in insulin resistance for both ethnic groups and body weight gain among white individuals. Those subjects who visited fast-food restaurants with a frequency of more than twice a week between baseline and follow-up gained an extra 4.5 kg of body weight and had a two-fold greater increase in insulin resistance than those that did not (Pereira et al., 2005). Findings from epidemiologic studies indicate that an increased consumption of SSB can lead to weight gain and increase the risk of chronic disease (Malik et al., 2006). Evidence for adverse effects on dyslipidemia, hypertension and other metabolic risk factors is also emerging. For example, the consumption of 1 or more soft drinks per day among participants in the Framingham Heart Study was associated with an increased likelihood of developing obesity, metabolic syndrome, impaired fasting glucose, higher blood pressure, hypertriglyceridemia and low HDL-C concentrations (Dhingra et al., 2007).

The evidence that associates dairy intake and obesity was recently reviewed in two papers (Dougkas et al., 2011, Abargouei et al., 2012). The review of epidemiological studies by Dougkas et al. (2011) suggests a modest negative association between dairy foods consumption and body weight. The systematic review and meta-analysis of randomized controlled trials by Abargouei et al. (2012) concluded that administering dairy products without energy restriction had no significant effect on weight change, whereas incorporating dairy products into energy restricted weight-loss diets significantly lowered body weight and body composition compared with subjects on conventional weight loss diets.

Some large epidemiologic studies have reported a link between high meat intake and obesity. National cross-sectional data from the US found a positive association between meat consumption and the risk of obesity and of central obesity. Subjects with high meat intakes were 27% more likely to be obese and 33% more likely to have central obesity compared to those with low meat intakes. Subjects with higher meat intakes also had higher energy intakes, which indicated that this association may be due to the higher energy and fat content of meat (Wang and Beydoun, 2009). Similar results were obtained in a longitudinal study on the European population. The EPIC study found that total meat, red meat, poultry, and

processed meat intakes were all positively associated with weight gain after a mean of 5 years of follow-up. According to the study, 250 grams of meat per day (e.g. one steak) would lead to an annual weight gain of 422 grams higher than the weight gain experienced by subjects consuming an isocaloric diet with lower meat content (Vergnaud et al., 2010).

The importance of fruit and vegetables in weight regulation has also been evaluated in recent large epidemiological studies and review articles. The Behavior Risk Factors Surveillance System of more than 400 000 individuals provided evidence that overweight and obese subjects consume lower amounts of fruit and vegetables than normal weight subjects after adjustment for demographic variables, SES and lifestyle factors (Heo et al., 2011). Buijsse (2009) assessed fruit and vegetable intake in relation to subsequent changes in body weight among 89 432 participants from 5 European countries. During the follow-up time of 6.5 years, fruit and vegetable intake was found to be significantly, albeit weakly inversely related to weight increase, though this association was stronger among smokers. A systematic review of 23 publications reported that higher levels of fruit and vegetable intakes were weakly associated with weight loss in adults in experimental studies. However, it was unclear whether this weak association was due to the intake of fruit and vegetables alone or to multiple other behaviors. In longitudinal studies, the weight of evidence for an association between fruit and vegetable consumption and less weight gain among adults was strong (Ledoux et al., 2011). Alinia et al. (2009) included only fruit intake in their review, from which they concluded that the majority of the evidence supports a possible inverse association between fruit intake and being overweight, but suggested that future studies were needed.

Few studies have attempted to analyze the effect of various food groups on weight change. In a comprehensive investigation of 3 separate US cohorts that included more than 120 000 men and women who were nonobese and free of chronic disease at baseline, 4-year weight gain was positively related with intakes of potato chips, potatoes, SSB, unprocessed and processed meat but inversely related with intakes of vegetables, whole grains, fruits, nuts and yoghurt (Mozaffarian et al., 2011). The EPIC-Potsdam study of German adults evaluated 24 food groups in relation to 2-year weight change. The consumption of food groups with a high energy density and fat content, such as butter, margarine, sauces and meat, significantly predicted large weight gain, while the consumption of cereals predicted large weight loss in women. Higher consumption of food items high in sugar by men predicted weight gain, whereas the consumption of milk and milk products predicted weight loss. Confounding factors, such as age, baseline BMI and lifestyle factors were controlled (Schulz et al., 2002).

2.3.6 Dietary patterns

Recently, dietary pattern analysis has emerged as a complementary approach to investigate the relationship between diet and chronic diseases (and their risk factors). In contrast to examining individual nutrients and foods, as described in previous chapters, dietary pattern analysis looks at the whole complexity of the diet. Several studies have suggested that dietary patterns derived by factor or cluster analysis predict CVD risk factors and mortality in different populations. The dietary intake patterns identified can, in most studies, be categorized as “western” (energy dense) and “prudent” (nutrient-dense or “healthy”). The “western” pattern is often characterized by a high intake of processed and red meat, refined

grains, certain desserts, fried foods and SSB. The prudent or healthy pattern usually entails high intakes of fruit, vegetables, whole-grain products and low-fat foods.

Kerver et al. (2003) examined the association between major dietary patterns and risk factors for CVD in a cross-sectional study of healthy US adult data derived from the NHANES II. Those authors identified 6 dietary patterns by factor analysis, of which the "western" and "healthy American" pattern were the most prominent. The "western" pattern associated positively with biomarkers for CVD risk, including serum C-peptide, serum insulin and glycated hemoglobin. The Swedish Intergene research program identified five food patterns that were related to CVD risk factors. One cluster was interpreted as "healthy" because of a more frequent consumption of high-fiber and low-fat foods and lower consumption of products rich in fat and sugar. The "traditional" and "fast energy" clusters had a significantly higher BMIs and waist-to-hip ratios than did the "healthy" cluster. The "fast energy" cluster was associated with higher serum TG concentrations, and the "traditional" and "fast energy" clusters were associated with lower serum HDL-C concentrations (Berg et al., 2008). Maskarinec et al. (2000) investigated the relationship between dietary patterns and BMI among 514 women with different ethnic backgrounds by using a factor analysis approach. The most significant dietary pattern, "meat," characterized by high intakes of processed and red meats, fish, poultry, eggs, fats and oils, and condiments was positively associated with BMI, whereas the other three patterns ("vegetable", "bean", "cold food") were negatively associated with BMI.

Prospective studies also point towards a protective role of adhering to a "prudent" dietary pattern in weight management as compared to a "western" pattern. Among the largest studies, the relationship between major dietary patterns and weight change was investigated in the Nurses' Health Study II (Schulze et al., 2006) and in the EPIC-Potsdam Cohort (Schulz et al., 2005). Among more than 50 000 women in the Nurses' Health Study II, those that increased their western pattern score gained significantly more weight than those that decreased their western pattern score over the 8-years follow-up period. Furthermore, women who increased their prudent pattern score, had smaller weight gains than the women who decreased their prudent pattern score over time, after adjusting for baseline lifestyle and dietary confounders and changes in confounders. The EPIC Potsdam Cohort data revealed that in about 25 000 middle-aged men and women, nonobese subjects who scored high for the high-fiber low fat pattern maintained their weight or gained significantly less weight over time compared with subjects with the opposite pattern. In particular, the high-fiber low fat pattern was characterized by high consumption of whole-grain bread, fruits, fruit juices, grain flakes/cereals, and raw vegetables, and by low consumption of processed meat, butter, high-fat cheese, margarine and meat.

Large prospective studies suggest that major dietary patterns derived from factor analysis predict the long-term risk of CHD in men and women. A significant inverse association between the prudent pattern and the risk of total CHD but a positive association between the western pattern and the risk of CHD was found in men from the Health Professionals Follow-up Study over the 8 year follow-up period, and also in women from the Nurse Health Study during a 12 year follow-up period (Fung et al., 2001; Hu et al., 2000).

During the past decade, 3 large comprehensive reviews were published on the evidence that related dietary pattern and macronutrient composition to obesity or weight gain. Togo et al. (2001) reviewed 30 observational studies of patterns of food intake and their associations with BMI or obesity. Those authors concluded that associations between food intake patterns, as assessed from diet index, factor analysis or cluster analysis and obesity were inconsistent. They also commented that the heterogeneity of food intake patterns identified by such analyses and the lack of a common gold standard complicated the comparison and the interpretation of the studies. Fogelholm et al. (2012) systematically reviewed the role of dietary macronutrient composition, food consumption and dietary patterns in predicting weight or WC changes. The review covered 43 prospective cohort, case-control and intervention studies published from the year 2000 onwards. The grading of evidence was based on a four-class grading: convincing (high), probable (moderate), suggestive (low) and no conclusion (insufficient). Probable evidence was found for high intakes of nuts in predicting less weight gain and for high intakes of meat in predicting more weight gain. Suggestive evidence was found for fiber, whole grains, high-fat dairy products, such as cheese and yoghurt, and high scores on a prudent dietary pattern in predicting less weight gain and for high energy density and high intakes of sweets and desserts in predicting more weight gain. Moreover, there was suggestive evidence for both high fiber and fruit intakes in protecting against increases in WC. The intake of total carbohydrates, fats and proteins did not show consistent associations with weight gain. Summerbell et al. (2009) conducted a comprehensive systematic review on the epidemiological evidence between foods, food groups, macronutrients, energy intake and physical activity and subsequent excess weight gain and obesity. Among all the dietary exposures examined, a higher consumption of fast food and non-caloric sweeteners were related with greater subsequent weight gain, whereas being breastfed was associated with lower subsequent weight gain, albeit the differences were small. Consumption of other foods, energy intake, nutrient intake and physical activity were not associated with subsequent excess weight gain or obesity. The authors suggested the evident underreporting of actual foods and drink intake and the overreporting of actual physical activity was greater among the overweight and the obese, and that this misreporting is a possible explanation for these somewhat unexpected findings.

2.4 Assessment of habitual diet and physical activity

2.4.1 Dietary and physical activity assessment methods

Accurate assessment of diet and physical activity is a major challenge in epidemiologic studies. Habitual dietary intake and physical activity are complex behaviors with large day-to-day variations, which makes it challenging to accurately measure these behaviors in free-living populations. However, even with the relatively crude measurements available, previous epidemiologic studies have demonstrated that many dietary factors and physical activity behaviors have significant associations with obesity and weight gain (Hu et al. 2008). Several methods that measure energy expenditure, diet composition and physical activity levels are available and the choice of the instrument depends on the research setting. Each approach has advantages and limitations (**Table 1**). Objective methods include biomarkers such as urinary nitrogen to estimate protein intake and accelerometer-based devices to monitor different dimensions of physical activity. The major drawback for the use of biomarkers for objectively assessing dietary intake is the lack of suitable biomarkers for most nutrients and food groups. Objective measurement of physical activity is often not feasible in large epidemiologic studies due its high financial cost. Thus, large epidemiologic studies have relied on self-reported methods including records and diaries, recalls and questionnaires, among which validated questionnaires of habitual diet (i.e. FFQs) and physical activity have become the main assessment tools for measuring long-term habitual patterns.

Given their widespread use, it is critical to consider the degree to which the questionnaires actually measure the aspect of diet and physical activity that they were designed to measure, i.e. the validity of the questionnaire (Willet et al. 2013). Correlations between energy-adjusted nutrients assessed by FFQ relative to other self-reported reference methods, most commonly food records, generally range between r=0.45-0.7. For most nutrients, validity correlations with biomarkers are lower, in the range of r=0.3-0.5. For physical activity questionnaires, the correlation coefficients of most physical activities are also in the range of r=0.3-0.5 compared to other self-reported or objective measurements of physical activity. The low to moderate validity of questionnaire reflects both the complexity of measuring habitual behaviors and the lack of a true gold standard for measuring diet and physical activity behavior. The rather low correlations with nutrient biomarkers may also result from technical errors related to measurement of biomarkers and to the homeostatic control of biomarker metabolism in addition to nondietary determinants of biomarkers (Hu et al. 2008, Willet et al. 2013).

Table 1. Overview of dietary and physical activity assessment methods and their advantages and limitations.

Method	Advantages	Limitations
1. Self-reported dietary and physical activity assessment methods		
Food and activity records or diaries Diet: Subjects record in detail the types and amounts (scale or household measures) of all foods and beverages consumed on several days, typically 3 to 7. Physical activity: Subjects record virtually all physical activity performed on several days, including type, duration and intensity.	• Gives quantitatively accurate information • Does not rely on memory • Could improve compliance in intervention studies, e.g. weight-loss studies • Multiple 7-day records are used as gold standards for validating other methods	• Literacy required • Relatively high burden for participants • High cost of data collection • Burdensome to code • The recording process itself may alter behavior because of the increased awareness and knowledge that recording everything is a demanding task
24-hour dietary recall Diet: Subjects are interviewed about their food and beverage consumption in the preceding 24 hours or the previous day. Much of the information is collected by probing questions, e.g. on cooking methods and portion sizes using food photography aids. Physical activity: similar to diet, but time frame may be up to 1 month	• Relatively accurate information • Appropriate for most populations including children • Relatively low burden on respondent for single recalls • Does not influence behavior if unannounced • A single recall sufficient for national surveys and estimating group means	• Requires highly trained interviewers • A single 24-hour recall is not representative of habitual diet or physical activity • Relies on respondents memory and perception of portion sizes
Habitual diet and physical activity questionnaires Diet: Food-frequency questionnaires: Subjects report their usual frequency of consumption for a list of foods and beverages for a period of time, usually the previous year Physical activity: Questionnaires are designed to assess multiple dimensions of physical activity, including e.g. types, frequency, duration and intensity.	• Main dietary and activity assessment tool in large epidemiologic studies • Measures long-term patterns, usually over the past year • Relatively inexpensive to administer and process • Used to rank individuals • Low burden for participants • Does not alter behavior	• Involves memory and cognitive estimation ability • Quantification of intake difficult due to lack of detail • Not suitable for estimating absolute intake (diet) • Inaccuracies due to incomplete listing of all foods and errors in frequency and portion size evaluation (diet)
Diet history method Combined method consisting of a face-to-face interview (usually 24-h recall), a food frequency questionnaire checklist and a 3-day food record.	• Collects more accurate quantitative data on long-term intakes than either of the 3 dietary assessment methods alone • Assesses meal patterns	• Not feasible in large epidemiologic studies because it is time consuming and expensive • Difficult to standardize

Table 1 (continued).

2. Objective dietary and physical activity assessment methods

Recovery biomarkers A biomarker for which a quantitative relationship exists between the biomarker and dietary intake in a specific time period, e.g. doubly labeled water and 24-hour urinary nitrogen, potassium or sodium	• Can be used to calculate intakes over a specific time period • Doubly labeled water is an objective and accurate measure of energy expenditure • Good measures of short-term intakes	• Only available for very few dietary factors • Doubly labeled water is for energy only and quite costly • Reflect absolute intakes, while some research questions require energy-adjustment • Multiple assessments needed to estimate long-term intakes
Concentration biomarkers Biochemical measurements of nutrient concentrations in blood or other tissues can be used when a correlation exists between dietary intakes and concentrations in biological samples. Useful as reference instruments in dietary validation studies and as markers of adherence in dietary intervention studies	• Biochemical measures are objective • Plasma concentration of 25-hydroxy Vitamin D is the most useful measure of Vitamin D status • For fatty acids, the best markers of dietary intake exist for fatty acids that cannot be endogenously synthesized such as alpha-linolenic acid and linoleic acid (but this depends on the blood fraction or tissue) • Alcylresorcinols for whole-grain intake	• Not available for many dietary exposures • Relation between nutrient intake and concentrations in biological samples is rarely linear due to homeostatic mechanisms • Other lifestyle and genetic factors also influence nutrient concentrations • Reverse causation: Disease onset may change concentration levels
Accelerometers A device that subjects typically attach to their waist can detect and record daily activities. They are useful for validating self-reported methods	• Provides real time monitoring of frequency, duration and intensity of activities • Portable, lightweight, noninvasive, allows for extended periods of recording	• Not suitable in large studies due to the high cost • Inaccurate assessment for a range of activities including upper-body movement and water-based activities
Pedometer A small device that measures steps when walking or running	• Simple to use • Inexpensive, noninvasive	• Inability to store data • Cannot distinguish different intensities
Indirect calorimetry Calculates energy expenditure from measurements of O2 and CO2 1) Closed collection systems (e.g. Douglas bag); 2) Open-circuit systems (expiratory collection with masks or transparent hoods or room calorimetry)	• Accurate • Precise • Some of the devices can be designed as portable	• Need for trained technicians • Devices may be impractical to wear • Room calorimetry is time-consuming and requires expensive laboratory set-up

Modifed from Hu, 2008

2.4.2 Misreporting of self-reported food intake and physical activity behavior

The plausibility of self-reported energy intake can be estimated by comparing energy intake with energy expenditure as measured by DLW or calculated via standard equations that contain the basal metabolic rate (BMR) and a physical activity level (Goldberg et al., 1991; Schofield, 1985). Under the condition of stable weight, energy intake should equal energy expenditure. Misreporting of food intake entails both under- and overreporting, but underreporting is a much greater problem and challenge in nutrition research than the overreporting of food intakes (Livingstone et al., 2003). Nearly 18% of the men and 28% of the women in NHANES III in the US population were classified as energy underreporters based on self-reported energy intakes that were less than 90% of the energy needed for basal requirements (Briefel et al., 1997). Livingstone and Black (2003) performed a comprehensive review of studies that evaluated the validity of reported energy intake by DLW and convincingly demonstrated widespread bias resulting in the underestimation of energy intake. The 43 studies that included 77 subgroups, found a mean energy intake to energy expenditure ratio of 0.83. As many as 53 (69%) out of the 77 subgroups had a reported mean energy intake of more than 10% below the mean energy expenditure. The psychosocial and behavioral characteristics related to energy misreporting have been extensively reviewed by Maurer and colleagues (2006) and are summarized in **Figure 2**.

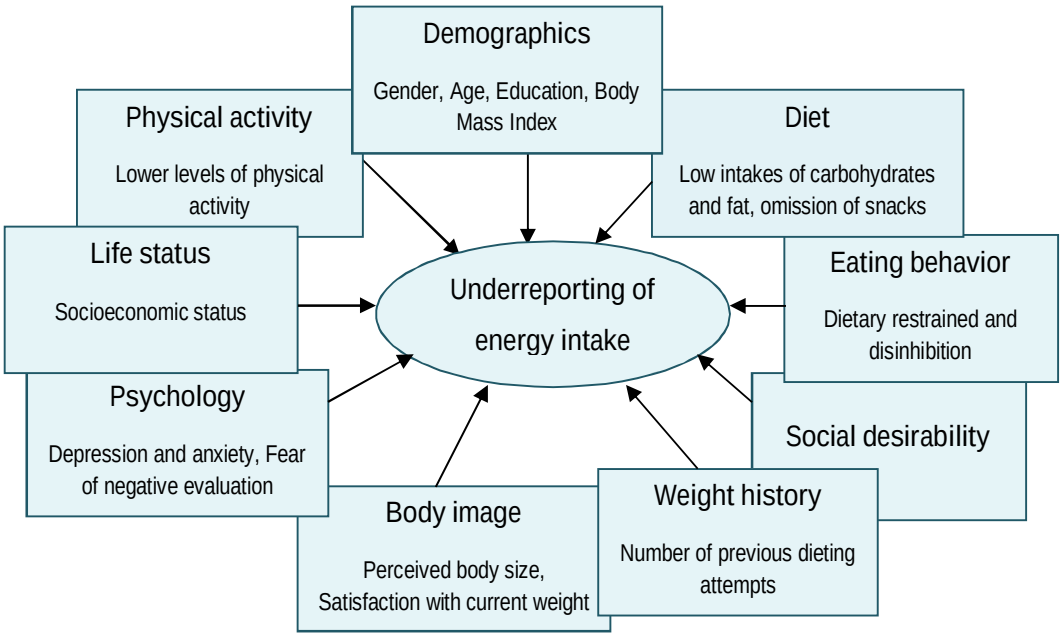

Figure 2. The psychosocial and behavioral factors related to misreporting of self-reported energy intake (modified from Maurer et al., 2006).

A number of studies have shown that the underreporting of energy intake is positively correlated with BMI and more prevalent among obese than normal-weight subjects (Goris et al., 2000; Lafay et al., 1997; Schoeller et al., 1990). A limited number of studies have also examined whether other metabolic variables differ between dietary underreporters and plausible energy reporters. In a cohort of overweight and obese postmenopausal women, underreporters had significantly higher fat mass, subcutenous fat tissue, visceral fat, C-reactive protein and lower levels of $VO2_{max}$ (Karelis et al., 2010). In another study among elderly men, underreporters were characterized by a higher WC, higher diastolic blood pressure, higher plasma insulin and a higher prevalence of the metabolic syndrome (Rosell et al., 2003).

As with self-reported dietary intake, physical activity questionnaires have well recognized limitations. Physical activity is a socially desirable behavior and its benefits are well known in the population. This may lead to overreporting of subject´s physical activity and underreporting of sedentary behaviors (Adams et al., 2005; Shephard, 2003). Similar to that found for diet, there is some evidence that the validity of physical activity questionnaires varies according to obesity status. Schmidt et al. (2003) compared estimates of time spent in physical activity using accelerometers with self-reported physical activity logs over a period of 7 days in 59 women. Correlations between the two measurements for total activity were fair to moderate. When those authors divided the sample according to BMI with a cut-off of 25 kg/m^2, there was a moderate significant correlation between total activity assessed by the two methods among the normal-weight women but not among the overweight and obese women (r=0.38 vs. 0.16) (Schmidt et al., 2003). Norman et al. (2001) assessed the validity of self-administered physical activity questionnaires against the respective activity records as a reference method in 111 middle aged and elderly men. The corresponding values differed significantly according to BMI status, with significantly higher validity correlations obtained from the lower BMI group (BMI<26 kg/m^2) compared to the heavier men (r =0.73 vs. 0.39). The overestimation of energy expenditure by activity records among 24 men on a controlled diet was greater with older age and with a higher percent of body fat as assessed by the DLW method (Irwin et al., 2001).

3 Aims of the Study

The overall objective of this thesis was to examine the cross-sectional associations of habitual dietary intake and physical activity with obesity measures and serum lipid-lipoprotein profiles in young adult twins. The study aimed to increase the accuracy of self-reported data by including objective measures of dietary intake and physical activity. In addition to analyzing twins as singletons, the co-twin control method was used to adjust for genetic background and unmeasured early environment shared by twins that usually confound these associations.

The specific aims of this thesis were as follows:

- To validate self-reported dietary intake and physical activity by using objective co-twin comparison assessments (I) and the DLW method (II);

- To investigate whether acquired eating and physical activity patterns are associated with obesity measures (I, II).

- To evaluate the association between dietary macronutrient composition and serum lipoprotein profile including HDL subclasses, and to estimate the potential effects of substituting one macronutrient for another (III).

- To examine whether acquired obesity and physical activity are associated with serum lipoprotein profile with a special focus on body fat distribution, i.e. subcutaneous, visceral and liver fat (IV).

- To study the relationship between habitual dietary intake (macronutrient intakes, dietary patterns and serum DHA) and serum lipoprotein subclass profile, and to examine how dietary patterns are related to anthropometric characteristics and nutrient intakes (V).

4 Materials and Methods

4.1 Study populations

This thesis is based on the FinnTwin16 and FinnTwin12 studies obtained from the Department of Public Health, University of Helsinki. **Figure 3** shows the data collection for those studies and the number of participants in the different substudies.

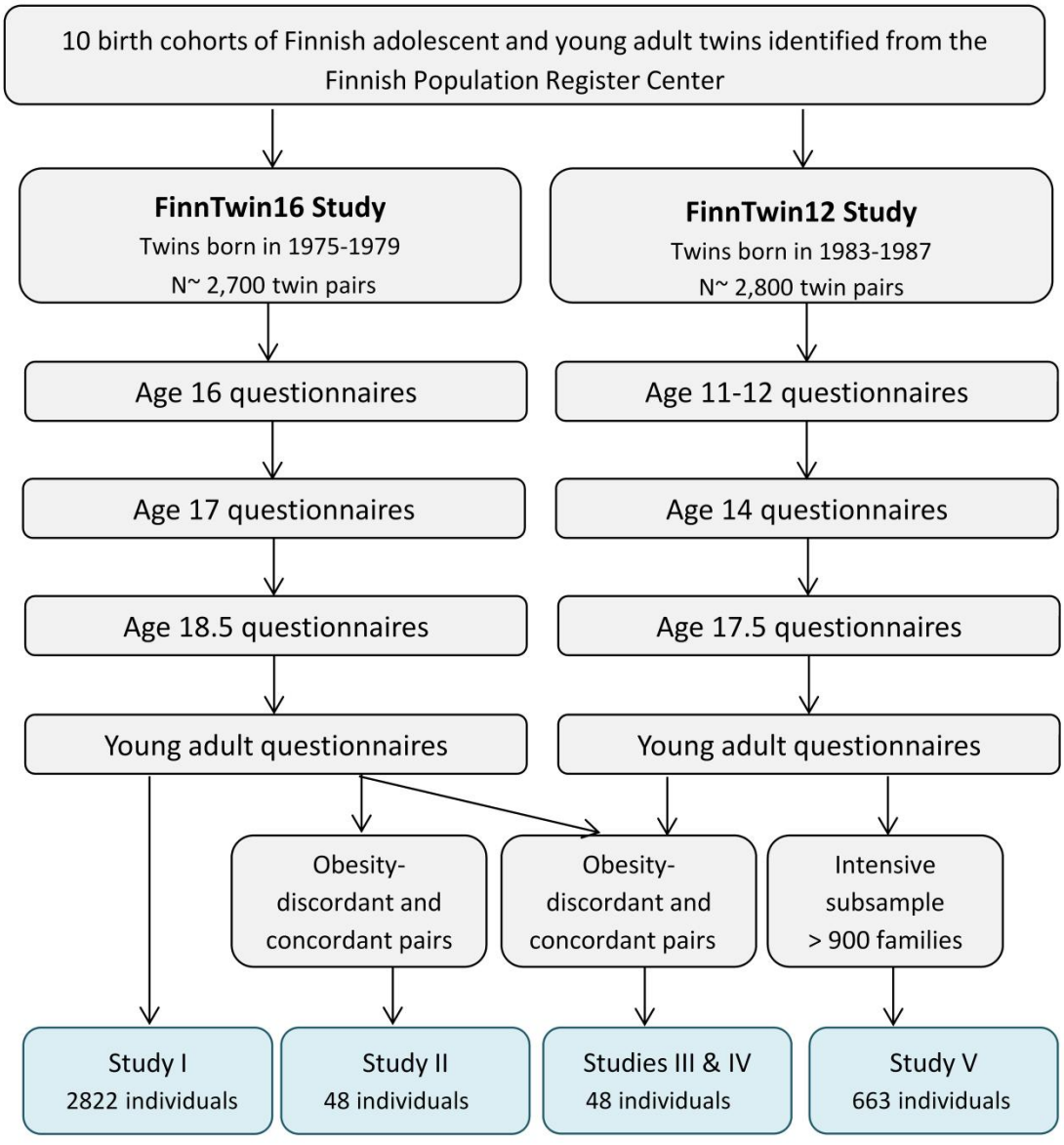

Figure 3. Two schematic charts of data collection in the FinnTwin12 and FinnTwin16 studies.

4.1.1 FinnTwin16 and FinnTwin12 cohorts

The participants were recruited from the FinnTwin12 and FinnTwin16 cohort studies, each consisting of five consecutive birth cohorts of Finnish twins that were ascertained from the Finnish Population Register Centre. Both studies are population-based, longitudinal investigations of behavioral development and health habits of Finnish twins who were initially enrolled during adolescence, and repeatedly assessed by questionnaires and interviews. The FinnTwin16 cohort includes Finnish twins born in 1975-1979. The baseline survey questionnaire was sent to all that cohort´s twins within 2 months after their 16th birthday (response rate of 88%) and individuals were mailed four follow-up questionnaires at 17, 18.5, ~24 years and ~35 years of age. The FinnTwin12 cohort includes Finnish twins born in 1983-1987. The twins received mailed questionnaires in the autumn of the year in which they reached the age of 11 (90% of the responses were received by the end of that year), and subsequent follow-up assessments were made at 14, 17.5 and ~22 years of age (Kaprio, 2013; Kaprio et al., 2002).

Study I includes all Finntwin16 subjects that responded to the fourth questionnaire. Opposite-sex twin pairs and twin individuals with missing co-twin information were excluded. Other exclusion criteria were unknown zygosity and missing data on height or weight. Study V includes a subsample of the FinnTwin12 subjects who visited the study center for one day, during which they filled out the FFQ and other questionnaires. A fasting blood sample was drawn on the same morning for the NMR spectroscopy analysis. Exclusion criteria were pregnancy, lipid-lowering medication, missing data for any of the covariates and implausible energy intakes (men: <800 or >5000 kcal/day; women: <600 or >4000 kcal/day). **Table 2** shows the number of twin individuals included in studies I and V and the general characteristics of the subjects.

Table 2. Descriptive statistics for age and measures of body size by zygosity.

	Study I (Finntwin16)		**Study V (Finntwin12)**	
	Monozygotic	Dizygotic	Monozygotic	Dizygotic
No. of twin individuals	1426	1396	267	411
No. of same-sex pairs	713	698	109	88
Females/males, n	564/862	646/750	166/101	195/216
Age, years	24.4 ± 0.9	24.4 ± 0.8	22.3 ± 0.6	22.4 ± 0.7
Weight, kg	66.9 ± 13.3	69.1 ± 13.7	67.4 ± 14.3	70.6 ± 15.6
Height, cm	171 ± 9	172 ± 9	171 ± 9	172 ± 9
BMI, kg/m^2	22.8 ± 3.4	23.1 ± 3.5	22.9 ± 3.6	23.5 ± 4.1
Waist circumference, cm	78.9 ± 10.8	79.9 ± 10.7	78.5 ± 9.5	80.1 ± 10.6

Data shown as mean values ± SD.

4.1.2 Obesity-discordant monozygotic twin pairs

The metabolic study on twins, TwinFat, started in 2002 with the identification of all obesity-discordant MZ pairs found at the last follow-up of FinnTwin16. After screening all MZ twin pairs, 18 pairs with a reported BMI difference of at least 3 kg/m^2 were identified. Of these, 14 pairs participated in study II (mean BMI difference ± standard deviation (SD): 5.2 ± 1.8 kg/m^2). For controls, 10 randomly selected weight-concordant MZ pairs were studied (BMI difference: 1.0 ± 0.7 kg/m^2). The obesity-discordant twin pairs were recontacted in 2008. Of the 14 pairs originally identified and examined in 2002, 9 were re-examined in 2008. Of these 9, only 5 pairs met the obesity-discordance criteria, and the remaining 4 pairs were no longer discordant. An additional 10 obesity-discordant MZ pairs were identified during the last follow-up of the FinnTwin12 at 22 years of age. A total of 5 pairs with reported or measured obesity discordance from the FinnTwin12 and FinnTwin16 databases were examined, although discordance criteria were no longer met. The resulting intensive samples in studies III-IV thus consisted of 15 obesity-discordant (BMI difference: 5.4 ± 1.8 kg/m^2) and 9 concordant (BMI difference: 1.8 ± 0.8 kg/m^2) MZ pairs. The discordant twin pairs represent the top 5% of the most discordant pairs of both cohorts. The twins were ascertained as being healthy based on their medical history and clinical examinations by the physician, Kirsi Pietiläinen. The twins were also normotensive, and did not take any medications other than oral contraceptives, with the exceptions of one obese co-twin (in studies III and IV) who had recently developed diabetes mellitus type 2 and was on insulin therapy and another obese co-twin who had an inactive ulcerative colitis and was receiving mesalazine and azathioprine medication. The twins' weights had been stable for at least 3 months preceding the study. None of the female subjects were pregnant or lactating. General characteristics of the weight-discordant and concordant MZ twins are shown in **Table 3**.

Table 3. Descriptive statistics for age and measures of body size in monozygotic co-twins discordant and concordant for obesity.

	Study II			**Studies III & IV**		
	Discordant pairs (n=14)		Concordant pairs (n=10)	Discordant pairs (n=15)		Concordant pairs (n=9)
	Heavier	Leaner	Both co-twins	Heavier	Leaner	Both co-twins
Females/males, n	6/8	6/8	10/10	9/6	9/6	8/10
Age, years	25.4 ± 1.8	30.6 ± 2.0	25.7 ± 1.2	26.8 ± 3.5	26.8 ± 3.5	30.1 ± 2.7
Weight, kg	88.8 ± 8.9	73.8 ± 8.7	80.2 ± 20.9	92.2 ± 19.5	75.7 ± 19.4	81.0 ± 12.7
Height, cm	170 ± 9	170 ± 9	173 ± 7	169 ± 7	168 ± 8	168 ± 7
BMI, kg/m^2	30.6 ± 2.0	25.4 ± 1.8	26.8 ± 7.1	30.3 ± 3.8	24.8 ± 1.0	28.4 ± 3.3
Body fat, %	38.3 ± 6.8	29.4 ± 8.8	28.2 ± 13.0	40.6 ± 7.7	32.2 ± 9.4	32.3 ± 8.5

Data shown as mean values ± SD.

4.1.3 Ethical considerations

The study protocols were approved by the institutional review boards of Indiana University, Bloomington, USA; the University of Helsinki, Finland and the Hospital District of Helsinki and Uusimaa, Finland. All participants gave their written informed consent.

4.2 Measures

An overview of the most important measures used in each study is given in **Table 4**. A more detailed description can be found in the following section.

Table 4. Measures used in studies I-V.

		Study				
		I	II	III	IV	V
Number of subjects		2822	48	48	48	663
Anthropometric assessment	Body mass index and waist circumference	x	x	x	x	x
	Body fat percentage		x	x	x	
	Liver fat			x		
Dietary assessment	Co-twin comparison questionnaire	x	x			
	Food-frequency questionnaire	x				x
	Three-day food diaries		x	x	x	
	Eating behavior questionnaire		x			
	Serum docosahexaenoic acid					x
Physical activity assessment	Self-reported frequency and intensity	x				
	Baecke questionnaire			x	x	x
	Three-day activity diaries		x			
	Double-labeled water		x			
Lipoprotein profile measurement	Enzymatic techniques, ultracentrifugation, gel electrophoresis			x	x	
	Serum nuclear magnetic resonance spectroscopy					x
Zygosity assessment	Validated zygosity questionnaire	x				x
	Genotyping of ten genetic markers		x	x	x	

4.2.1 Measures of obesity and body fat

FinnTwin16 and FinnTwin12 cohorts

In study I (Finntwin16), height, weight and WC were self-reported and subjects received a tape and instructions on how to self-measure WC by mail. In study V (Finntwin12), these anthropometric characteristics were measured by a study nurse. WC was measured midway between the spina iliaca superior and the lower rib with a nonflexible tape.

Obesity-discordant monozygotic pairs

In the subsample of obesity-discordant pairs (studies II-IV), weight and height were measured in the fasting state, with subjects being barefoot and in light clothing. Body composition was measured using whole body dual x-ray absorptiometry (DXA) scans (Lunar Prodigy, Madison, WI, software version 8.8). Whole body fat percentage was calculated as fat mass/(fat mass + lean mass + bone mineral content). The magnetic resonance imaging and spectroscopy measurements in study IV for determining the amounts of subcutaneous adipose tissue, visceral adipose tissue and liver fat were performed on a clinical 1.5-T imager (Avanto, Siemens, Erlangen, Germany) (Lundbom et al., 2010). Visceral and subcutaneous fat volumes of 16 slices, each 1 cm, centered at the L4/L5 intervertebral disk were measured by the SliceOmatic (TomoVision, Quebec, Canada using 4.3 segmentation software). Point-resolved spectroscopic localization technique was used to obtain nonsuppressed liver spectra. The localization of the voxel of interest was chosen within the right lobe of the liver avoiding vascular structures and contaminating signals from subcutaneous fat and the gallbladder. Liver spectra were analyzed by using jMRUI (Claude Bernard University of Lyon, Lyon, France) 3.0 software. Spectroscopic intra-cellular triglyceride content was expressed as methylene/(water methylene) signal area x 100. This measurement of the percentage of hepatic fat determined by proton spectroscopy has been validated against the lipid content of liver biopsies in humans (Ryysy et al., 2000).

4.2.2 Assessment of diet, physical activity and smoking

Co-twin comparison of eating and physical activity behavior

In studies I and II, co-twin comparisons of eating behaviors and physical activities were assessed by questionnaire, in which the twins were asked to compare their behavior with their corresponding co-twin's behavior during the last 12 months (**Appendix 1**). A total of 13 co-twin comparison questions were asked. For example "Which one of you eats more" with four response alternatives "me", "my co-twin", "there is no difference between us" and "I do not know".

Dietary assessment by food-frequency questionnaire

In study I, usual dietary habits during the previous 12 months were assessed by a quantitative 24-item FFQ (**Appendix 2**). The questionnaires were designed to be self-administered and

respondents were asked how often they consumed certain foods listed using five frequency response categories that ranged from never to several times per day. The answer categories were recoded into weekly consumption frequencies. In a previous study, factor analysis identified the following 4 dietary patterns using this questionnaire: healthy, high-fat foods, sweets and meat (Keskitalo et al., 2008).

In study V, information on habitual diet was estimated using a qualitative FFQ that incorporated 52 food and non-alcoholic beverage items that are common in the Finnish diet, 1 question on fat spreads and 3 questions on the type of bread consumed (**Appendix 3**). The question on fat spreads asked the respondent to describe the amount of fat spread per slice of bread by choosing one of these 5 response categories: "I do not use", "very little, a very thin layer", "little, a thin layer", "moderately" and "quite much". This question included 4 pictures that showed the different amounts of fat spreads on a slice of bread to help subjects to estimate their usual portion size. 3 additional questions asked about the number of slices of rye bread, whole grain bread or white (wheat) bread eaten on a typical day.

The 52-item FFQ was modified from a previous validated national FFQ and covered the main food groups (Pietinen et al., 2010; Silventoinen et al., 2009). Participants were asked to estimate how often they had consumed each of the food or beverage items ("never", "a few times per year or rarely", "a few of times per month", "a few times per week", "once a day", "several times a day") during the previous 12 months. The number of alcoholic beverages consumed during a typical week was assessed by a detailed interview as part of the Semi-Structured Assessment of Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994). First, participants were asked whether they consumed alcoholic beverages during the previous week, and, if yes, the number of standard drinks they consumed on each day categorized as beer, wine, spirits, and other alcoholic beverages. If participants reported the previous week to be unusual in terms of alcohol consumption, they were further asked to specify the number of drinks of beer, wine, spirits, and other alcoholic beverages they consumed during each day of a typical week. The number of drinks per week of beer, wine, liquor and other alcoholic beverages was then calculated by summing up the reported number of beverages consumed for each day during the previous week (if typical) or during a typical week (if the previous weeks consumption was atypical).

Nutrient intake calculation

The online version of the national computer-based food-composition database of the National Public Health Institute (Fineli) was used to convert food and beverage consumption data to energy and nutrient intakes (www.fineli.fi). First, a database was established containing all appropriate foods for each line item. The value for each line item was derived and weighted by the frequency of consumption in the food group considered. The most frequent foods consumed in Finland were derived from the Findiet 2002 (Mannistö et al., 2003). Standard portion sizes were assigned for each food item using standard portion size tables (Sääksjärvi and Reinivuo, 2004). Sex-specific portion sizes were assigned for the main dishes. The nutrient content for each composite line item was weighted according to the frequency of consumption of the respective single food items. The weekly consumption frequency was computed by recoding the response categories as follows: "never" = 0, "a few times per year

or rarely" = 0.25, "a few times per month" = 1, "a few times per week" = 3.5, "once a day" = 7 and "several times a day" = 17.5. The question on the amount of fat spread was recoded into the amounts in grams as follows: "I do not use" = 0, "very little, a very thin layer" = 2.25, "little, a thin layer" = 4.5, "moderately" = 6.75 and "quite much" = 9. Total energy and nutrient intake were calculated for each individual by multiplying the nutrient and energy contents of the specified portion of each food item by the frequency of its consumption and then summing all the items. Thus, an estimate of each subject's total nutrient daily intake was obtained. Macronutrients were expressed as percentages of total energy intake. The mean nutrient intakes of the study subjects are reported according to gender in **Table 5**.

Table 5. Energy and nutrient intakes (median and interquartile range) of the subjects.

Variable	Men (n=297)	Women (n=382)
Total energy intake (kcal/d)	2471 (2059, 2896)	1880 (1599, 2212)
Carbohydrates (E%)	44.9 (41.7)	47.9 (45.0, 51.4)
Protein (E%)	16.7 (15.3, 18.4)	15.7 (14.3, 17.5)
Total fat (E%)	31.3 (28.4, 34.0)	29.4 (26.4, 32.6)
Alcohol (E%)	4.4 (2.0, 6.3)	3.1 (1.6, 4.9)
Saturated fatty acids (E%)	11.2 (9.8, 12.6)	11.1 (9.5, 12.3)
Sucrose (E%)	7.5 (5.9, 9.4)	10.3 (8.1, 12.7)
Fiber (g/d)	21.2 (16.4, 26.6)	20.1 (15.6, 25.6)
Vitamin C (g/d)	85.1 (59.2, 121.8)	86.6 (62.6, 129.5)
Vitamin D (g/d)	7.5 (5.7, 9.7)	5.0 (3.8, 6.8)
Calcium (g/d)	1182 (882, 1484)	1018 (734, 1273)
Iron (g/d)	14.2 (12.0, 17.2)	11.9 (9.9, 14.0)

E%, percentage of energy intake.

Dietary assessment by three-day food diaries

In studies II-IV, total energy and macronutrient intake data that were estimated from 3-day food diaries were used. The subjects were instructed by a registered dietician to record all foods and beverages they consumed for 3 days using household measures and to adhere to their usual diet during the recording period. The days were predefined and consecutive (two working and one non-working day). The conversion of data from the records into nutrient values was performed by using the program DIET32, which incorporates the national Finnish database for food composition (Aivo Finland Oy). Daily energy intake was expressed in kcal and macronutrient intakes as percentages of total energy intake. Dietary intake data of total n-3 PUFA, including ALA, EPA, and DHA were assessed. Intake data on supplements were not included in the nutrient intake calculation. Mean food and nutrient intakes were calculated by taking the mean of the 3 days.

Eating behavior questionnaires

In study II, obesity-related eating habits and behaviors were obtained by questionnaire. The participants were asked to choose one of four options that best characterizes their overall

eating style (“normal”, overeating, restrictive eating or alternating overeating and restricting) and answer a short 12-item questionnaire with 5 items assessing snacking styles, 3 items for assessing health-conscious eating, 2 items for assessing emotional eating, one item to assess externally cued eating (eating triggered by seeing food or advertisements of food, etc.) and one item about night eating) with the response alternatives “usually”, “often”, “sometimes” or “rarely” (Keski-Rahkonen et al., 2007). The answer categories “usually” and “often” were combined in the analysis as was “sometimes” and “rarely”. Body dissatisfaction, drive for thinness and bulimia subscales obtained from the Eating Disorder Inventory were used to evaluate body image and psychological and behavioral aspects of eating (Garner, 1991). An abbreviated 18-item version of TFEQ was used to assess the cognitive aspects of eating and eating behavior (Stunkard and Messick, 1985).

Physical activity assessment

In study I, the weekly hours of physical activity computed were based on 2 questions. The first question was “How often do you exercise in your leisure time?” The response alternatives were “Not at all”, “Less than once a month”, “1–2 times a month”, “About once a week”, “2–3 times a week”, “4–5 times a week” and “Every day”. The second question was “How long do you exercise per occasion?” The response alternatives were “Less than 30 minutes”, “Half an hour to under one hour”, “One hour to under 2 hours” and “Two hours or more”. The assessment of the intensity of physical activity was based on the following question: “Is your physical activity during leisure-time about as strenuous on average as: walking, alternately walking and jogging (slow running), jogging or running?” The following MET numerical values were assigned to the categories: 4 (for exercise intensity corresponding to walking), 6 (alternately walking and jogging), 10 (jogging), and 13 (running).

In studies II-V, the Baecke physical activity questionnaire was used (Baecke et al., 1982). The Baecke questionnaire apportions separate indices for physical activity at work (work index), sports activities during leisure time (sport index) and physical activity during leisure time excluding sports (leisure time index). The total physical activity index was obtained by summing up the three indices.

In study II, physical activity was also assessed by 3-day activity records. Each day was divided into 96 periods of 15 min each. For every 15 min period, the subjects were asked to assign the dominant activity performed to one out of eight categories. Energy costs were assigned to each category in the activity diaries as follows: 1 (sleeping or resting), 1.2 (sitting), 1.4 (standing), 1.6 (working at a very low intensity), 2.8 (working at a low intensity), 3.8 (working or exercising at a moderate intensity), 5.1 (working or exercising at a vigorous intensity) and 6.7 (working or exercising at a very vigorous intensity). The BMR of the individual subjects was estimated by recommended predictive equations (WHO 1985). The amount of time spent in each activity category in hours was multiplied by the energy cost of that specified activity, each of which were summed and divided by 24 to obtain the total mean physical activity level. Total energy expenditure (TEE) was estimated by multiplying the BMR by the total physical activity level. Activity energy expenditure was calculated as TEE− [BMR + 0.1 TEE)].

Assessment of energy intake underreporting

In study II, energy intake of each individual was validated against TEE measured by DLW (Westerterp et al., 1995). The measurement was concurrent with the 3-day food and physical activity diary collections and performed in a free-living setting for 14 consecutive days after leaving the study center. Underreporting of actual energy intake was defined as TEE (DLW)-reported energy intake and overreporting of physical activity as reported TEE (activity diaries)-TEE (DLW).

Assessment of smoking habits

Smoking habits were assessed by the following question: "Which of the following best describes your smoking habits?" The response alternatives were: 1) "I smoke 20 cigarettes or more a day"; 2) "I smoke 10-19 cigarettes a day"; 3) "I smoke 1-9 cigarettes a day"; 4) "I smoke once a week or more often but not daily"; 5) "I smoke less than once a week"; 6) "I have quit smoking"; 7) "I have experimented with smoking, but I don't smoke" and 8)" I have never smoked". In studies III and IV, the response categories 1-3 were combined as daily smokers and all others as non-smokers. In study V, the response categories 1-3 were combined as daily smokers, 4 and 5 as occasional smokers, 6 as former smokers, and 7 and 8 as never smokers.

4.2.3 Biochemical analysis of serum lipid profiles

Studies III and IV used biochemical analysis of serum lipid profiles. Venous fasting blood samples were drawn after an overnight fast within one week before the collection of the food and activity diaries and DLW measurements. Serum concentration of total cholesterol, TG and HDL-C were measured in fresh samples using enzymatic methods (Roche Diagnostics Hitachi; Hitachi, Tokyo, Japan). LDL-C was calculated using the Friedewald equation (LDL = total cholesterol-(HDL-C)-TG/2.2) (Friedewald et al., 1972). HDL was isolated by ultracentrifugation of a 0.5 ml volume of serum (Taskinen et al., 1988). Distribution of HDL 2b, 2a, 3a, 3b, and 3c subspecies and mean particle size of HDL were determined by native GEE (Blanche et al., 1981; Pérusse et al., 2001). The molecular size intervals for HDL subspecies 2b, 2a, 3a, 3b, and 3c were used, and for each subspecies, the relative area under the densitometric scan is reported. Mean HDL particle size was calculated by multiplying the mean size of each HDL subclass by its relative area under the densitometric scan. The LDL peak particle diameters were determined using 1 mm 2% to 10% linear nondenaturating polyacrylamide gradient gels as the gradient matrices (Vakkilainen et al., 2002).

In study V, lipoprotein subclasses and concentrations were measured by proton NMR spectroscopy (Ala-Korpela, 2008; Soininen et al., 2009). This NMR platform has recently been used in various extensive epidemiological and genetics studies (Inouye et al., 2010; Kujala et al., 2013; Stančáková et al., 2011). Concentrations of the subclasses were as follows: chylomicrons and extremely large VLDL particles (with particle diameters from ~75 nm upwards), very large VLDL (mean particle diameter of 64.0 nm), large VLDL (53.6 nm), medium VLDL (44.5 nm), small VLDL (36.8 nm), very small VLDL (31.3 nm); intermediate-density lipoprotein (IDL) (28.6 nm), large LDL (25.5 nm), medium LDL (23.0

nm), and small LDL (18.7); very large HDL (14.3 nm), large HDL (12.1 nm), medium HDL (10.9 nm), and small HDL (8.7 nm) (**Figure 4**). We grouped "extremely large", "very large" and "large VLDL" to "large VLDL", "small VLDL" and "very small VLDL" to "small VLDL", "IDL" and "large LDL" to "large LDL" and "very large HDL" and "large HDL" to "large HDL". Thus, 3 subclasses (large, medium and small) of VLDL, LDL and HDL were analyzed. The concentrations of individual VLDL, LDL and HDL subclasses were summed to obtain total particle concentrations. The mean particle sizes for VLDL, LDL and HDL particles were calculated by weighting the corresponding subclass diameters with their particle concentrations. IDL particles were included in the LDL measure. Apolipoprotein B (ApoB) and Apolipoprotein A1 (ApoA1) were calculated from an extended version of the Friedewald formula (Niemi et al., 2009).

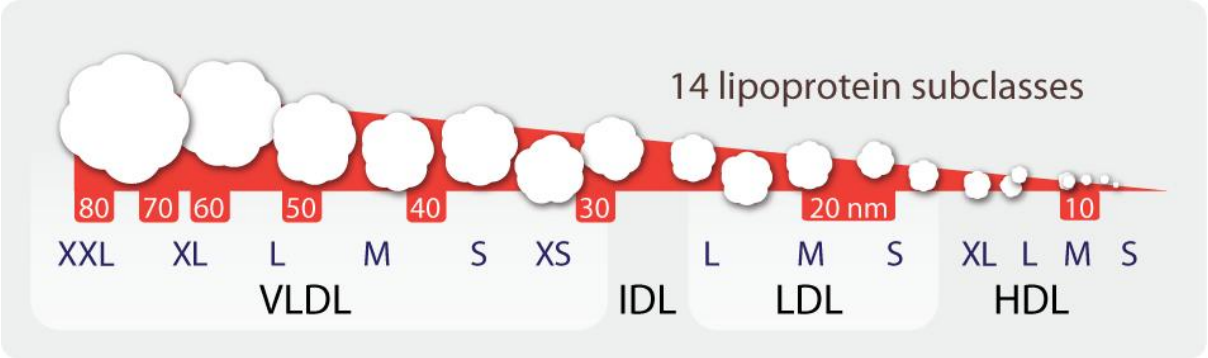

Figure 4. Particle size of 14 lipoprotein subclasses measured by proton NMR spectroscopy. This figure is a courtesy of Antti J. Kangas.

4.2.4 Zygosity

The zygosity status was determined by a validated questionnaire (Sarna et al., 1978). In some unclear cases, questionnaire information was supplemented by additional information from photographs, fingerprints, and DNA marker studies (Kaprio et al., 2002; Sarna et al., 1978). In the obesity-discordant pairs, zygosity was confirmed by genotyping of ten informative genetic markers by the Paternity Testing laboratory, National Public Health Institute, Helsinki, Finland (Pietiläinen et al., 2004).

4.3 Statistical methods

All statistical analyses were carried out using the Stata statistical software package (release 9.0 or 11.0; Stata Corporation, College Station, Texas). The descriptive characteristics of the sample are shown as mean ± SD and results as mean ± standard error (SE), unless specified otherwise.

6.3.1 Individual-level analyses

When analyzing twins as individuals, the effect of the clustering of individuals within twin pairs on standard errors and P-values was taken into account by survey methods (Williams, 2000). Non-normally distributed variables were log-transformed before parametric analyses. Differences between men and women were evaluated by the adjusted Wald test (t-test adapted for clustered twin data) for independent samples for continuous variables and by the chi-square test for categorical variables. Pearson correlations or Spearman correlations were used to determine correlations between the dietary intakes and lipid profiles. The association between sex-specific tertiles of macronutrients and NMR-derived lipoprotein subclass profile was also analyzed by Analysis of covariance (ANCOVA). A test for a linear trend across increasing tertiles of nutrients was performed by assigning the median value of each nutrient for each category and treating these as a continuous variable. Interaction terms were added to the multivariate models to test whether the association between diet and lipoprotein profile was similar in men and women. When the interaction terms were significant, the results were presented separately according to sex. Non-normally distributed variables were log-transformed and back-transformed to present the geometric means and their 95% CIs. Pearson partial correlations and multivariate models were adjusted for confounding factors, including age, sex, BMI, smoking status and physical activity (if not indicated otherwise).

In study V, factor analysis was conducted using factor loadings that were extracted by the principal component analysis (PCA) and rotation by an orthogonal transformation (varimax rotation) to derive dietary patterns. Daily foods and beverage intake were log-transformed and expressed as nutrient densities (grams per 1000 kcal) before entry to PCA to adjust for energy intake. Extracted factors were assessed by eigenvalue, scree-plot analysis and theoretical interpretability. A 5-factor solution was selected. Finally, dietary pattern scores were calculated by summing the food intakes that were weighted by the factor loading and thus, each subject received a factor score for each pattern (Kim and Mueller, 1978). Food patterns were named according to the foods that loaded most positively on the factor. Pearson's correlation coefficients were calculated to assess associations between the dietary pattern scores and anthropometric characteristics and also nutrient intakes.

6.3.2 Multivariate nutrient density model

Total energy intake is associated with the intake of most nutrients. It may also be associated with serum lipid concentrations and other potentially confounding variables, such as body size and physical activity. These associations can introduce confounding and might influence the results of correlation analysis. Therefore, multivariate nutrient density models were also built (Willett et al., 1997). This model can be interpreted as the association between the nutrient compositions of the diet with the serum lipid profile when total energy is constant. Thus, this is an isocaloric analysis that adjusts for confounding by total energy intake, where an increase in the intake of one macronutrient corresponds to a reduced intake of (an)other macronutrient(s). For example, if carbohydrates are excluded and all others macronutrients are included in the model, the β coefficient for each macronutrient in the model represents the effect of replacing carbohydrates by an equal amount of energy from that particular

macronutrient. This model was used to evaluate the replacement of 5% of energy from carbohydrates by protein or total fat. In a model that included just one type of fat (1% of energy intake) along with total fat and total energy intake (i.e. the fat substitution model) (Willett, 2013), we estimated the effect of substituting that fat for the same amount energy from other type of fats in the diet. Potential confounding factors were included in the models.

6.3.3 Within-pair analyses

Co-twin comparison of eating and physical activity behavior was analyzed by the paired *t* test in pairs when both co-twins gave the same answer (both co-twins reported on the same individual in the pair). In those pairs with internally consistent answers, differences between co-twins in self-reported food intake and physical activity behaviors were also analyzed by the paired *t* test. Using all twin pairs, multivariate regression analysis was carried out by using the intrapair difference in BMI or WC as the dependent variable and the eating and physical activity-related behaviors as the independent variables. The twin pairs for which the co-twin with the higher BMI or WC exhibited the behavior and the co-twin with the lower BMI or WC did not was coded as 1. The twin pairs for which the co-twin with the lower BMI or WC exhibited the behavior and the co-twin with the higher BMI or WC did not was coded as –1. All other pairs i.e. those with no differences in BMI or WC, those with inconsistent answers or those for which one or both members of the pairs did not know were coded as 0. In the obesity-discordant pairs, differences between obese and lean co-twins were tested by the Wilcoxon's Matched-Pairs Signed-Ranks Test for continuous variables and by the McNemar's Test of Symmetry for categorical variables. The Wilcoxon’s test was also used to analyze whether underreporting was significantly different from zero. The Mann-Whitney U test was used to test for differences between twin pairs discordant and concordant for liver fat. Twin similarity was assessed using sex-adjusted intra-class correlations. Associations of dietary intakes and obesity with the serum lipid profile within twin pairs were analyzed using Pearson partial correlations and multivariate analysis adjusted for potential confounding variables. The same multivariate nutrient density models that were used in individual twins were also applied to intrapair differences.

5 Results

5.1 Co-twin comparison of eating and physical activity habits (I)

Study I aimed to investigate whether acquired eating and physical activity patterns are associated with obesity measures and to compare objective co-twin comparison assessments against those of self-reported food intake in 1411 twin pairs. MZ and DZ twins did not differ from each other in the food group intakes. Men consumed "healthy" foods (mean ± SE: 27.7 ± 0.4 vs. 38.4 ± 0.7 servings/week) and sweet and fatty delicacies (6.1 ± 0.1 vs. 7.4 ± 0.1) less frequently and fatty foods (8.4 ± 0.2 vs. 6.1 ± 0.1) and meat (9.6 ± 0.2 vs. 6.2 ± 0.1) more frequently than women ($p<0.001$ for all). Men and women did not differ from each other in the weekly hours of physical activity (3.3 ± 0.1 vs. 3.4 ± 0.1 hours/week), but men exercised at higher intensities than women (9.4 ± 0.1 vs. 7.7 ± 0.1 MET, $p<0.001$). 13% of the young adult MZ and 8% of the DZ twins still lived in the same household. About three quarters (74%) of the MZ and half (48%) of the DZ twins reported meeting or being daily or almost daily contact and 22.5% of the MZ and 43% of the DZ twins had contact at least weekly. Only 7% of the twins (3.5% MZ, 9% DZ) reported to be in contact with the co-twin less than once a week.

In individual-level analysis, self-reported intake of "healthy foods" per week was weakly inversely correlated with BMI and WC ($r=-0.10$, $p<0.001$ and $r=-0.13$, respectively, $p<0.001$) in men. Self-reported intake of meat per week was weakly positively correlated with BMI and WC ($r=0.07$, $p=0.004$ and $r=0.11$, $p<0.001$, respectively) in women. Intrapair differences in the intake of "healthy" foods, fatty foods, sweet and fatty foods or meat were not significantly correlated with intrapair differences in BMI or WC in MZ or DZ twins for either sex.

The pairwise response frequencies to eating and physical activity-related behavior are given in **Table 6**. Co-twin reports revealed that 33.7% of the MZ and 46.9% of the DZ twin pairs agreed that they differ in their exercise behavior from each other. A substantial proportion of the twins also agreed on which of the twin eats more overall, 29.6% and 38.1% of the MZ and DZ pairs, respectively. The agreement was weaker for dieting and non-exercise activities, such as fidgeting. A larger proportion of the DZ twins reported that they differ in the specific behavior compared to MZ twin pairs. In contrast, MZ twin pairs more often reported that there is no difference between them in the specific behavior compared to DZ twin pairs (Table 6).

For some behaviors, such as exercising, male and female twin pairs agreed to a similar extent on which twin exhibits the behavior more (35% of MZ male pairs and 32% of MZ female pairs, 47% of DZ male pairs, 46% of DZ female pairs). For some behaviors, male twins reported more often that there is a notable difference between them and their respective co-twins than female twins. For example, 21% of MZ and 30% of DZ men reported to differ in the intake of sweet and fatty delicacies, whereas the corresponding percentages in women were lower 11% and 22%, respectively.

Table 6. Number and percentage of twin pairs with consistent and inconsistent answers for 13 eating and physical activity-related behaviors.

Behavior		Twin A answers							
Which one of you…?	Twin B answers	Me		My co-twin		There is no difference between us		I do not know	
		MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
Eats more? N=710 MZ, 693 DZ	Me My co-twin No difference I do not know	33 (4.7) **210 (29.6)** 104 (14.7) 40 (5.6)	62 (9.0) **264 (38.1)** 111 (16.0) 69 (10.0)	 8 (1.1) 66 (9.3) 23 (3.2)	 15 (2.2) 55 (7.9) 24 (3.5)	 145 (20.4) 63 (8.9)	 54 (7.8) 29 (4.2)	 18 (2.5)	 10 (1.4)
Eats more snacks? N=709 MZ, 693 DZ	Me My co-twin No difference I do not know	21 (3.0) **155 (21.9)** 94 (13.3) 47 (6.6)	52 (7.5) **214 (30.9)** 100 (14.4) 62 (9.0)	 17 (2.4) 83 (11.7) 35 (4.9)	 38(5.5) 63 (9.1) 50 (7.2)	 164 (23.1) 66 (9.3)	 50 (7.2) 37 (5.3)	 27 (3.8)	 27 (3.9)
Eats more fatty foods? N=708 MZ, 695 DZ	Me My co-twin No difference I do not know	11 (1.6) **113 (16.0)** 90 (12.7) 29 (4.1)	22 (3.2) **172 (24.8)** 79 (11.4) 40 (5.8)	 6 (0.9) 126 (17.8) 34 (4.8)	 36 (5.2) 87 (12.5) 58 (8.4)	 200 (28.3) 83 (11.7)	 111 (16.0) 63 (9.1)	 16 (2.3)	 27 (3.9)
Eats more sweet and fatty delicacies (e.g. chocolate, pastries, ice cream) N=711 MZ, 693 DZ	Me My co-twin No difference I do not know	17 (2.4) **119 (16.7)** 104 (14.6) 34 (4.8)	33 (4.8) **183 (26.4)** 97 (14.0) 62 (9.0)	 13 (1.8) 98 (13.8) 34 (4.8)	 28 (4.0) 76 (11.0) 45 (6.5)	 198 (27.9) 76 (10.7)	 111 (16.0) 63 (9.1)	 18 (2.5)	 27 (3.9)
Selects food more according to healthiness? N=709 MZ, 689 DZ	Me My co-twin No difference I do not know	17 (2.4) **148 (20.9)** 115 (16.2) 27 (3.8)	34 (4.9) **212 (30.8)** 105 (15.2) 43 (6.2)	 13 (1.8) 88 (11.4) 24 (3.4)	 16 (2.3) 70 (10.2) 28 (4.1)	 196 (27.6) 68 (9.6)	 119 (17.3) 49 (7.1)	 13 (1.8)	 13 (1.9)
Eats more sweets (e.g. candies or jellies)? N=710 MZ, 693 DZ	Me My co-twin No difference I do not know	30 (4.2) **133 (18.7)** 112 (15.8) 36 (5.1)	50 (7.2) **215 (31.0)** 85 (12.3) 57 (8.2)	 10 (1.4) 101 (14.2) 26 (3.7)	 21 (3.0) 87 (12.6) 33 (4.8)	 175 (24.7) 69 (9.7)	 76 (11.0) 44 (6.4)	 18 (2.5)	 25 (3.6)
Eats more slowly? N=710 MZ, 693 DZ	Me My co-twin No difference I do not know	13 (1.8) **135 (19.0)** 80 (11.3) 22 (3.1)	25 (3.6) **202 (29.2)** 60 (8.7) 34 (4.9)	 20 (2.8) 107 (15.1) 31 (4.4)	 37 (5.3) 105 (15.2) 59 (8.5)	 179 (25.2) 95 (13.4)	 77 (11.1) 68 (9.8)	 28 (3.9)	 25 (3.6)

Table 6 (continued). Number and percentage of twin pairs with consistent and inconsistent answers for 13 eating and physical activity-related behaviors.

Behavior		Twin A answers							
	Twin B answers	Me		My co-twin		There is no difference between us		I do not know	
		MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
Eats more regularly?	Me	17 (2.4)	33 (4.8)						
N=712 MZ, 693 DZ	My co-twin	**195 (27.4)**	**233 (33.6)**	22 (3.1)	26 (3.8)				
	No difference	97 (13.6)	95 (13.7)	85 (11.9)	90 (13.0)	192 (27.0)	70 (10.1)		
	I do not know	27 (3.8)	52 (7.5)	23 (3.2)	41 (5.9)	42 (5.9)	37 (5.3)	12 (1.7)	16 (2.3)
Is more worried about appearance?	Me	27 (3.8)	25 (3.6)						
N=707 MZ, 695 DZ	My co-twin	**118 (16.7)**	**188 (27.1)**	9 (1.3)	23 (3.3)				
	No difference	99 (14.0)	81 (11.7)	101 (14.3)	89 (12.8)	189 (26.7)	93 (13.4)		
	I do not know	47 (6.6)	53 (7.6)	35 (5.0)	34 (4.9)	78 (11.0)	83 (11.9)	26 (3.7)	26 (3.7)
Goes on diets more often?	Me	11 (1.6)	10 (1.5)						
N=707 MZ, 687 DZ	My co-twin	**91 (12.9)**	**137 (19.9)**	5 (0.7)	8 (1.2)				
	No difference	65 (9.2)	47 (6.8)	69 (9.8)	77 (11.2)	275 (38.9)	153 (22.3)		
	I do not know	21 (3.0)	32 (4.7)	33 (4.7)	45 (6.6)	103 (14.6)	129 (18.9)	34 (4.8)	49 (7.1)
Exercises more?	Me	14 (2.0)	24 (3.5)						
N=710 MZ, 689 DZ	My co-twin	**239 (33.7)**	**323 (46.9)**	10 (1.4)	9 (1.3)				
	No difference	128 (18.0)	99 (14.4)	81 (11.4)	64 (9.3)	182 (25.6)	89 (12.9)		
	I do not know	12 (1.7)	28 (4.1)	10 (1.4)	20 (2.9)	29 (4.1)	27 (3.9)	5 (0.7)	6 (0.9)
Walks instead of taking a car or elevator, or makes other 'active' choices in daily life?	Me	22 (3.1)	27 (3.9)						
	My co-twin	**123 (17.3)**	**192 (27.6)**	5 (0.7)	8 (1.2)				
	No difference	147 (20.7)	131 (18.9)	75 (10.6)	66 (9.5)	217 (30.6)	121 (17.4)		
N=710 MZ, 695 DZ	I do not know	30 (4.2)	47 (6.8)	24 (3.4)	29 (4.2)	56 (7.9)	60 (8.6)	11 (1.6)	14 (2.0)
Makes more movement during normal non-exercise activities (i.e., fidgeting)?	Me	35 (4.9)	65 (9.4)						
	My co-twin	**97 (13.7)**	**158 (22.8)**	12 (1.7)	9 (1.3)				
	No difference	154 (21.7)	167 (24.1)	55 (7.8)	41 (5.9)	189 (26.6)	66 (9.5)		
N=710 MZ, 693 DZ	I do not know	54 (7.6)	78 (11.3)	16 (2.3)	21 (3.0)	74 (10.4)	64 (9.2)	24 (3.4)	24 (3.5)

Abbreviations: MZ, monozygotic; DZ, dizygotic; N, number of twin pairs. Percentages are shown in brackets. The number of twin pairs with differences in eating or physical-activity-related behaviors based on consistent answers of both co-twins is shown in bold.

We obtained a high degree of accuracy in those cases where both co-twins gave the same internally consistent answer of there being a difference between them, when we analyzed which of them had the larger BMI or WC. In both MZ and DZ pairs, the co-twins who ate more, ate more snacks, ate more fatty foods, ate more sweet and fatty delicacies (chocolate, pastries, ice cream), ate faster, selected food less according to its perceived healthiness and made less active choice in daily life, had significantly higher BMI and WC than their co-twins. The P-values ranged from 0.05 to 0.0001 (**Figure 5**). In addition, MZ and DZ twin pair members who had more movement during normal non-exercise activities (i.e. fidgeting) had lower WC values (intrapair difference (Δ) mean ± SE: -2.1 ± 0.8 cm, p=0.009 for MZ and -3.7 ± 1.0 cm, p=0.0003 for DZ pairs) and lower BMI (Δ: -1.36 ± 0.35 kg/m^2, p=0.0001) in DZ pairs. Being more worried about appearance was associated with having a lower BMI and WC in DZ pairs (ΔWC: -3.1 ± 0.9 cm, p=0.001 and ΔBMI: -0.7 ± 0.3 kg/m^2, p=0.047). Dieting was associated with a greater BMI (Δ: 1.5 ± 0.4 kg/m^2, p=0.0001), but not with a greater WC, in DZ pairs. Eating regularly and eating more sweets (candies or jellies) were not associated with differences in BMI or WC between co-twins.

MZ and DZ twin pair members who ate less fatty foods overall than their co-twins had the lowest BMI, 21.7 ± 0.3 kg/m^2 and 22.3 ± 0.2 kg/m^2, respectively. MZ and DZ twins who ate fewer fatty delicacies had the lowest WC, 74.7 ± 0.8 cm and 76.5 ± 0.7 cm, respectively. Twin pair members who ate more (overall) than their co-twins had the highest BMIs (MZ: 23.9 ± 0.3 kg/m^2, DZ: 25.0 ± 0.3 kg/m^2) and WCs (MZ: 82.0 ± 0.9 cm, DZ: 85.3 ± 0.8 cm). The biggest BMI and WC differences were observed between the twins that differed in the amount of food consumed in both zygosity groups. MZ twins who ate more than their co-twins had 1.9 ± 0.1 kg/m^2 higher BMI and 5.5 ± 0.6 cm larger WC than their corresponding co-twin. Among DZ twin pairs the differences were larger, 2.9 ± 0.2 kg/m^2 and 7.5 ± 0.7 cm, respectively.

The intrapair differences in BMI and WC in those co-twins who provided internal consistent answers were generally similar between male and female twin pairs. For example, male MZ twin pair members who ate more than their twin brothers had a BMI that was 1.9 ± 0.2 kg/m^2 higher and a WC that was larger by 4.9 ± 0.7 cm. The corresponding differences in MZ female twins were 1.9 ± 0.3 kg/m^2 and 6.5 ± 0.9 cm, respectively. Male DZ twin pair members who ate more than their twin brothers had a BMI that was higher by 3.0 ± 0.4 kg/m^2 and a WC that was larger by 7.9 ± 0.9 cm. The corresponding differences in DZ female twins were 2.7 ± 0.3 kg/m^2 and 7.1 ± 1.1 cm, respectively (p<0.0001 for all).

A small number of twin pairs (n=19 MZ and 17 DZ) differed in 5 of the eating behaviors related to food choice. The co-twins who ate more, ate more snacks, ate more fatty foods, ate more sweet and fatty delicacies and selected food less according to healthiness had a 3.4 ± 0.7 kg/m^2 higher BMI and a 9.2 ± 2.3 cm larger WC than their co-twins (p<0.001). The corresponding differences for DZ twins were 5.3 ± 1.4 kg/m^2 and 12.5 ± 3.1 cm, respectively (p<0.01) (Figure 5). An intrapair difference of more than 3 BMI units was found for 11 out of these 19 MZ and 12 out of these 17 DZ twin pairs (Bogl LH et al., unpublished results).

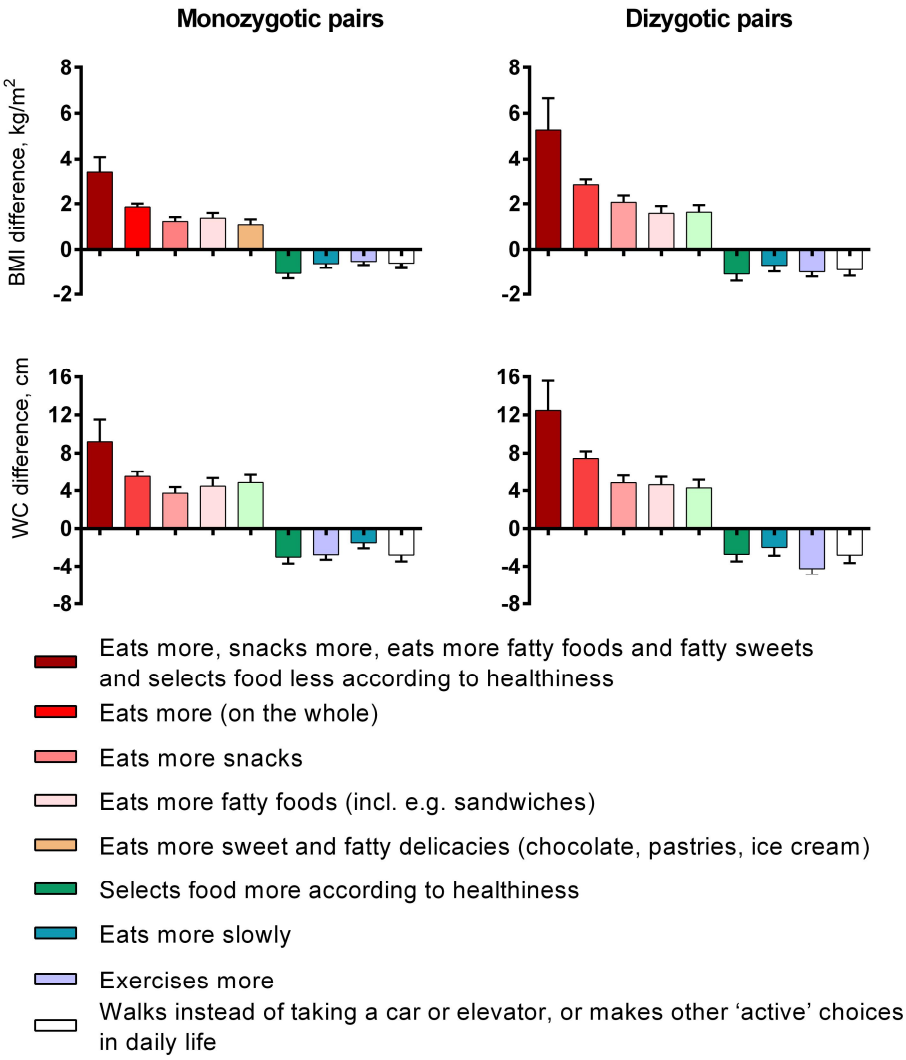

Figure 5. Intrapair differences in body mass index (BMI) and waist circumference (WC) in double-respondent co-twins who differ in eating and physical activity-related behaviors. All intrapair differences shown are significant at least at $p<0.05$ by paired t-test. The co-twins were asked to rate themselves in relation to their co-twins. For example: "Which one of you eats more". Only co-twins with consistent answers and differences in the behavior are included. n=113-239 pairs for MZ and 172-323 pairs for DZ twins. 19 MZ and 17 DZ pairs differed in 5 of the eating behaviors simultaneously (shown in dark red). Data are shown as mean values ± SE.

The 13 behaviors listed in Table 6 are likely to be intercorrelated to some extent. Therefore, multiple linear regressions were performed with intrapair differences in which all 13 behaviors were entered simultaneously into the regression model. Intrapair differences in the amounts of food consumed between co-twins were the strongest independent predictor of differences in BMI and WC among both zygosity groups (MZ: β: 0.63 ± 0.13 for BMI and 1.52 ± 0.46 for WC, DZ: β: 1.21 ± 0.19 for BMI and 3.53 ± 0.54 for WC, $p<0.001$ for all). In addition, differences in the intake of sweet and fatty delicacies and exercise habits between co-twins remained independent predictors of intrapair differences in BMI and WC for both

twin types. In the MZ pairs, co-twin differences in the eating rate were associated with differences in BMI, differences in snacking with BMI and WC, and differences in making active choices in daily life with WC. In the DZ pairs, co-twin differences in dieting behavior, being worried about appearance and fidgeting were associated with differences in BMI. In the same model, there was an inverse association between sweets (e.g. candies or jellies) and BMI and WC within co-twins, although this difference was only seen in MZ pairs.

Mean self-reported food group consumption of double-respondent MZ and DZ pairs (combined) with consistent answers is shown in **Table 7**. The intakes of only a few food items were found to differ between the twins who differed in the amount of food they consumed. Co-twin comparison questions on which one of the pair eats more snacks, more fatty foods, more sweet and fatty delicacies and selects food more according to healthiness corresponded well with self-reported servings of foods obtained from the FFQ. Twin pair members, for whom both co-twins agreed that this identified twin eats more snacks, reported a higher consumption of sweet pastries, chocolate, other candies and salty snacks, and lower intakes of dark bread and porridge, muesli and cereals. Twins who reported that they ate more fatty foods than their co-twins reported a higher consumption of white bread, fried potatoes or French fries, high-fat cheese, sausages, chocolate, other candies, salty snacks, pizza, hamburger, fried foods, creamy foods, regular soft drinks, butter-vegetable oil mixtures and margarine. Twins who reported that they ate more sweet and fatty delicacies than their co-twins, ate more fried potatoes or French fries, sweet pastries or ice cream, chocolate, other candy, salty snacks and fried foods. The consumption of healthy food items, such as fruit and vegetables and dark bread were significantly lower in the co-twins who snacked more, ate more fatty foods or sweet and fatty delicacies. The twins of pairs for whom both co-twins agreed that this specified twin eats healthier had a higher self-reported intake of dark bread, rice or pasta, porridge, muesli or cereals, yoghurt, fish, fresh vegetables, cooked vegetables, fruit, berries, skimmed milk or sour milk, tea and oil. In addition, they had lower self-reported intakes of various fatty and sweet and fatty foods.

Twins who exercise more based on the co-twin comparison questions also had a higher self-reported physical activity (4.8 vs. 2.1 hours per week, $p<0.001$) and exercised at higher intensities (9.8 vs. 7.5 MET, $p<0.001$) than their co-twins. Furthermore, being physically active was associated with making healthier food choices. The more active twin member consumed more dark bread ($p<0.001$), rice and pasta ($p<0.001$), porridge, muesli or cereals ($p<0.001$), yoghurt ($p<0.001$), chicken ($p<0.05$), fresh vegetables ($p<0.01$), cooked vegetables ($p<0.001$), fruits ($p<0.001$), pizza ($p<0.05$), skimmed milk or sour milk ($p<0.05$) and tea ($p<0.05$) and less amounts of potatoes ($p<0.05$), sausages ($p<0.05$), creamy foods or fried foods ($p<0.01$) salty snacks ($p<0.01$), whole milk or sour milk ($p<0.01$), soft drinks ($p<0.05$) and coffee ($p<0.001$).

Table 7. Food intake in double-respondent monozygotic and dizygotic twin pairs with consistent answers to the behavior claims.

Food and beverage items	Behavior claim for which both twins agree that Twin 1 exhibits the behavior more									
	eats more (overall) (n = 474)		eats more snacks (n=369)		eats more fatty foods (n=285)		eats more sweet and fatty delicacies (n=302)		selects food according to healthiness (n=360)	
	Twin		Twin		Twin		Twin		Twin	
	1	2	1	2	1	2	1	2	1	2
Bread (slices per day)										
Dark bread	2.6	2.4*	2.4	2.7*	2.3	2.7**	2.3	2.6**	2.7	2.2***
Whole-meal bread	1.6	1.6	1.6	1.5	1.8	1.6$^{(*)}$	1.6	1.5	1.5	1.7$^{(*)}$
White bread	1.1	1.2	1.1	1.0	1.2	0.9**	1.1	1.0	0.9	1.2**
FFQ items (servings/week)										
Potatoes, baked/mashed	3.4	3.2$^{(*)}$	3.3	3.4	3.3	3.1	3.2	3.3	3.2	3.2
Potatoes, fried, French fries	1.3	1.3	1.3	1.2	1.4	1.0***	1.4	1.1**	1.0	1.3***
Rice or pasta	3.2	2.9**	3.1	3.2	3.1	3.4**	3.0	3.2$^{(*)}$	3.5	3.1*
Porridge, muesli, cereals	3.0	3.1	2.9	3.6**	2.6	4.2***	3.1	3.8**	4.4	2.7***
Yoghurt	4.5	4.5	4.8	5.2	4.2	5.3***	4.6	5.0	5.3	4.5**
Cheese, low fat	3.0	2.8	3.2	3.8	2.9	3.8*	3.1	4.5	4.2	2.9
Cheese, high fat	6.0	5.7	5.3	5.7	6.3	5.3*	5.8	5.2	5.5	6.0
Fish	1.5	1.4	1.4	1.6	1.3	1.5	1.5	1.4	1.6	1.4*
Chicken	2.5	2.2*	2.5	2.4	2.3	2.5	2.2	2.3	2.6	2.4
Meats	4.5	4.2	4.1	4.5	4.2	3.9	4.2	4.6	3.9	4.1
Sausages	2.9	3.1	3.0	2.8	3.3	2.4***	3.2	3.1	2.3	3.3***
Eggs (boiled, fried, omelet)	1.6	1.6	1.4	1.6	1.7	1.5	1.5	1.5	1.5	1.5
Fresh vegetables	5.8	5.5	5.1	6.0**	4.8	6.6***	5.0	6.3***	7.2	4.9***
Cooked vegetables	2.8	2.7	2.5	3.1***	2.7	3.3***	2.4	3.1***	3.4	2.4***
Fruits	6.0	5.7	6.2	5.9	5.4	7.0	6.0	6.2	7.3	5.5***
Berries	2.0	1.9	2.0	2.0	1.8	2.1	1.9	2.1	2.3	1.9*
Sweet pastries, ice cream, etc.	2.5	2.3	2.6	2.0***	2.4	2.3	3.3	1.9***	2.3	2.5$^{(*)}$
Chocolate	1.8	2.0	2.1	1.5***	1.9	1.6*	2.5	1.5***	1.7	2.0**
Other candy	2.4	2.5	2.8	2.2***	2.7	2.3*	2.9	2.2***	2.5	2.7$^{(*)}$
Salty snacks	1.3	1.3	1.5	1.1***	1.6	1.1***	1.4	1.1***	1.1	1.4***
Pizza	1.2	1.3	1.1	1.1	1.2	1.0**	1.1	1.1	0.9	1.2***
Hamburgers	1.0	1.0	1.0	0.9$^{(*)}$	1.1	0.8***	1.0	1.0	0.8	1.0***
Fried food	1.3	1.3	1.3	1.2	1.5	1.0***	1.4	1.1**	1.0	1.5***
Creamy foods	1.2	1.1	1.2	1.3	1.4	1.0***	1.3	1.1$^{(*)}$	1.0	1.3***
Salad dressing	1.6	1.7	1.3	1.4	1.5	1.5	1.4	1.5	1.5	1.5
Skimmed milk or sour milk	6.1	5.6	6.0	5.8	5.5	6.0	6.2	5.7	6.1	5.1*
1-1.5% milk or sour milk	4.3	4.4	3.7	4.4$^{(*)}$	4.2	3.8	4.0	4.5	3.7	4.3
Whole milk or sour milk	0.7	1.1*	0.6	0.6	0.7	0.5	0.8	0.6	0.6	0.8
Juice	5.2	5.2	4.6	5.2	4.9	5.2	5.0	5.1	5.1	5.3
Regular soft drinks	2.6	2.8	2.6	2.6	2.8	2.1**	2.9	2.7	2.2	3.1**
Diet soft drinks	2.6	2.3	2.7	2.4	2.4	2.5	2.5	2.4	2.6	2.7
Coffee	10.0	10.6	9.8	10.2	9.4	9.4	9.7	10.4	9.1	9.9
Tea	3.8	4.3$^{(*)}$	3.9	4.5	3.7	4.7**	3.6	4.3*	4.8	3.7**
Butter	1.4	1.3	1.3	1.2	1.4	1.0	1.3	1.2	1.1	1.4
Butter-vegetable oil-mixture	2.5	2.7	2.7	2.7	3.2	1.8***	3.1	2.6	2.1	3.4***
Margarine, <65 % fat	5.0	4.5	5.1	4.8	5.3	4.0**	5.2	4.4	3.9	4.7
Margarine, 70–80 % fat	3.2	3.7	3.6	3.4	4.2	2.8**	4.1	3.6	2.9	3.9*
Vegetable-stanol spread	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.1
Oil	2.4	2.1$^{(*)}$	2.0	2.3	2.4	2.7	2.1	2.5*	2.8	2.3*
Food groups (servings/week)										
Healthy foods (total)	34.1	32.4$^{(*)}$	33.3	37.0***	30.5	39.3***	32.4	36.7***	41.3	31.5***
Fatty foods (total)	7.3	7.0	7.5	6.7**	8.2	5.9***	7.6	6.5***	5.8	7.7***
Fatty and sweet foods (total)	6.8	6.7	7.5	5.7***	7.1	6.3*	8.7	5.6***	6.5	7.3**
Meat (total)	7.4	7.2	7.2	7.3	7.6	6.4**	7.4	7.7	6.2	7.4**

Significant differences between co-twins (paired t-test): ***p<0.001, **p<0.01, *p<0.05, $^{(*)}$p<0.08.
Data are shown as mean values. n refers to the number of twin pairs.

5.2 Diet and physical activity in obesity-discordant monozygotic twin pairs (II)

The aims of study II were to investigate whether obesity-discordant twin pairs differ in their eating and physical activity patterns and to validate self-reported energy intake from 3-day food diaries by DLW. As much as 61% of the obese co-twins reported eating too much, whereas only 21% of non-obese co-twins reported doing so (p=0.014). Attempts to maintain healthy eating patterns tended to be less frequent in obese than in non-obese co-twins (50% vs. 86%, p=0.059). Body Dissatisfaction (10.2 ± 1.8 vs. 5.8 ± 2.1, p=0.013) and Drive for Thinness scores (4.1 ± 1.0 vs. 2.1 ± 0.8, p=0.02) were higher in obese than in non-obese co-twins. Total energy intake did not differ between the obese and non-obese co-twins (**Table 8**). Total fat intake was similar in the co-twins but obese twin pair members had a lower percentage of energy from dietary unsaturated fatty acids than their non-obese siblings. They also reported a significantly lower consumption of sweet (3.4 ± 1.3 vs. 5.7 ± 1.8 g/MJ, p=0.046) and sweet and fatty delicacies (3.2 ± 0.9 vs. 6.6 ± 1.2 g/MJ, p=046) than non-obese co-twins and they also tended to consume less fruits and berries (8.1 ± 3.0 vs. 11.8 ± 3.6 g/MJ, p=0.08). Dietary intake was similar in the concordant co-twins (mean intakes shown in Table 8).

Table 8. Energy intake, energy expenditure and macronutrient intakes in monozygotic co-twins discordant (n=14) and concordant (n=9) for obesity.

	Obesity-discordant pairs		Weight-concordant pairs
	Obese	Non-obese	Both co-twins
Total energy expenditure (MJ/d)	12.4 ± 0.4	11.5 ± 0.7	NA
Total energy intake (MJ/d)	9.6 ± 1.0	9.8 ± 1.1	8.2 ± 0.8
Fat (g/d)	88.6 ± 12.7	74.3 ± 5.0	59.7 ± 8.8
Saturated fatty acids (g/d)	33.4 ± 5.8	29.0 ± 2.1	24.8 ± 4.0
Monounsaturated fatty acids (g/d)	26.0 ± 3.6	20.6 ± 1.3	17.9 ± 2.7
Polyunsaturated fatty acids (g/d)	12.9 ± 1.9	9.9 ± 1.7	7.5 ± 1.0
Carbohydrates (g/d)	279.8 ± 31.8	275.6 ± 30.5	217.1 ± 24.9
Protein (g/d)	82.9 ± 9.1	90.7 ± 14.3	82.3 ± 8.4
Fat (E%)	30.8 ± 2.0	33.9 ± 1.3	31.2 ± 1.9
Saturated fatty acids (E%)	12.2 ± 1.1	12.7 ± 0.8	12.6 ± 1.1
Monounsaturated fatty acids (E%)	8.6 ± 0.5	10.0 ± 0.4*	9.3 ± 0.5
Polyunsaturated fatty acids (E%)	3.8 ± 0.3	4.9 ± 0.5*	4.0 ± 0.2
Carbohydrates (E%)	48.9 ± 1.6	49.1 ± 1.3	48.9 ± 2.5
Protein (E%)	15.7 ± 1.1	14.6 ± 0.6	17.8 ± 0.6

Data are mean ± SE. NA, not assessed. 8 obesity-discordant pairs had total energy expenditure measurements. The same pairs were selected for calculating energy intake the food diaries. Wilcoxon's test between the obese and non-obese co-twins: *p<0.05.

The activity patterns of the obesity-discordant and concordant co-twins from the physical activity diaries are shown in **Figure 6**. The mean activity score (2.0 ± 0.1 vs. 2.1 ± 0.1) and activity energy expenditure (4.6 ± 0.2 vs. 4.4 ± 0.3 MJ/d) did not differ significantly between the obese and non-obese co-twins. All twins spent most of their days in sedentary activities (categories 1 and 2). Analysis of the time spent in different activity categories per day showed that non-obese co-twins exercised 7 ± 5 minutes daily at the very vigorous intensity (category 8), whereas none of the obese co-twins reported any engagement in very vigorous intensity exercise (p=0.08). Obese co-twins exercised for 16 more minutes daily at a moderate intensity than their co-twins (category 6; p=0.04).

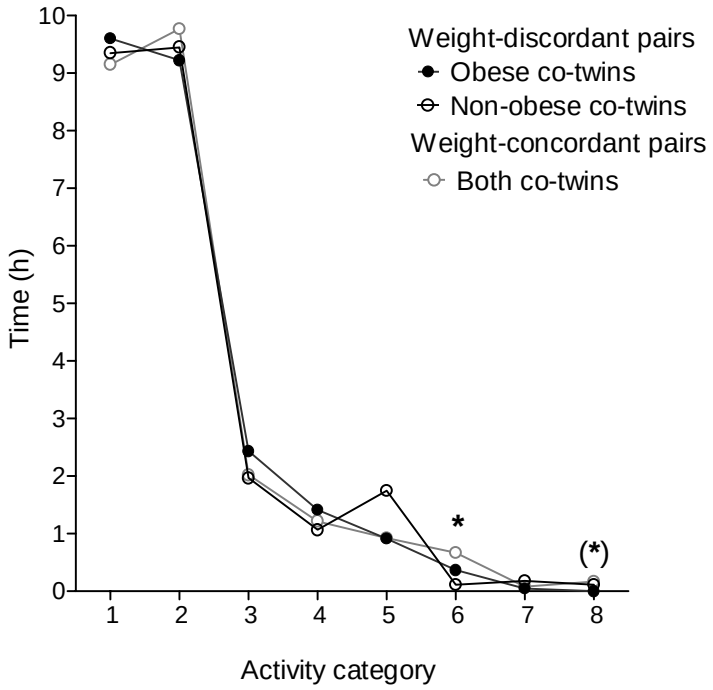

Figure 6. Mean time spent in different categories of physical activity in obesity-discordant (n=14) and weight-concordant (n=10) monozygotic twin pairs. Activity categories: 1=sleeping or resting; 2=sitting; 3=light activity standing; 4=working at a very low intensity, 5=working at a low intensity, 6=working or exercising at a moderate intensity; 7=working or exercising at a vigorous intensity; 8= working or exercising at a very vigorous intensity. Wilcoxon's test between the obese and non-obese co-twins: *p<0.05, $^{(*)}$ p=0.08.

Obese co-twins underreported their actual daily energy intake by 3.2 ± 1.1 MJ/d (p=0.036), which corresponds to 24.7% of TEE. In lean co-twins, TEE and reported energy intake (0.8 MJ/d, 8.4% of TEE, p=0.26) did not differ significantly. Overreporting of actual physical activity was significant for obese twins (1.8 ± 0.8 MJ/d, p=0.049), but not for their respective lean co-twins (1.1 ± 0.6, p=0.12) (**Figure 7**). The degree of underreporting (TEE-EI) correlated with BMI in the obese twins (r=0.70, p=0.05), but not for their non-obese co-twins (r=-0.26, p=0.54). Underreporting of energy intake was correlated with all macronutrients in grams per day (fat: r=-0.79, p=0.006; carbohydrates: r=-0.84, p=0.001; protein: r=-0.54, p=0.049), but not significantly with macronutrients as a percentage of energy intake (fat: r=-0.21; carbohydrates: r=0.23; protein: r=0.31).

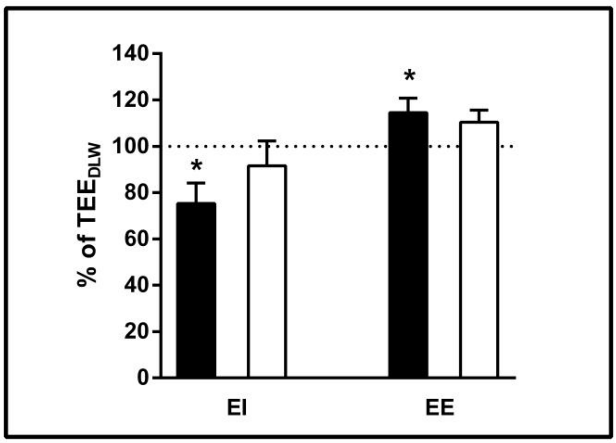

Figure 7. Mean ± SE reported energy intake (EI) from food diaries and energy expenditure (EE) from activity diaries in obese (solid bars) and non-obese (open bars) co-twins expressed as % of total energy expenditure (TEE) measured by doubly labeled water. *p<0.05, significantly different from TEE_{DLW} as evaluated by the Wilcoxon's test.

The finding of significant underreporting of energy intake by the obese co-twins is supported by the results of the co-twin comparison questions, for which most twins mutually reported that the obese co-twins habitually eat more (71%, n=10) than their leaner co-twin siblings (**Figure 8**). As many as 64% (n=9) of the leaner co-twins reported that they exercised more than their obese co-twins, but only 36% (n=5) of the obese co-twins reported that the lean co-twin exercises more. In addition, 29% (n=4) of the obese co-twins reported that they exercised more than their leaner siblings, but only 1 (7%) of the lean co-twins confirmed this. The co-twin assessments further suggest that obese co-twins underestimate or underreport their intakes of high-fat foods as half (n=7) of the leaner co-twins reported that their obese co-twin siblings ate more fatty foods, whereas only 21% (n=3) of the obese co-twins reported to do so. Moreover, 50% (n=7) of the leaner co-twins reported they ate healthier than their obese co-twin, and 43% (n=6) of the obese co-twins agreed with that statement. None of the obese co-twins reported that they ate healthier than their leaner co-twins and none of the leaner co-twins reported that their obese co-twins ate healthier. For some co-twin comparison questions, a substantial number of twins reported that they did not differ in the specified behavior. For example, only 2 (14%) lean and 1 (7%) obese subject reported that they ate more slowly than their co-twins, whereas 50% (n=7) of the lean and 43% (n=6) of the obese co-twins reported that there is no difference between them.

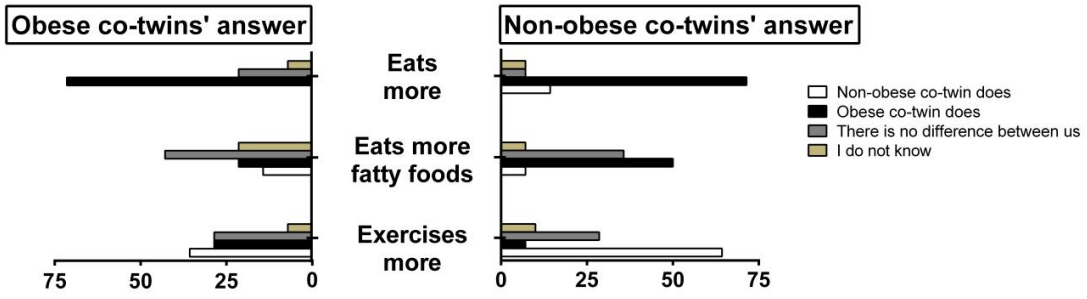

Figure 8. Within-pair comparison of 2 eating patterns and exercise habits among obesity-discordant monozygotic twin pairs (n=14). The co-twins were asked to rate themselves in relation to their co-twins: 'Which one of you…'. The values represent percentages.

5.3 Associations between macronutrient intake and serum lipoprotein profile (III)

The aims of study III were to investigate the associations between habitual macronutrient intake, as assessed by 3-day food diaries, and serum lipoprotein profiles in 48 twin individuals (24 twin pairs). The correlations between dietary macronutrients (expressed as percentages of energy) and HDL subspecies were first studied by partial individual-level correlation analysis that was adjusted for sex, BMI and Baecke physical activity index. Serum HDL-C concentrations were significantly correlated with total dietary PUFAs (r=0.34, p=0.02), total n-6 PUFAs (r=0.31, p=0.035) and total n-3 PUFAs (r=0.32, p=0.028) among twin individuals. The percentage of energy from total dietary n-3 PUFAs correlated significantly with HDL particle size and most of the HDL subclasses studied after adjusting for these covariates (**Figure 9a**). Total n-3 PUFAs also tended to be associated with lower ApoB concentrations (r=-0.29, p=0.05) and a higher HDL mass (r=0.28, p=0.06), but was not significantly correlated with TGs, LDL peak particle size or ApoA1. HDL mean particle size did not correlate with total PUFAs (r=0.14, p=0.36) or total n-6 PUFAs (r=0.08, p=0.58).

A partial within-pair correlation analysis that was adjusted for sex, BMI and Baecke physical activity index revealed that protein intake was significantly correlated with ApoA1 (r=0.43, p=0.05), HDL particle size (r=0.45, p=0.04), percentages of HDL2a (r=0.55, p=0.01) and HDL3b (r=-0.68, p<0.0001) within MZ twin pairs. Intrapair differences in total n-3 PUFA intakes were significantly correlated with differences in HDL particle size and subspecies after adjusting for covariates (**Figure 9b**). Intrapair differences in total n-6 PUFA intake were also positively correlated with those in HDL particle size (r=0.47, p=0.009), HDL2b (r=0.52, p=0.005) but inversely with HDL3b (r=-0.44, p=0.011), although correlation coefficients were somewhat lower than those for total n-3 PUFAs. Different types of dietary fatty acids were significantly correlated with one another as shown in **Table 9**.

Table 9. Correlations between dietary fatty acids in individual twins and within twin pairs.

	SFAs	MUFAs	PUFAs	n-6 PUFAs
Individual twins (n=48)				
n-3 PUFAs	0.19	0.51***	0.72***	0.65***
n-6 PUFAs	0.29*	0.70***	0.98***	
PUFAs	0.31*	0.73***		
MUFAs	0.73***			
Within twin pairs (n=24)				
n-3 PUFAs	0.07	0.31	0.62**	0.49*
n-6 PUFAs	-0.10	0.47*	0.98***	
PUFAs	-0.06	0.51*		
MUFAs	0.54**			

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n-3 PUFAs, omega-3 polyunsaturated fatty acids; n-6 PUFAs, omega-6 polyunsaturated fatty acids ***p<0.001, **p<0.01, *p<0.05.

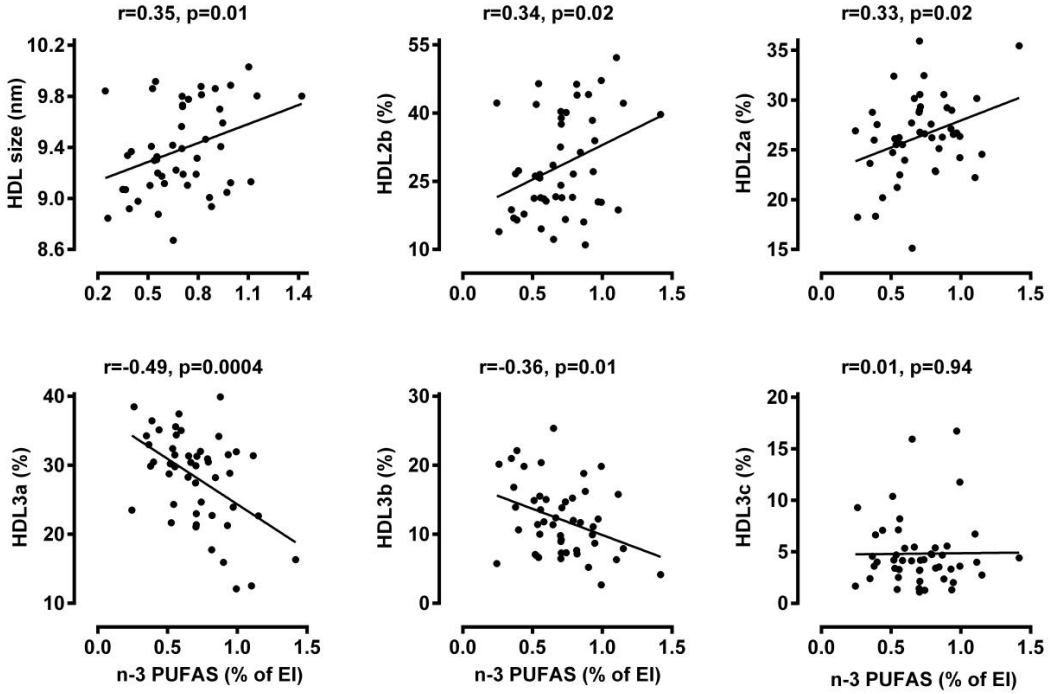

Figure 9a. Pearson partial correlations (adjusted for sex, body mass index and physical activity) between total omega-3 (n-3) PUFA intake and HDL particle size and subspecies in 48 twin individuals.

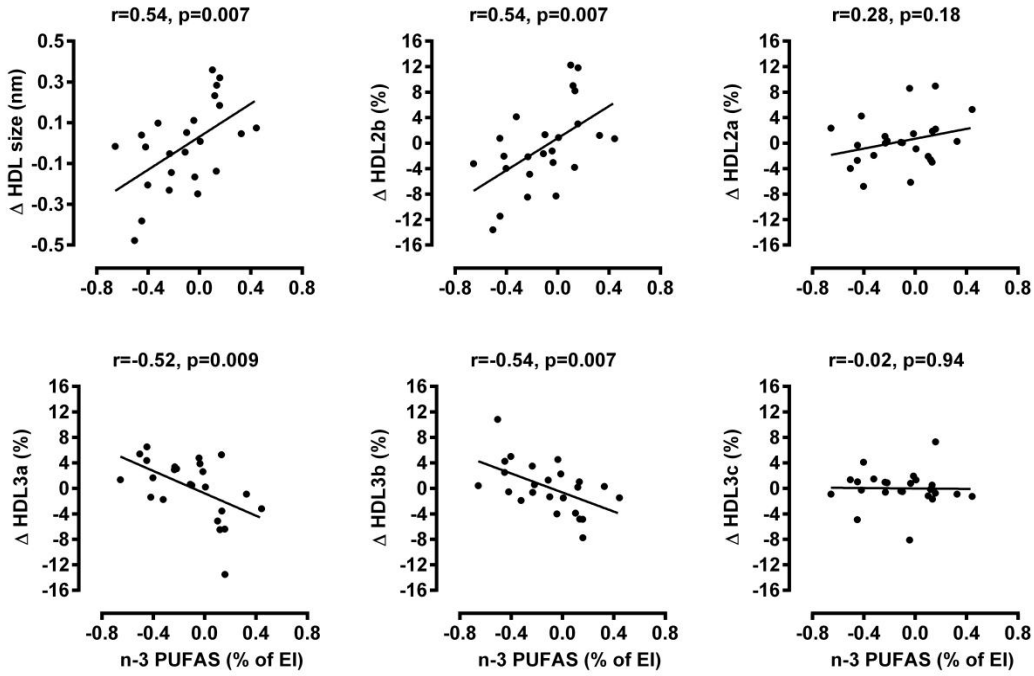

Figure 9b. Pearson partial correlations (adjusted for sex, intrapair differences (Δ) in body mass index and Δphysical activity) between Δ in total omega-3 (n-3) PUFAs and HDL particle size and subspecies in 24 monozygotic twin pairs.

A more thorough examination of the associations between different dietary fatty acids and lipid variables was achieved when a series of fat substitution models were carried out for both individual-level and intrapair difference analyses that were adjusted for sex, age, BMI, smoking status, the percentage of energy from alcohol and Baecke physical activity index.

In twin individuals, a 1% increase in the percentage of energy from n-3 PUFAs with a corresponding decrease in the percentage of energy from other type of fats in the diet was related to increased concentrations of HDL-C (β ± SE: 0.5 ± 0.1 mg/dl, p=0.001) and apoA1 (β ± SE: 27 ± 11 mg/dl, p=0.02), increased proportions of HDL2b (β ± SE: 20 ± 6%, p=0.002) a higher mean HDL particle size (β ± SE: 0.6 ± 0.2 nm, p=0.002) and HDL mass (β ± SE: 92 ± 35, p=0.02), decreased concentrations of serum TG (β ± SE: -0.7 ± 0.3 mg/dl, p=0.03), ApoB (β ± SE: -24 ± 8 mg/dl, p=0.004) and lower proportions of HDL3a (β ± SE: -16 ± 4%, p=0.001) and HDL3b (β ± SE: -10 ± 3%, p=0.001).

Within twin pairs, consuming 1% energy more from n-3 PUFAs with a corresponding reduction in the amount of energy from other type of fats in the diet was related to increased proportions of HDL2b (β ± SE: 13 ± 5 %, p=0.02), a higher mean HDL particle size (β ± SE: 0.4 ± 0.1 nm, p=0.02) and lower proportions of HDL3a (β ± SE: -9 ± 4%, p=0.04) and HDL3b (β ± SE: -6 ± 3 nm, p=0.04). In contrast, a 1% increase in the percentage of energy from n-6 PUFAs at the expense of other type of fats in the diet was not significantly related to HDL mean particle size or any of the HDL subspecies within twin pairs.

Within each twin pair, the twin that had the higher percentage of energy from n-3 PUFAs (intrapair difference: 0.24 ± 0.04% of energy intake) had a significantly larger mean HDL particle size (9.5 ± 0.1 vs. 9.3 ± 0.1 nm, p=0.046), higher percentages of HDL2b (31 ± 3 vs. 26 ± 03%, p=0.04) and lower percentages of HDL3a (27 ± 1.5 vs. 30 ± 1.5%, p=0.02) and HDL3b (11 ± 1.2 vs. 13 ± 1.3%, p=0.02). However, there were no differences in BMI or Baecke physical activity index between these co-twins. Twins who consumed more energy from n-3 PUFAs than their co-twins also consumed more energy from n-6 PUFAs (4.2 ± 1.3 vs. 3.4 ±1.2%, p=0.002). However, the HDL mean particle size and subspecies distribution between the twins with the higher and lower intakes of n-6 PUFAs were similar.

5.4 Associations between obesity and lipoprotein profile (IV)

The objective of study IV was to determine whether obesity and physical activity are associated with serum lipoprotein profiles with a special focus on body fat distribution, i.e. subcutaneous, visceral and liver fat in a sample of 15 BMI-discordant and 9 BMI-concordant MZ twin pairs. The lipid profile among BMI-concordant pairs did not differ significantly. In contrast, the heavier co-twins in the discordant pairs had a more adverse lipid profile. This was characterized by higher concentrations of total cholesterol (**Figure 10**), higher concentrations of ApoB (mean ± SE: 81.1 ± 4.0 i.e. 4.5 ± 0.2 mmol/l vs. 69.8 ± 3.3 mg/dl i.e. 3.9 ± 0.2 mmol/l, p=0.01), LDL-C (110 ± 6 i.e. 6.1 ± 0.3 mmol/l vs. 98 ± 6 mg/dl i.e. 5.4 ± 0.3 mmol/l, p=0.05) and proportions of HDL3c (5.4 ± 1.1 vs. 3.5 ± 0.6%, p=0.003), lower percentages of HDL2b (29 ± 3 vs. 36 ± 3 mg/dl, p=0.01) and a reduced HDL mean particle size (9.4 ± 0.1 vs. 9.6 ± 0.1 nm, p=0.01).

There were no differences in concentrations of total TG, VLDL-TG, ApoA1, Apolipoprotein C3, VLDL-C and HDL3-C, LDL peak particle size, VLDL or HDL masses between the obesity-discordant or concordant co-twins.

Splitting the obesity-discordant twin pairs on the basis of the median for intrapair differences in liver fat (2.6%) revealed two groups of obesity-discordant pairs. Seven obesity-discordant pairs (intrapair difference in weight of 16 kg) had no differences in their liver fat (intrapair difference in liver fat: 0-0.2%, p=0.40). In this group, both co-twins had low liver fat percentages (range: 0.1-0.6%). The other eight obesity-discordant pairs (intrapair difference in weight of 17 kg) differed significantly in the percentage of liver fat (intrapair difference in liver fat: 2.6-9.0%, p=0.012). In this group, the heavier co-twins' liver fat percentages ranged from 3.8% to 9.4%. Despite the discordance in body weight of both groups, only those obese co-twins who also had high liver fat presented higher concentrations of total cholesterol, ApoB, LDL-C, LDL mass, IDL-C, HDL3c percentages and lower concentrations of HDL-C. In BMI-discordant but liver fat concordant twin pairs, the lipid profiles were very similar. Figure 10 illustrates this for total serum cholesterol concentration.

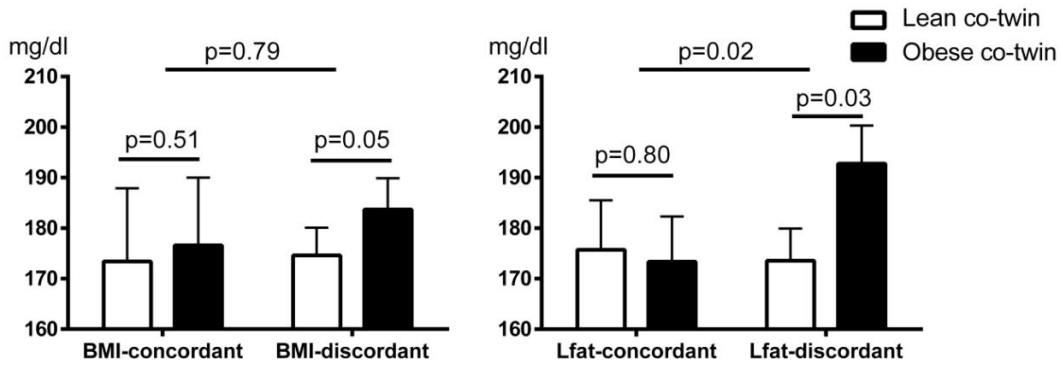

Figure 10. Total cholesterol in BMI-concordant (n=9) and discordant (n=15) and in liver fat-concordant (Lfat-concordant, n=7) and discordant (Lfat-discordant n=8) monozygotic twin pairs. Wilcoxon signed rank test between the leaner vs. heavier co-twin (p values immediately above the bars). Mann-Whitney U test of the intrapair differences between the concordant and discordant groups (p values on top of the figure). Data are shown as mean values ± SE.

Intrapair differences in liver fat were strongly correlated with concentrations of total cholesterol, ApoB, and LDL-C and LDL mass (**Figure 11)**. These correlations remained significant but became weaker after being adjusted for sex, smoking status and Baecke physical activity index (total cholesterol: r=0.53, ApoB: r=0.56, LDL-C: r=0.40, LDL-mass: r=0.44, p=0.01-0.08). Intrapair correlations were weaker for intra-abdominal (total cholesterol: r=0.23, ApoB: r=0.49, LDL-C: r=0.12, LDL-mass: r=0.27, only ApoB $p<0.05$) and subcutaneous fat (total cholesterol: r=0.30, ApoB: r=0.44, LDL-C: r=0.24, LDL-mass: r=0.36, only ApoB $p<0.05$).

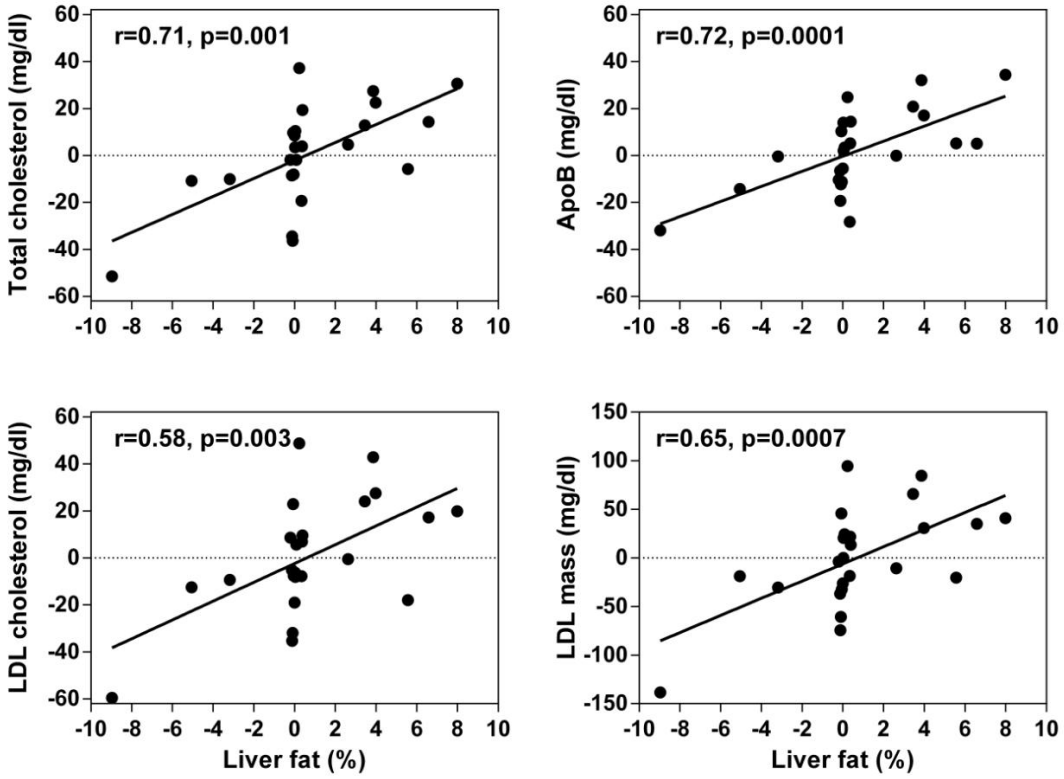

Figure 11. Spearman correlations between intrapair differences in liver fat percentages and intrapair differences in selected lipid parameters in 24 monozygotic twin pairs.

In contrast, intrapair differences in the Baecke physical activity index that were adjusted for sex, smoking status and BMI, were strongly correlated with a favorable lipid profile: ApoB: r=-0.53, LDL-C: r=-0.54, HDL-C: r=0.47, LDL mass: r=-0.48, HDL mass: r=0.46, HDL3b: r=-0.45 ($p<0.05$ for all).

Intrapair differences in intra-abdominal fat were strongly correlated with those in subcutaneous fat (r=0.62, p=0.002) and liver fat (r=0.52, p=0.01). Thus, the independent contributions of body fat distribution variables were assessed by using multivariate regression analyses adjusted for sex, smoking status and intrapair differences in physical activity. Intrapair differences in liver fat, but not those in intra-abdominal or subcutaneous fat were independently associated with concentrations of ApoB ($\beta=2.2 \pm 1.0$, $p=0.05$; $R^2=0.45$), total cholesterol ($\beta=3.6 \pm 1.4$, $p=0.02$; $R^2=0.37$), LDL-C ($\beta=2.9 \pm 1.3$, $p=0.04$; $R^2=0.56$), HDL3-C ($\beta=0.7 \pm 0.3$, $p=0.04$; $R^2=0.08$) and ΔLDL mass at borderline significance ($\beta=5.7 \pm 2.9$, $p=0.07$; $R^2=0.48$).

5.5 Associations between dietary patterns and lipoprotein profile (V)

Study V assessed the relationship between habitual diet (i.e. intakes of macronutrients as assessed by FFQ, dietary patterns and serum n-3 PUFAs) and NMR-derived serum lipoprotein subclass profile in 679 twin individuals (197 complete pairs). Factor analysis identified five distinct dietary patterns that together explained 22.2% of the variance.

Figure 12 shows the factor loadings of the five dietary patterns for factor loadings of at least 0.3. The dietary patterns were labeled according to the food items that loaded highly: “Fruit and Vegetables”, “Meat”, Sweets and Desserts”, “Junk food” and “Fish”. The “Fruit and Vegetable” pattern was highly positively correlated with Vitamin C ($r=0.69$, $p<0.001$), as was physical activity (0.28, $p<0.001$), dietary fiber (0.35, $p<0.001$), and serum n-3 PUFA ratio (0.21, $p<0.001$). However, “Fruit and Vegetable” was negatively correlated with the percentage of energy from SFAs ($r=-0.12$, $p=0.004$) and alcohol ($r=-0.18$, $p<0.001$). The “Meat” pattern correlated positively with the percentage of energy from fat ($r=0.26$, $p<0.001$) and protein ($r=0.23$, $p<0.001$) but negatively with the percentage of energy from carbohydrates ($r=-0.27$, $p<0.001$) and dietary fiber ($r=-0.14$, $p=0.002$). The “Sweets and Desserts” pattern was most strongly correlated with sucrose ($r=-0.28$, $p<0.001$) and also with other macronutrients as a percentage of energy but inversely with micronutrients (e.g. $r=-0.18$, $p<0.001$ for calcium). The “Junk food” pattern correlated positively, albeit weakly, with BMI ($r=0.08$, $p=0.03$) and WC ($r=0.12$, $p=0.001$), and this association remained significant after additional adjustment for physical activity ($r=0.08$, $p=0.04$ for BMI and $r=0.11$, $p=0.003$ for WC). This dietary pattern was also correlated positively with total fat ($r=0.22$, $p<0.001$), sucrose ($r=0.17$, $p<0.001$), alcohol ($r=0.09$, $p=0.04$) and negatively with dietary fiber ($r=-0.29$, $p=0.001$) and all micronutrients (e.g. $r=-0.16$, $p<0.001$ for Vitamin C). As anticipated, the “Fish” dietary pattern correlated positively with intakes of protein ($r=0.30$, $p<0.001$), vitamin D ($r=0.69$, $p<0.001$) and serum n-3 PUFAs (e.g. $r=0.34$, $p<0.001$ for serum DHA ratio).

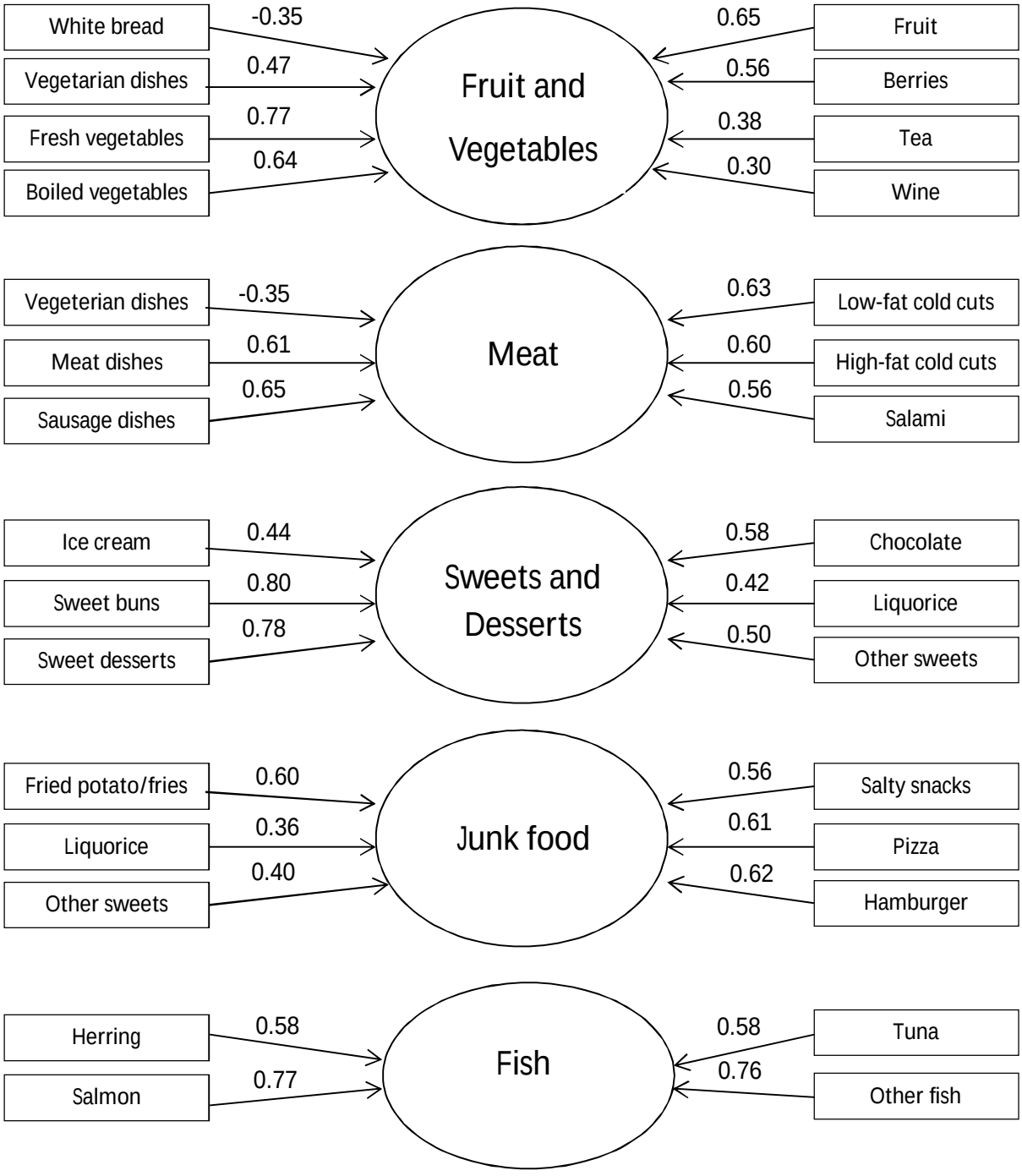

Figure 12. Factor loadings for the five dietary patterns identified from the food frequency questionnaire (n =679). The patterns were identified using factor analysis with the principal-component factor method. Factor loadings of ≥ 0.3 are shown.

Pearson partial correlation coefficients between habitual dietary intakes (percent of energy from macronutrients and dietary pattern scores) and lipoprotein particle profile in twin individuals and within MZ and DZ twin pairs (Bogl LH et al., unpublished results) are shown in **Figure 13**. The results are shown for men and women combined as the associations did not differ significantly by sex.

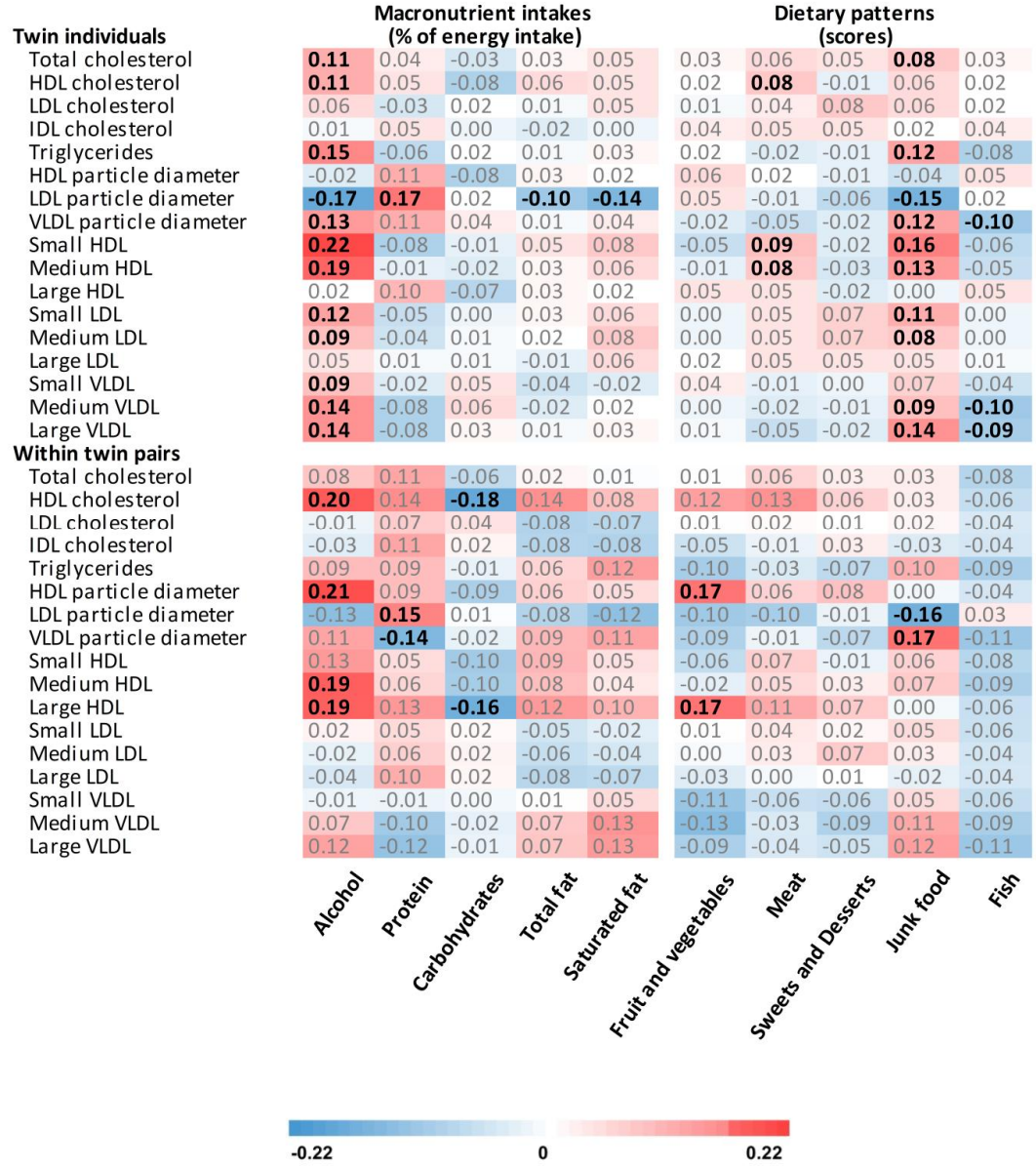

Figure 13. Pearson partial correlation coefficients between intakes of macronutrient and dietary patterns with lipoprotein particle profile (n=679 individuals and 197 twin pairs). Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; VLDL, very-low-density lipoprotein. Plasma lipids (mmol/l), lipoprotein particle diameter (nm), Particle concentrations: HDL (µmol/l), LDL and VLDL (nmol/l). Partial correlations are adjusted for sex, age, body mass index, waist circumference, physical activity, smoking status, and the percentage of energy from alcohol (except for alcohol). Significant correlations are shown in bold: p<0.001-0.05.

The associations of macronutrients with LDL particle diameter and with total LDL particle concentration were also assessed by the nutrient density model (carbohydrate substitution model) (**Table 10**). Replacing 5% of energy from carbohydrates with protein was significantly related to an increased LDL particle diameter, whereas replacing the same energy amount with alcohol and SFAs was related to a reduced LDL particle diameter after adjusting for covariates. Age and WC were inversely related to LDL particle diameter in the same model. Replacing 5% of energy from carbohydrates with energy from alcohol was significantly related to an increased total LDL particle concentration. BMI, female sex and age were also positively associated with LDL particle concentration in the same model. The association between protein intake and LDL particle diameter remained significant in intrapair analyses (β ± SE=0.05 ± 0.03 nm, p=0.04 within MZ and DZ twin pairs and 0.09 ± 0.04, p=0.02 within MZ pairs only).

Table 10. Parameter estimates (β ± SE) from a multivariate nutrient density model evaluating the effect of replacing carbohydrates with other macronutrients on LDL particle size and concentration (n=679).

	LDL particle diameter (nm)		LDL particle concentration (nmol/l)	
	β ± SE[a]	P value	β ± SE[a]	P value[b]
Protein intake (5 E%)	0.039 ± 0.013	**0.003**	2.8 ± 9.9	0.92
SFA intake (5 E%)	-0.044 ± 0.019	**0.019**	23.3 ± 14.1	0.07
MUFA + PUFA intake (5 E%)	-0.001 ± 0.014	0.96	-16.1 ± 10.4	0.11
Alcohol intake (5 E%)	-0.034 ± 0.009	**<0.001**	11.2 ± 6.4	**0.044**
Total energy intake (100 kcal/d)	-0.001 ± 0.001	0.14	5.2 ± 0.7	0.62
Sex				
Men (Reference)				
Women	0.024 ± 0.020	0.18	27.8 ± 11.9	**0.029**
Age (years)	-0.040 ± 0.010	**<0.001**	24.2 ± 8.2	**0.005**
Total Baecke index (1 unit)	-0.009 ± 0.005	0.09	-3.4 ± 3.4	0.45
BMI (kg/m2)	0.006 ± 0.004	0.19	7.5 ± 3.0	**0.006**
Waist circumference (cm)	-0.004 ± 0.002	**0.033**	-0.2 ± 1.2	0.68
Smoking status				
Never smokers (Reference)				
Former	-0.001 ± 0.022	0.96	11.9 ± 16.0	0.41
Occasional	0.003 ± 0.021	0.90	-3.3 ± 15.3	0.69
Daily	-0.023 ± 0.014	0.10	14.4 ± 10.3	0.15

Abbreviations: EI, energy intake; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. [a]The β coefficient for macronutrients can be interpreted as the increase in the dependent variable resulting from a replacement of 5% of energy from carbohydrates with that macronutrient. [b]P-value is from analysis using the log-transformed dependent variable.

5.6 Associations between serum docosahexaenoic acid and lipoprotein profile (V)

A higher serum DHA to total fatty acid ratio was positively associated with the concentrations of total cholesterol, IDL-C, LDL particle diameter and negatively associated with concentrations of TGs, medium and large VLDL and VLDL particle diameters. The association between the serum DHA ratio and HDL variables differed significantly by sex (sex interaction $p<0.05$). In women, the serum DHA ratio was related to increased concentrations of HDL-C and large HDL and an increased HDL particle diameter. In men, the correlation between DHA ratio and HDL particle diameter was lower than in women and the observed increase in particle diameter was mainly due to reduced concentrations of small and medium HDL. Many of the associations remained significant in the intrapair analysis within MZ and DZ twin pairs and thus controlling for age, sex, shared environment factors and partially DNA sequence (Bogl LH et al., unpublished results) (**Table 11)**.

Table 11. Pearson partial correlation between serum docosahexaenoic acid ratio and lipoprotein profile

	Individual-level (n=663)			Within twin pairs (n=188 pairs)		
	Subjects	r	P-value	Subjects	r	P-value
Total cholesterol (mmol/l)	All	0.10	**0.02**	All	0.16	**0.03**
LDL cholesterol (mmol/l)	All	0.03	0.46	All	0.12	0.11
IDL cholesterol (mmol/l)	All	0.11	**0.005**	All	0.15	**0.04**
Triglycerides (mmol/l)	All	-0.09	**0.04**	All	-0.13	0.08
Small LDL (nmol/l)	All	0.02	0.69	All	0.12	0.11
Medium LDL (nmol/l)	All	0.03	0.50	All	0.13	0.08
Large LDL (nmol/l)	All	0.07	0.10	All	0.16	**0.03**
LDL particle diameter (nm)	All	0.12	**0.003**	All	0.07	0.32
Small VLDL (nmol/l)	All	-0.01	0.90	All	-0.04	0.61
Medium VLDL (nmol/l)	All	-0.15	**0.001**	All	-0.20	**0.007**
Large VLDL (nmol/l)	All	-0.15	**0.001**	All	-0.19	**0.009**
VLDL particle diameter (nm)	All	-0.20	**<0.001**	All	-0.30	**<0.001**
HDL cholesterol (mmol/l)	Men[a]	0.04	0.53	All	0.21	**0.004**
	Women	0.19	**<0.001**	-	-	-
Small HDL (µmol/l)	Men[a]	-0.18	**0.001**	Men[a]	-0.19	0.10
	Women	-0.01	0.91	Women	0.07	0.51
Medium HDL (µmol/l)	Men[a]	-0.17	**0.002**	Men[a]	-0.23	0.05
	Women	0.06	0.28	Women	0.08	0.41
Large HDL (µmol/l)	Men[a]	0.11	0.07	All	0.24	**0.001**
	Women	0.22	**<0.001**	-	-	-
HDL particle diameter (nm)	Men[a]	0.12	**0.04**	All	0.23	**0.002**
	Women	0.22	**<0.001**	-	-	-

Abbreviations: LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein, VLDL; very-low-density lipoprotein; HDL, high-density lipoprotein. Analyses were adjusted for sex, age, body mass index, waist circumference, physical activity, smoking status and the percentage of energy from alcohol. [a]P-value for sex interaction <0.05.

6 Discussion

6.1 Summary of the main findings

The measurement of habitual dietary intake and physical activity has previously relied upon subjective self-reports that are prone to misreporting, which particularly complicates studies on the relationship between diet and obesity. We attempted to overcome this limitation in the present study by utilized mutual responses of twin pairs and the data show that several acquired eating and physical activity behaviors are significantly associated with measures of obesity and abdominal obesity. The identified co-twins for whom both twin pair members concordantly answered that this twin eats more (overall), snacks more, eats more fatty foods and sweet and fatty delicacies, eats faster or chooses less healthy foods, had significantly higher BMIs and WCs. Leisure-time physical activity was associated with healthier dietary choices and decreased BMI and WC within twin pairs. Eating more overall was the strongest independent predictor of having an increased BMI and WC independent of genetic predisposition. In the obesity-discordant twin pairs, daily energy intakes and physical activity levels as assessed by 3-day diaries did not significantly differ between the obese and non-obese co-twins. This was due to considerable underreporting of actual energy intake and due to overreporting of actual physical activity by the obese co-twins, but not by the non-obese co-twins. Underreporting of energy intake was associated with significantly lower reported intakes of all macronutrients in grams per day, but not with macronutrients as proportions of energy intake.

Habitual macronutrient intake and dietary patterns were related to the serum lipoprotein profile independent of potential confounding factors. Specifically, a higher proportion of n-3 PUFAs in the diet was associated with a favorable HDL subspecies profile and an increased HDL mean particle size. Alcohol consumption was associated with increased concentrations of serum TGs, HDL-C, ApoA1, small LDL, small and medium HDL and also large VLDL particles. Protein intake correlated positively with LDL particle diameter. An energy-dense, nutrient poor dietary pattern (“Junk food”) was positively related to concentrations of small, dense HDL and serum TGs, a shift in the subclass distribution of VLDL towards larger particles and a change in LDL towards smaller particles. The serum DHA to total fatty acid ratio, which is a biomarker of habitual fish intake was associated with higher concentrations of IDL-C, lower TG concentrations, decreased VLDL particle diameter, increased HDL particle diameter and a favorable HDL subclass distribution. Most of these associations persisted under the analyses within twin pairs, but some associations became substantially weaker or non-significant, which suggests that shared genetic and environmental factors do partly account for some of the associations between dietary intake and serum lipoprotein particle profile. Besides diet, acquired obesity was associated with an unfavorable lipid profile independent of genetic effects. The obese co-twins had higher concentrations of ApoB, IDL-cholesterol, LDL-C, percentages of HDL3a and HDL3c and lower concentrations of HDL-C, HDL2-C and percentages of large HDL2b as compared to their leaner twin siblings. In particular, the accumulation of liver fat was associated with increased concentrations of atherogenic lipids.

6.2 Comparison with previous studies

6.2.1 Co-twin comparisons of eating and physical activity habits

The present study used comparative measures between twin pairs to increase the accuracy of responses to questions about obesity-related behaviors. This allowed the identification of several obesogenic eating and physical activity patterns that were associated with increased BMI and WC values. The results were very consistent in both MZ and DZ twins. However, MZ twins had slightly lower within-pair differences for BMI and WC than the DZ twins, which is probably due to the greater genetic and environmental similarities in MZ than in DZ twins.

Those twin pair members for whom both co-twins agreed that the identified twin eats more food overall were characterized by higher BMI and WC values. As obesity is the result of an energy imbalance in which energy intake exceeds energy expenditure, it would be expected that obese subjects consume larger amounts of foods and thus, more energy than lean subjects. However, in observational studies it has been inherently difficult to show any consistent associations between macronutrient intake or food intake patterns and obesity (Fogelholm et al., 2012; Summerbell et al., 2009; Togo et al., 2001). This may be partly due to the fact that current dietary assessment methods are not well suited for accurately estimating energy intake, which is an important determinant of obesity. FFQs are not designed for measuring an individual´s absolute intake but are useful for ranking individuals according to their food or nutrient intakes. Food records are more accurate than FFQs. However, the process of recording increases the subject´s awareness of foods eaten, which may lead to undereating during the recording period (Goris et al., 2000), and therefore will not give a true picture of a person's habitual eating patterns. Thus, both methods are prone to conscious or unconscious misreporting. The findings of previous reports (Goris et al., 2000; Lafay et al., 1997; Schoeller et al., 1990) are confirmed in the present study, i.e. that underreporting during a study is especially common among obese subjects. This phenomenon is likely to weaken any diet and disease relationships, which is particularly troublesome when looking for dietary determinants of obesity (Livingstone and Black, 2003). In the present study, bias due to underreporting of actual energy intake may be reduced because we included only twin pairs with clear differences in health-related behaviors as observed by the twins themselves and which were mutually confirmed by them. The results of this study therefore suggest that the amount of food consumed does indeed play a major role in weight maintenance.

The co-twin comparison questions further revealed that those co-twins who consumed more snacks, fatty foods, sweet and fatty delicacies and less healthy foods had significantly higher BMIs and WCs. Thus, it is not the amount of food alone that is the main contributor to the overconsumption of energy, but rather the consumption of large portions of energy-dense foods that leads to increased energy intake. This is in line with studies on self-selected or self-reported portion-sizes that show that obese individuals or those with a higher BMI consume larger portions of main meals (Berg et al., 2008), especially food of high energy-density, snacks, and high-carbohydrate foods (Burger et al., 2007).

Co-twins who snacked more than their twin counterparts had significantly higher BMIs and WCs. Our results are in accordance with a previous Swedish study (Bertéus Forslund et al., 2005), which showed that sweet and fatty food groups were associated with snacking and contributed considerably to daily energy intake. In the same study, obese men and women were found to be more frequent snackers when compared to a reference population. However, other researches, did not observe a difference in snack food intake between normal weight and overweight or obese adolescents (Kerr et al., 2009) or differences in BMI between adult snackers and never snackers (Hampl et al., 2003). The authors were aware that this might be attributable to considerable misreporting of obese subjects (Kerr et al., 2009). The association between high snack food intake and BMI is plausible, because the energy-density of snacks and also the portion sizes of snacks consumed by children and young adults have increased markedly (Zizza et al., 2001). Our data showed that the co-twins, who snacked more, self-reported significantly more salty snacks, chocolate, sweet pastries, ice cream and sweets in the FFQ. This is in line with a previous Finnish study, which found that sweet bakery goods are the most commonly selected snacks among men, whereas for women sweets and chocolate, followed by sweet bakery goods (Ovaskainen et al., 2006). Likewise, Zizza et al. (2001) reported that in young U.S. adults the main food items that contributed to caloric intake from snacks were desserts and salty snacks. In contrast Hampl et al. (2003) reported that multiple snackers had the most optimal diet quality and females who never snacked had the highest intake of desserts. However, it has been noted that there is no agreement on the definition of snacking and thus, research outcomes may vary according to the definition used (Gregori and Maffeis, 2007).

The co-twins who ate more fatty foods and more sweet and fatty delicacies, such as chocolate, pastries and ice cream were characterized by higher BMIs and WCs. This finding is in accordance with an earlier study among 23 obesity-discordant twins, in which the preference for fatty food was reported by 52% of the obese but only by 17% of the lean twin counterparts. Moreover, when the twins were asked to recall their preference for fat at the time the twins left their parental homes, the obese and lean co-twins consistently recalled that the obese twin had the greater preference for fatty foods. The obese members of the discordant co-twins also reported a tendency to overeat. The overconsumption by the respective obese twins was significant with regard to sandwiches, pastries and pies, ice cream and desserts, and alcoholic beverages but not for sweet cakes, candies and soft drinks (Rissanen et al., 2002). In agreement with this early twin study, obesity measures in the present study did not differ between twin pairs who reported differences in their intakes of candies and jellies in the present study. In the multivariate regression model analysis, the intake of sweets became inversely related to obesity measures. However, this may be due to correlations between the intake of sweets and sweet and fatty delicacies in the model. Moreover, sweet foods are often accompanied by fat, and it was found that a preference for sweet and fatty delicacies was significantly related to increased BMI and WC. As reviewed by Hill and Prentice (1995), most epidemiologic studies find either no association or even an inverse association between a high-carbohydrate, high-sugar diet and body weight. A large sample of over 11 000 children and adolescents participating in the 1999–2004 NHANES Survey revealed that candy consumers were less likely to be overweight and obese than non-candy consumers, but no differences were found between the groups for blood pressure, blood lipid levels, and cardiovascular risk factors (O'Neil et al., 2011). Energy intake was higher in

candy consumers than non-candy consumers, which suggests that candy is an important source of calories that results in excess energy intake. The inverse association between candy eating and body weight became apparent after energy adjustment. However the adjustment may not be appropriate as energy intake is an intermediate variable. Moreover, reverse causation may explain these somewhat surprising findings (Fogelholm and Tetens, 2011).

Large prospective studies report that a Western-style diet that contains many high-fat foods (e.g. red and processed meat, sweets and dessert, French fries, high-fat dairy products, fast food) is associated with higher BMI (Hu et al., 2000; Slattery et al., 1998). Furthermore, a high consumption of fried foods (Guallar-Castillón et al., 2007) and fast food (Pereira et al., 2005) has been suggested to increase the obesity risk. The Québec Family study showed that a lower consumption of dietary fat and lower fatty food intake was associated with a less pronounced weight gain and lower increases in WC among adults over a 6-year period (Drapeau et al., 2003).

In the present study, the co-twins who ate healthier relative to his/her respective co-twin had lower BMI and WC values. Most previous studies relating healthy eating patterns that have emerged by factor analysis, cluster analysis or dietary indexes and adiposity or body weight gain over time report an inverse relationship (Gao et al., 2008; Heidemann et al., 2008; Hu et al., 2000; McCullough et al., 2000; Newby et al., 2003). On the other hand, some studies show no relationship between healthy or prudent eating patterns and BMI (Huijbregts et al., 1995; Kennedy et al., 1995). The co-twins who exercised more were leaner and had healthier dietary choices, i.e. they consumed more dark bread, fresh and cooked vegetables, fruit and less sausages, salty snacks, creamy and fried foods and soft drinks. Interestingly, twins who ate at a slower pace relative to their co-twins had lower BMIs and WCs. In line, self-reported faster eating rates have been associated with increased BMI in two studies in the Japanese population (Otsuka et al., 2006; Sasaki et al., 2003). Possible physiologic mechanisms include hormonal regulators of satiety, such as a depressed response of the gut hormones PYY and GLP-1 (Kokkinos et al., 2010).

Earlier twin studies found it challenging to demonstrate that the heavier twins consumed more energy or consumed different dietary compositions than their lean co-twins when they used self-reported dietary intake data. Samaras et al. (1998) studied the association between dietary intake assessed by FFQ, and body fat in a sample of 90 middle-aged female MZ pairs from the TwinsUK cohort after excluding underreporters. After controlling for genetic and shared environmental factors within twin pairs, the intake of fat and protein was not related to BMI or percent body fat. They also observed an inverse correlation between total carbohydrate intake and total fat mass. In pair-wise analyses, co-twins with the higher sugar intakes had a lower total body and central fat percentage. The authors concluded that the role of dietary factors in influencing body fat had been overestimated in previous cross-sectional studies. De Castro (2004) investigated the influence of dietary and psychological factors on energy intake and body size among 110 MZ twin pairs using the 7-day food diary method. Differences in energy density and eating rate within twin pairs were positively related to differences in daily energy intakes, but not with differences in body weight or BMI. Hasselbalch et al. (2010) conducted a study in which they examined intrapair differences in habitual dietary intake. Dietary intake was assessed by an extensive FFQ in relation to intrapair differences in anthropometric measures in 153 male and 158 female twin pairs of the Danish twin Registry.

There was a positive cross-sectional correlation between the energy-adjusted intake of SSB and BMI and WC within male pairs and with hip circumference within female pairs. In men, recent weight gain was also positively correlated with SSB consumption within twin pairs. Those authors also revealed that there was a negative correlation between energy intake and BMI, fat mass index and hip circumference in women. The authors speculated that the women in particular may have underreported their dietary intakes in the FFQ.

6.2.2 Energy underreporting in obesity-discordant monozygotic twin pairs

The findings of the obesity-discordant pairs confirm that obese and non-obese co-twins differ significantly in their eating patterns and physical activity behaviors. In addition, many of the obese but only few of the leaner co-twins reported that they often eat more than they actually need. Obese co-twins reported higher body dissatisfaction and had a higher drive for thinness scores than their non-obese co-twins. Few differences in dietary composition were found between the obesity-discordant pairs according to their self-reporting. The obese twins consumed less energy from dietary MUFAs, PUFAs, less sweet and fatty delicacies and they also tended to consume less fruits and berries than their corresponding co-twins. However, energy intake did not differ significantly between the obese and lean co-twin pairs, despite the fact that both twin members independently reported that the heavier of the two eats more and chooses less healthy foods than their lean co-twins.

In an earlier study of 14 female and 9 male overweight-discordant twin pairs of the Finnish Twin Cohort Study, Hakala et al. (1999) ascertained that overweight co-twins had higher disinhibition and hunger scores, and a tendency to emotional eating and binge eating. Among women, energy intake was significantly higher in the overweight twins than in their leaner co-twins, whereas no differences were observed in the male twin pairs. However, it must be noted that the twins of that study were older (age range: 35-60y) than those of the present study and the diet was assessed by a detailed retrospective dietary history, which dealt with the subject´s normal diet during the previous year. Thus, the dietary assessment method used in that study may be more reflective of a subject's usual eating pattern as compared to the 3-day food diary, which was used in this present study. Nevertheless, macronutrients as a percentage of energy did not differ between the twin pair, which is in agreement with our study.

In the present study, DLW revealed that reported energy intakes were significantly lower than energy expenditure in the obese co-twins and amounted to only 75% of expenditure in the obese co-twins and 92% of expenditure in their leaner counterparts. This magnitude of underreporting of energy intake is generally in agreement with previous research that compared the self-reported energy intakes of adults using food records with energy expenditure measured by DLW (Hill and Davies, 2001), although a higher degree of underreporting has also been observed. For example, in the extreme case reported by Buhl et al. (1995), obese adults underreported their daily energy intake by 59% using the 14 day food record technique.

Goris and Westerterp (1999) designed a study to distinguish between the two errors that contribute to underreporting, i.e. underrecording (failure to record all foods in a food diary) and undereating (eating less than usual or less than required to maintain body weight) during the study period. The sample comprised 24 lean female dieticians who were familiar with the weighted food record method. Energy expenditure was estimated from resting metabolic rate and physical activity was measured by accelerometers. Underrecording was estimated from the discrepancy between total water intake and water loss measured by the elimination of deuterium. Undereating during the study was determined by the energy balance monitored by the measurement of body weight. Specifically, body mass was measured once a week before the beginning of the study, at the start of the study and at the end of the recording period which lasted for 7 days. The main finding was that in this sample of highly motivated lean women, the mean percentage of underreporting was 16%, which was entirely explained by undereating. The same authors also examined the contributions of undereating and underrecording to underreporting among 30 obese men using similar methods. Obese men underreported their energy intake, on average, by 37%. This was explained by both undereating (26%) and underrecording (12%) (Goris et al., 2000). Thus, from the two studies it can be concluded that underreporting of food intake does not necessarily mean that subjects are consciously lying about what they eat, but that they also reduce their food intake during the recording period, which may happen consciously or unconsciously.

In the present study, underreporting occurred at the whole-diet level with lower self-reported intakes of all macronutrients in absolute grams per day. Proportional intakes of energy from macronutrients were not significantly affected by underreporting. This is a minor problem in epidemiologic studies of diet and health outcomes because energy adjustment largely corrects for that problem (Willett, 2013). However, some evidence suggests that underreporting is selective for some foods and nutrients (Goris et al., 2000; Heitmann and Lissner, 1995; Heitmann et al., 2000; Johansson et al., 1998; Rosell et al., 2003). In a population of 323 Danish adults who reported their habitual diet by dietary interview, underreporting of energy and protein intake was assessed by comparing data from a dietary interview with estimated energy expenditure and 24 hour nitrogen outputs. Underreporting was proportionally less for protein than for other energy sources, especially in the obese, which suggests that in this subpopulation fatty foods and foods rich in carbohydrates were underreported more than total dietary intake (Heitmann and Lissner, 1995). In a Norwegian dietary survey of 3144 randomly selected men and women aged 16–79 years, underreporters identified from FFQs were more likely to be obese and expressed increased desire for losing weight than those subjects who did not underreport. The underreporting was characterized by reporting lower intakes of foods rich in fat and sugar and higher intakes of fiber and vitamin C than subjects with plausible energy reports. Among foods, under-reporters recorded lower intakes of cakes, potato chips, edible fats, chocolate and sweets, and SSB, and higher intakes of potatoes, meat, fish, and nonalcoholic beverages (Johansson et al., 1998). Similar results were obtained in a cross-sectional study of 301 healthy Swedish men aged 63 y in which diet was assessed by 7 day records. Under-reporters professed lower intakes of fat and carbohydrates and lower intakes of saccharides than subjects with reliable energy reports. Foods with low social desirability, including butter, margarine, buns and pastry, chips and snacks were recorded less, whereas bread, potatoes, meat, poultry and fish were recorded higher in underreporters (Rosell et al., 2003). Thus, accumulating evidence suggests that there is a systematic tendency

for study participant to underreport the consumption of undesirable foods such as those high in fat or simple carbohydrates, rather than underreport all types of foods. Although, the proportions of the macronutrients were not affected by the underreporting in the present study, the observation that obese co-twins self-reported eating only half of the amount of sweet and fatty delicacies as compared to lean co-twins suggests selective underreporting of some foods.

6.2.3 Associations between dietary factors and serum lipoprotein profile

Consistent with a meta-analysis of 42 experimental studies (Rimm et al., 1999), our results show that alcohol intake is associated with increased concentrations of HDL-C, ApoA1, and TGs. Further, ethanol intake has been related to increased lipoprotein particle sizes in older adults (Mukamal et al., 2007; Muth et al., 2010). In the present study, alcohol intake was associated with a decreased LDL particle size and an increased VLDL particle size, in addition to increased concentrations of small and medium-sized HDL, medium-sized VLDL and small-sized LDL particles. Thus, our findings suggest that associations between alcohol consumption and lipoproteins are not uniformly favorable. Regarding other macronutrients, a recent cross-sectional Swedish study reported the atherogenic lipid phenotype to be associated with a higher intake of disaccharides and a lower intake of protein and alcohol, which were assessed by the diet history method (Sonestedt et al., 2012). In the present study, we also observed significant associations with protein and alcohol intake and a dietary pattern high in fat and sucrose, which suggests that intakes of these macronutrients play a significant role in lipoprotein metabolism.

Most of the evidence on the effects of diet on lipoprotein subfractions, however, comes from interventional studies, which extensively focused on the role of low-carbohydrate diets in atherogenic dyslipidemia. Beneficial effects of carbohydrate restriction on serum lipoprotein subclass distribution have been reported in both isocaloric (Dreon et al., 1998; Krauss and Dreon, 1995) and energy-reduced diets (Sharman et al., 2002; Wood et al., 2006). Comparing low and high-carbohydrate (26% vs. 54% of energy) diets, Krauss et al. (2006) reported that low-carbohydrate diets either high or low in SFAs (15% vs. ~8% of energy) reduced concentrations of TGs and ApoB, small LDL mass, and total:HDL cholesterol but increased LDL peak diameter in weight stable subjects. The effects of the low-carbohydrate diets were similar for the low and high SFA content, except for LDL-C concentration, which decreased less with the higher saturated fat intake because of an increase in mass of large LDL. Their earlier studies showed that SFAs, particularly myristic and palmitic, but not stearic acid increase concentrations of larger, cholesterol-enriched LDL and this occurs in association with decreased HL activity (Dreon et al., 1998).

Our finding that dietary SFA intake is related to increased concentrations of small LDL and inversely to LDL particle size can be seen as contradictory to these earlier intervention studies. However, our results are difficult to compare with those study findings as we did not look at carbohydrate restriction, but at dietary fat intakes as part of the usual diet in which carbohydrate intake is at recommended levels. In support of our observation, increases in

LDL particle size were observed among adolescents following the National Cholesterol Education Program (NCEP) step II diet, which was lower in SFAs (7% vs. 15% of energy) and higher in MUFAs (14% vs. 9% of energy) than the control diet (Azadbakht et al., 2007). Adams et al. (2010) also reported that the consumption of 5 weekly servings of beef hamburger patties high in SFAs and trans fats over a 5-week period led to significant reductions in LDL particle diameter in 10 mildly hypercholesterolemic men. Thus, the findings regarding dietary fat and LDL particle size are somewhat conflicting. However, it has been reported that dietary SFAs may interact with other components of the diet on lipoprotein metabolism. For example, a recent study by Mangravite et al. (2011) found that plasma total cholesterol, LDL-C, non-HDL-C and ApoB concentrations were improved by replacement of carbohydrate with protein derived primarily from beef only in the context of the low SFAs diet. A higher SFA diet in combinations with higher beef intake tended to increase HL activity, and small apoCIII containing LDL (Faghihnia et al., 2012).

In the present study, we found few associations between individual dietary macronutrients and serum lipoprotein profile, but consistent associations between a dietary pattern high in sucrose and fat and an unfavorable serum lipoprotein profile. This suggests that the combination of simple sugars and fats, or other aspects of this dietary pattern, potentiate to produce the adverse effect. The “Junk food” dietary pattern correlated significantly with sugar intake, despite an overall inverse correlation with carbohydrates and dietary fiber. Accumulating evidence suggests that fructose, which is bound to glucose as a component of the molecule sucrose, adversely influences the serum lipoprotein profile (Stanhope et al., 2013). Simple sugars and high-glycemic index starches promote increased hepatic secretion and reduce peripheral clearance of TG-rich lipoproteins, which in turn, generate small, dense LDL and HDL particles (Hellerstein, 2002). Dietary fiber has been reported to offset the carbohydrate induced hypertriglyceridemia (Anderson, 2000). Our findings of a positive association between a dietary pattern high in sucrose and refined carbohydrates and low in fiber and micronutrients, which led to an adverse lipoprotein profile, could be explained by this biology.

In the present thesis, dietary intake of n-3 PUFAs was examined in relation to serum lipoprotein subclass profile in study III (total n-3 PUFAs) and in study V (serum DHA). Using native GEE to determine the proportions of HDL subclasses, we found moderate and strong positive correlations between n-3 PUFA intakes and proportions of HDL2b and HDL2a and negative correlations with percentages of HDL3a and HDL3b. These profiles remained significant in analysis within twin pairs, which is a finding that supports a possible causal role of dietary n-3 PUFAs on HDL metabolism. Associations with fish intake as part of the usual diet have not been reported previously, but earlier studies have examined supplementation with n-3 PUFAs. In agreement with our results, previous supplementation trials have shown that relatively large doses of n-3 PUFAs increase the HDL2/HDL3 ratio in healthy volunteers (Franceschini et al., 1991), in hypercholesterolemic subjects (Abbey et al., 1990) and also in patients with familial combined hyperlipidemia (Calabresi et al., 2004). The increase in the HDL2/HDL3 ratio has mainly been attributed to a selective increase in HDL2 and no change or only a small reduction in HDL3. Wooten et al. (2009) examined the combined and independent effects of n-3 PUFAs supplementation and aerobic exercise on serum lipids and lipoproteins concentrations. Four weeks of n-3 PUFAs supplementation

resulted in a shift in HDL from smaller, denser particles (HDL3a+3b) to larger, more buoyant particles (HDL2a+2b) without significant changes in lipid and lipoprotein-cholesterol concentrations. Despite the relatively high dosage of n-3 PUFAs supplementation of 4.55g per day for a period of 42 days, the authors did not observe changes in concentrations of total cholesterol, LDL-C, VLDL-C or TGs, which suggests that the dosage or duration of n-3 supplementation may have been below the threshold to reduce TG synthesis and increase LDL-C. This is also a likely explanation for the lack of such associations in the present study, whereby the n-3 PUFA intake was part of the twins´ usual diet and which were substantially lower than those used in the above mentioned intervention studies.

Using NMR to determine serum fatty acids and lipoprotein subclasses, we further investigated these associations using serum DHA as a biomarker of DHA intake. DHA is a long-chain n-3 PUFA that consists of 22 carbons and 6 double bonds. It can be either obtained from the diet or synthesized endogenously from alpha-linolenic acid. The conversion of alpha-linolenic acid to EPA is very limited (<8%) and to DHA marginal (<4%) (Emken, 2004). In women, this conversion is more efficient than in men (Burdge and Wootton, 2002). A higher intake of EPA and DHA in the diet reduces the conversion of alpha-linolenic acid to long-chain PUFAs, probably by product inhibition of the desaturase enzymes (Burdge et al, 2003). Previous studies that compared serum fatty acids with fatty acid intake have found the highest correlations for DHA and EPA ranging from as low as 0.20 to as high as 0.78 in different studies (Andersen et al., 1996; Hjartåker et al., 1997; Hodge et al., 2007; Ma et al., 1995). Fatty fish such as salmon, mackerel and herring have the highest content of EPA and DHA (Kris-Etherton et al., 2003). In the current study, the correlation between serum DHA and the dietary pattern high in fish was 0.34. Both the fish dietary pattern derived from the FFQ and the biomarker were significantly associated with lower concentrations of large and medium VLDL and a reduced VLDL particle size, though correlations with the biomarker were stronger, probably because of the larger measurement error associated with the FFQ. Serum DHA levels were significantly related to an increased HDL particle size and to reduced TG concentrations, which confirmed our earlier observations. In men, the increase in HDL size was due to a proportionally larger increase in small HDL as compared to large HDL. In women, the increase in HDL size was mainly due to a selective increase in large HDL but no associations with small or medium HDL particles were found.

We are aware of only one other study that examined the association between habitual dietary intake of n-3 PUFAs and serum lipoprotein profiles in an observational setting. In a population-based sample from the Norton Sound Region of Alaska, the intake of n-3 PUFAs as measured by FFQ was significantly associated with fewer large VLDL particles, a smaller VLDL size, more large HDL particles and a larger HDL size (Annuzzi et al., 2012). The authors adjusted their analyses for covariates including carbohydrate and sugar intakes. Dietary n-3 PUFAs were not associated with total serum LDL particle number. Therefore the authors suggested that the benefits of n-3 PUFAs may be related to cardiovascular processes other than those that affect atherosclerosis. We found very similar associations and came to similar conclusions using an objective biomarker for assessing habitual fish intake.

The observed association between increasing n-3 PUFA intakes and lipoprotein subclasses towards smaller VLDL particle sizes and increased HDL particle size may be the result of a reduced production of VLDL in the liver due to reduced hepatic TG synthesis or enhanced

fatty acid beta-oxidation (Chan et al., 2002; Price et al., 2000). This, in turn, may lead to a reduced CETP-mediated cholesteryl ester transfer out of HDL and the accumulation of larger more buoyant HDL particles (Griffin and Packard, 1994). Moreover, cholesteryl ester-rich TG-poor HDL particles are poor substrates for HL, and their presence may therefore prevent the conversion of HDL2 to HDL3 and lead to a selective retention of HDL2 in the plasma (Beisiegel, 1996).

6.2.4 Association between obesity and serum lipoprotein profile

Studies of twins discordant for obesity have contributed to a better understanding of the metabolic effects of acquired obesity (Naukkarinen et al., 2012). In the present study, we studied acquired obesity in relation to the serum lipoprotein subclass profile. Other studies have found that obesity correlates positively with concentrations of large VLDL particles, small LDL particles and inversely with large HDL particles in agreement with our results (James et al., 1997; Magkos et al., 2008). However, those earlier studies were not able to address the possibility of genetic confounding. We studied MZ twins discordant for obesity, and therefore verified that the genomic sequence and shared familial factors do not underlie the associations between obesity and a pro-atherogenic lipoprotein profile.

Although there is a strong link between obesity and metabolic complications, such as dyslipidemia, insulin resistance and hypertension, it is now well recognized that not every obese patient has the expected metabolic abnormalities or increased CVD risk. A large prospective study by Hamer and Stamatakis (2012) of over 22 000 men and women without a known history of CVD at baseline were followed up for more than 7 years for cause-specific mortality. Metabolically healthy obese individuals were not at an elevated risk of CVD and all-cause mortality and their risks were comparable to the metabolically healthy nonobese participants. However, in this large epidemiological study, obesity was measured by BMI, and there is now accumulating evidence that body fat distribution is more important in determining cardio-metabolic risk than overall obesity. Abdominal and gluteofemoral fat have been shown to have independent and opposite associations with glucose levels, dyslipidemia, insulin resistance, and type 2 diabetes in several studies, which have been extensively reviewed by Manolopoulos et al. (2010).

There are important differences in the metabolic and hormonal characteristics between subcutaneous and visceral fat tissue. Abdominal and visceral fat has been found to be related to higher HL activity and lower lipoprotein lipase activity (Bos et al., 2005; Després et al., 2000). Compared with subcutaneous fat cells, abdominal visceral adipocytes are more sensitive to catecholamine-induced lipolysis and poorly sensitive to insulin (Arner, 1999). Macrophages are more prevalent in visceral than in subcutaneous fat. The inflammatory cells secrete Interleukin-6 and Tumor necrosis factor alpha both of which increase the C-reactive protein levels that lead to a chronic inflammatory state with increased CVD risk (Fantuzzi, 2005). It has been suggested that subcutaneous adipose tissue acts as a “metabolic sink” where excess free fatty acids are stored as TGs in the adipocytes. However, the storage capacity of subcutaneous fat is limited, and once it is exceeded, fat begins to accumulate in the visceral fat depots (Frayn, 2002).

In the present study, we found marked differences in the levels of pro-atherogenic serum lipids between the obesity-discordant group with liver fat discordance, but not in the obesity-discordant group without liver fat concordance. This finding cannot be confounded by sex, age, familial factors or DNA sequence. Multivariate analysis revealed that intrapair differences in liver fat, but not those in intra-abdominal or subcutaneous fat were independently associated with ApoB, LDL-C and total cholesterol. This suggests that liver fat may be a better marker of the lipid abnormalities with obesity than intra-abdominal fat. The same obesity-discordant twin sample recently showed that the obese co-twins with higher levels of liver fat had significant alterations in their insulin and glucose responses during an oral glucose tolerance test (OGTT), greater tendency for hypertension, a downregulation of mitochondrial oxidative phosphorylation, branched-chain amino acid catabolism, fatty acid oxidation and adipocyte differentiation pathways and an upregulation of chronic inflammation (Naukkarinen et al., 2013).

Both visceral (intra-abdominal) and hepatic fat have been previously identified as the key drivers of cardio-metabolic risk. However, they are strongly correlated, thus it is difficult to differentiate their independent effects. Kotronen et al. (2007) examined the role of intra-abdominal and liver fat in explaining variation in cardiometabolic risk factors in 356 subjects by using multivariate models. In the models that included liver fat, intra-abdominal fat and other covariates, both types of fat depots explained the variation in TGs, HDL-C, insulin and hepatic insulin sensitivity independent of each other (Kotronen et al., 2007). The findings by Fabbrini et al. (2009) point towards liver fat as the culprit of the metabolic sequelae associated with obesity, which agrees with our findings. Those authors investigated groups of obese subjects who differed in visceral adiposity but matched for hepatic fat content or differed in hepatic fat content but matched for visceral adiposity. They used stable isotopes to trace VLDL secretion rates and the euglycemic hyperinsulinemic clamp to measure insulin sensitivity. Hepatic, adipose tissue and muscle insulin sensitivity were substantially lower and VLDL secretion rate almost doubled in subjects with high hepatic fat content matched for visceral adipose tissue, whereas no differences were seen in the other group. Serum TGs in obese subjects are known to increase by the combination of increased secretion and severely impaired clearance of TG-rich VLDL particles (Taskinen et al., 2011). Large VLDL particles are excellent substrates for CETP that facilitates the exchange of cholesterol ester and TGs between triglyceride and lipoproteins. This interaction yields TG-rich LDL and HDL that are hydrolyzed by HL, which facilitates the formation of atherogenic small, dense LDL and decreased large HDL (Barter et al., 2003).

Familial aggregation studies (Abdelmalek et al., 2006), twin studies (Schwimmer et al., 2009), candidate gene studies (Di Rosa and Malaguarnera, 2012) and GWAs studies (Romeo et al., 2008) all suggest that genetic factors play a role in determining the susceptibility to developing fatty liver and nonalcoholic steatohepatitis. In GWA studies, patatin-like phospholipase domain-containing protein 3 (PNPLA3) has been found to be the strongest signal so far. Interestingly, FTO, the strongest known common genetic risk factor for obesity has not been associated with liver fat (Hernaez, 2012). Lifestyle factors probably interact with genetic factors in determining disease risk. Physical activity and the level of fitness have been shown to improve liver enzymes independent of changes in weight (St George et al., 2009). Dietary sugars, particularly fructose, are suspected to lead to excess fat accumulation in the

liver and ectopic fat deposition in muscles, but definite evidence is missing (Bravo et al., 2013). It is not clear why in our study some of the obesity-discordant twin pairs showed a large difference in their liver fat content while the other twin pairs had an almost identical liver fat content despite their marked difference in body weight. The liver fat discordant pairs may have differed from the liver-fat concordant pairs in their lifestyles or their genetic susceptibility in response to weight gain. Importantly, the fact that the leaner twins of the liver fat discordant pairs had normal liver fat contents suggests that predisposed individuals can be protected from fattening of the liver and the accompanied lipid abnormalities by keeping a normal body weight and by pursuing a healthy lifestyle.

6.3 Methodological considerations

6.3.1 Cross-sectional design

A major limitation of the analysis used in this study is the cross-sectional nature of the data, which do not allow conclusions about causality or the directionality of the reported associations to be made. Associations observed in cross-sectional studies can be affected by reverse causality, by which some individuals in studies of diet and obesity may change their diet as a result of their increased body weight. Thus, it is not possible to determine whether the differences in eating and physical activity behaviors arose before or after the weight differences between co-twins emerged. For example, overweight or obese individuals might consume more low-fat products or less candy and SSBs in an attempt to reduce their body weight. Nevertheless, cross-sectional studies yield hypotheses about dietary factors that can be tested more rigorously in prospective settings or by intervention studies.

6.3.2 Confounding

Confounding refers to a situation in which a factor is related to both the exposure and the outcome variable and does not lie in the causal pathway between them (Rothman, 2002). The present study used multivariate analysis to control for known confounding factors, such as smoking, physical activity and alcohol consumption and for unknown genetic factors in the analysis within twin pairs. However, it is not possible to remove all potential confounding factors through statistical correction and the possibility for residual confounding remains. Some studies (Gillman et al., 2001; Quintiliani et al., 2010), including the present investigation, have suggested that healthy and unhealthy lifestyle behaviors tend to cluster. Thus, it is difficult to disentangle the effects of the dietary factor of interest from those of other aspects of the diet and the overall lifestyle. For example, many nutrients are highly correlated within a particular food, which makes it challenging to pinpoint a single dietary factor. The collinearity of nutrients was advantageously used in study IV to examine the association between the overall dietary pattern and the serum lipoprotein profile. The matched MZ design was utilized as another study design to control for confounding. The obese and non-obese twins used in this study were not only inherently matched for age and gender, but

also for the intrauterine environmental (e.g. maternal diet) and childhood environmental factors.

6.3.3 Self-reported anthropometric measures

In Finntwin16 (Study I), height, weight and WC were self-reported and used to compute BMI. Validity studies suggest that self-reported weight, height and WC is reasonably accurate (Lawlor et al., 2002; Niedhammer et al., 2000; Rimm et al., 1990). Overestimation of height and underestimation of weight tends to increase with age and with increasing BMI (Madrigal et al., 2000; Nyholm et al., 2007). Considering that the heavier twins may have underreported their body weight to a greater extent than their leaner co-twins, the use of self-reported measures may have underestimated the true intrapair difference in BMI between the behaviorally discordant twin pairs.

In the Finntwin16 study, the validity of self-reported and measured anthropometric measures was determined in 566 twins for a mean interval of 663 days after the completion of the questionnaire. The intra-class correlation was 0.94 for BMI and 0.73 for WC. The kappa value for obesity was 0.66 (95% CI 0.58 to 0.74) and that for abdominal obesity 0.60 (0.52 to 0.69) (Saarni et al., 2009).

6.3.4 Diet and physical activity assessment methods

Methodological difficulties in accurately assessing habitual dietary intake have hampered previous studies on dietary determinants of obesity. In Studies I and II, the cross-twin evaluations served as an important source of internal validation. The particular option of mutual responses from twin pair members has been previously used in case-control studies on breast cancer, with the specific purpose of attaining information on anthropometric and childhood risk factors for breast cancer. The authors concluded that twins are generally well-informed about their co-twins reproductive histories, but also noticed that this knowledge decreases when more detailed information is requested (Hamilton and Mack, 2000).

It is uncertain whether the results of this rather unique study can be applied directly to other studies in which proxy data are used. Twins may be better proxy respondents for each other on lifestyle behaviors than other relatives such as nearly same aged non-twin siblings or long-standing spouses. Despite this, the use of relatives as proxy informants could be explored as a methodological approach to improve the inherently poor self-reporting of dietary and physical activity behaviors that have previously relied on single informants. The study design used in this investigation might not be suitable in studies for which more detailed nutritional information is required. However, the design provides some unique advantages when looking at obesity-related behaviors, because it does not solely rely on the subject's self-report or recall of foods and portion sizes.

In Studies II, III and V, self-reported dietary data were used to examine the association between dietary factors and lipoprotein profile, despite their known limitations concerning underreporting. We adjusted for energy intake and other characteristics related to underreporting, such as age, sex and BMI to minimize this problem. In addition, subjects in

study V with extremely low or high energy intakes were excluded. We confirm some well-known associations between dietary intake and serum lipids which demonstrate the validity of our dietary data. Study III used 3-day food diaries to assess the association between dietary n-3 PUFAs and HDL subclasses. It is uncertain whether the 3-day records accurately reflect habitual intake because of day-to-day variation in food intake among free-living subjects. However, the validity of the food records is increased when the days are predefined and consecutive (Willett, 2013). The fact that the association between dietary n-3 PUFAs and HDL particle size was also seen in study V using serum measures of DHA lends substantial credibility to our results.

The FFQ that was used to derive dietary patterns was rather short compared to other FFQs used in large population-based studies. However, the derived patterns and the variance explained by them were comparable with previous studies. For example, earlier twin studies in the US and UK populations have identified a dietary pattern high in sugar and fat (similar to our "Junk food" pattern) and a healthy dietary pattern (similar to our fruit and vegetable pattern) (van den Bree et al., 1999; Teucher et al., 2007). In the present study, the 5 identified dietary patterns explained 22% of the total variance, which is similar to that found in others studies that used factor analysis to derive dietary patterns from FFQs (van Dam et al., 2003; Teucher et al., 2007).

6.3.5 Generalizability

A further methodological issue arises in the potential limitation of the generalizability of the results to new populations and settings. First, our sample included only Caucasian young adults and therefore the results may not be generalizable to other age groups or other ethnicities. Second, twins may differ from singletons in several health and lifestyle aspects and therefore the results may not generalize to the population as a whole. It has been well documented that twins are shorter and lighter at birth than are singletons (Liu and Blair, 2002; Wilson, 1979) because of their intrauterine retardation of growth and often preterm births. The differences in height, weight and BMI between twins and singletons remain but decline within the first few years of life (van Dommelen et al., 2008). Studies on adolescence or adults report that twins have similar or only slightly lower weight, height and BMIs than singletons (Andrew et al., 2001; Eriksen et al., 2013; Luke et al., 1995; Pietiläinen et al., 1999). In a study from the UK twin registry, lifestyle and metabolic variables of the twins were compared with a population-based study of women. The only difference between twins and singletons was found for weight, for which MZ twins were found to be lighter than singletons and also DZ twins. Twins were found to be representative of the general population for other characteristics, such as height, bone mineral density, blood pressure, current alcohol or tobacco use (Andrew et al., 2001). In addition, no differences in lipid levels were observed between twins and singletons in Scottish adults despite the lower birth weight and postnatal catch-up growth of twins (Tuya et al., 2006). Studies from the Danish twin registry based on large sample sizes have not found differences in diabetes prevalence (Petersen et al., 2011) or adult mortality (Christensen et al., 1995) between twins and singletons, or between MZ and DZ pairs. The mean macronutrient intake in our twins was comparable to that obtained from the national Findiet 2007 Survey (Pietinen et al., 2010). The differences between twins and

singletons appear small or nonexistent and the findings of this thesis are in accordance with earlier studies based on unrelated individuals. Therefore, it is likely that the conclusions drawn from this twin population can be generalized to the non-twin population.

6.3.6 Genetic and epigenetic discordance in MZ twins

The logic of using the MZ discordant twin design rests on the assumption that MZ twins are fully matched for their shared environment and genetic background, and therefore any differences between them can be attributed to environmental experiences unique to each twin (Vitaro et al., 2009). MZ twins develop from one single zygote and have the same chromosomal DNA sequence, and therefore for the most part single nucleotide polymorphism cannot be important in explaining discordance between MZ twins. However, apart from their unshared environmental experiences, phenotypic discordance between MZ twins can arise from somatic mutations that lead to mosaicism, epigenetic modifications and other genetic changes in one member of the twin pair. According to an extensive review by Machin (1996), mosaic chromosomal abnormalities in MZ twins have been reported for trisomy 21, trisomy 13 and monosomy 21. If one of a 46XY male MZ pair loses the Y chromosome, then the twin pair shows sex discrepancy, i.e. one twin being female with Turner´s Syndrome, characterized by having a short stature and lack of ovarian development and the other twin being a healthy male. There are only a few case reports of a point mutation that underlies the phenotypic discordance of MZ twins. In one case, a nonsense mutation in the Interferon regulatory factor 6 was found in the affected twin of a pair of MZ twins discordant for Van der Woude syndrome, which was subsequently confirmed in 45 additional unrelated families affected with the syndrome (Kondo et al., 2002). A mitochondrial DNA mutation has also been reported in one MZ twin pair clinically discordant for Leber´s disease. This mutation was not detected in their mother, which suggests that it was a *de novo* mutation (Biousse et al., 1997).

Epigenetic mechanisms, such as X-chromosome inactivation and imprinting may also underlie the phenotypic discordance of MZ twins. Skewed X-chromosome inactivation, in which either the paternal or maternally derived X chromosome is silenced, has been described in a number of studies in female twins discordant for Duchenne muscular dystrophy (Richards et al., 1990), bipolar disorder (Kuratomi et al., 2008) or mental retardation (Plenge et al., 2002). Fraga et al. (2005) found that 19% of healthy female MZ twins had skewed X-chromosome methylation patterns that differed between the twins. Differences in genomic imprinting could also explain the phenotypic discordance in MZ twins, as has been described for the Beckwith-Wiedemann syndrome (Weksberg et al., 2003). In addition to epigenetic variations associated with X-inactivation and imprinting, a number of studies have found significant differences in DNA methylation and histone modification profiles in specific genomic regions and more recently, whole-genome wide scans in MZ twin pairs (Bell and Spector, 2011). Copy number variation differences in MZ twins discordant for CHD also reveal the existence of somatic mosaicism in tissues arising from the same zygote (Bruder et al., 2008).

Chromosomal abnormalities in one MZ twin are extremely rare, and therefore unlikely to play a role in the present study. However, when present, they can advance the understanding of the relevant clinical problem. It is possible that epigenetic changes or copy number variation

differences associate with the obesity-discordance in the present study´s MZ twins. If such differences do exist, longitudinal twin data will make it possible to examine whether they are the cause or consequence of the obesity discordance. Alternatively, they could be the result of an effect such as diet or physical activity that may trigger the weight gain and the associated epigenetic change in one twin.

6.3.7 Other methodological considerations

The strengths of our study include the use of a large representative population sample of twins and also a smaller sample of obesity-discordant twin pairs, the combination of both presents an ideal model to study acquired obesity independent of genetic factors. However, MZ twin pairs discordant for obesity are extremely rare, and despite screening 10 yearly twin birth cohorts at the age of 23-33 years, only a relatively small number of obesity-discordant MZ twin pairs was identified and participated in this study. Therefore, the analysis of obesity-discordant pairs is likely to be underpowered and therefore prone to type 2 error. It is possible that individual discordant twin pairs had different reasons behind their weight discordance but the small sample size did not allow us to identify these patterns. Another limitation is that the food groups derived from the food diaries may have been too broad to accurately capture obesity-specific food patterns. The purpose of the FFQ was to capture habitual dietary intakes and rank individuals according to their intake of foods and nutrients. However, the lengths of the food list may have been too short to capture energy values. A definite strength of the study is the accumulation of evidence from multiple methodological approaches to research, including biomarkers of energy and nutrient intake, self-reported dietary intake and co-twin control comparisons.

7 Conclusions and future directions

In summary, this thesis provides convincing evidence that acquired eating and physical activity patterns are important determinants of obesity and lipid disturbances in young adults. However, misreporting of these behaviors by obese subjects can lead to inappropriate conclusions about potential behavioral contributors (e.g. high-fat food intake and physical inactivity) to obesity and dyslipidemia. Further, the results of this thesis lend support for a beneficial role of regular fish consumption on lipid and lipoprotein risk factors for CVD.

The main conclusions of this thesis can be summarized as follows:

- Obesity is associated with eating more, particularly more energy-dense foods and with exercising less at high intensities (I, II, V).

- Twin pairs who differed in the amount of food they consumed displayed the largest intrapair difference in BMI and WC and eating more (overall) was the strongest predictor of intrapair differences in obesity measures as indicated by multivariate regression analysis (I).

- Simple questions about co-twin comparisons of eating and physical activity behaviors showed highly significant associations with obesogenic dietary and activity behaviors, but there were few associations with these behaviors as assessed by the FFQs or by the 3-day diaries. Thus, the inclusion of mutual responses of twins, and possibly by other proxy respondents may improve the accuracy of the assessment of these behaviors that have previously relied on self-reports (I & II).

- Obesity-related underreporting of habitual energy intake and overreporting of physical activity can conceal real differences in true energy intake and exercise behaviors between obese and lean subjects (II).

- Higher dietary n-3 PUFA intake is related to reduced serum TG concentrations and a favorable lipoprotein subclass distribution in terms of VLDL and HDL (III &V).

- Higher scores on a dietary pattern that loaded highly on pizza, hamburger, fried potatoes or French fries, salty snacks, sweets and licorice were associated with higher serum TGs concentrations, smaller VLDL particles, smaller LDL particles and higher concentrations of small HDL particles independent of adiposity and other lifestyle factors, which can be seen as unfavorable in terms of CVD risk (V).

- Obesity is an important determinant of an atherogenic lipid and lipoprotein subclass profile. The accumulation of liver fat was particularly associated with increased concentrations of total cholesterol, LDL-C and ApoB (IV).

- Within MZ twin pair analysis revealed that many of the observed associations were independent of the potential confounding effects of genotype and shared environmental factors (I-V).

This study calls for continued efforts to improve dietary assessment methods, especially in the estimation of habitually consumed portion sizes. The expanding field of metabolomics may advance the development of nutrient biomarkers and possibly advance nutritional epidemiological research to assess diet and disease relationships. Intervention studies are required to confirm our findings regarding the diet and lipoprotein profile associations. Research is also required to understand the underlying cardioprotective mechanisms of certain nutrients. In addition, the effects of diet on CVD risk involve pathways other than plasma lipoproteins and therefore it will be important to examine the influence of dietary factors on other indicators of metabolic and cardiovascular health. As we learn more about the genetic and environmental determinants of obesity and adverse serum lipid profiles, the major challenge in the future will be to understand how dietary factors interact with the genetic makeup to modulate obesity and plasma lipids, and other CVD risk factors.

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Appendices

Appendix 1: Co-twin comparison questions in the FinnTwin16 study (unofficial translation to English)

Next we ask you to compare yourself with your co-twin sibling. Think about the past 12 months. Which one of you: you or your co-twin usually

	Me	My co-twin	There is no difference between us	I do not know
Eats more regularly	1	2	3	4
Eats more snacks	1	2	3	4
Eats more (on the whole)	1	2	3	4
Eats more slowly	1	2	3	4
Selects food more according to healthiness	1	2	3	4
Eats more fatty foods (incl. e.g. sandwiches)	1	2	3	4
Eats more sweet and fatty delicacies (chocolate, pastries, ice cream)	1	2	3	4
Eats more sweets (candies or jellies)	1	2	3	4
Is more worried about appearance	1	2	3	4
Goes on diets more often	1	2	3	4
Exercises more	1	2	3	4
Walks instead of taking a car or elevator, or makes other “active” choices in daily life	1	2	3	4

Appendix 2: Short food-frequency questionnaire in the FinnTwin16 study (unofficial translation to English)

In the following we ask you about the foods and drinks you have consumed during the past year (12 months).

How many slices of bread do you eat in a day (mark 0 if none?)

Dark bread (examples)	_____slices per day
Whole-meal bread (examples)	_____slices per day
White bread (examples)	_____slices per day

How often do you usually use the following foods (think about the past 12 months)?

	Never	A few times per month or less	A few times per week	Once a day	Several times a day
Potatoes, baked / mashed	1	2	3	4	5
Potatoes, fried, French fries	1	2	3	4	5
Rice or pasta	1	2	3	4	5
Porridge, muesli, cereals	1	2	3	4	5
Yoghurt	1	2	3	4	5
Cheese, low fat (examples)	1	2	3	4	5
Cheese, high fat (examples)	1	2	3	4	5
Fish (in foods or cold cuts)	1	2	3	4	5
Chicken (in foods or cold cuts)	1	2	3	4	5
Meats (in foods or cold cuts)	1	2	3	4	5
Sausages (in foods or cold cuts)	1	2	3	4	5
Eggs (boiled, fried, in omelets)	1	2	3	4	5
Fresh vegetables	1	2	3	4	5
Cooked vegetables	1	2	3	4	5
Fruits	1	2	3	4	5
Berries	1	2	3	4	5
Sweet pastries, ice cream, etc.	1	2	3	4	5
Chocolate	1	2	3	4	5
Other candy	1	2	3	4	5
Salty snacks (chips, popcorn, nuts)	1	2	3	4	5
Pizza	1	2	3	4	5
Hamburgers	1	2	3	4	5
Fried food	1	2	3	4	5
Creamy food	1	2	3	4	5
Salad dressing	1	2	3	4	5

How often do you usually use the following drinks (think about the past 12 months)?

	Never	A few times per month or less	A few times per week	Once a day	Several times a day
Skimmed milk or sour milk	1	2	3	4	5
1-1.5% milk or sour milk (examples)	1	2	3	4	5
Whole milk or sour milk (examples)	1	2	3	4	5
Juice	1	2	3	4	5
Regular soft drinks	1	2	3	4	5
Diet soft drinks	1	2	3	4	5
Coffee	1	2	3	4	5
Tea	1	2	3	4	5

How often do you usually use the following dietary fats (on bread, in cooking, in baking)?

	Never	A few times per month or less	A few times per week	Once a day	Several times a day
Butter	1	2	3	4	5
Butter-vegetable oil-mixture (examples)	1	2	3	4	5
Margarine, fat content under 65% (examples)	1	2	3	4	5
Margarine, fat content, 70-80% (examples)	1	2	3	4	5
Vegetable-stanol spread (Benecol)	1	2	3	4	5
Oil	1	2	3	4	5

Appendix 3: Food-frequency questionnaire in the FinnTwin12 study (unofficial translation to English)

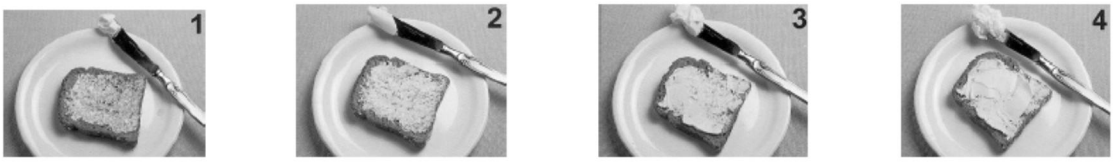
In the following we ask you about the foods and drinks you have consumed during the past year (12 months).

How many slices of bread do you eat in a day (mark 0 if none?)

Dark bread (examples)	_____slices per day
Whole-meal bread (examples)	_____slices per day
White bread (examples)	_____slices per day

How much fat spreads do you put on your bread?

1 Very, little, a very thin layer
2 Little, a thin layer
3 Moderately
4 Quite much
5 I do not use



How often do you usually use the following foods (think about the past 12 months)?

	Never	A few times per year or rarely	A few times per month	A few times per week	Once a day	Several times a day
Potatoes, boiled / mashed	1	2	3	4	5	6
Potatoes, fried, French fries	1	2	3	4	5	6
Rice or pasta	1	2	3	4	5	6
Porridge	1	2	3	4	5	6
Muesli, cereals	1	2	3	4	5	6
Yoghurt, cultured milk	1	2	3	4	5	6
Cheese, low fat (examples)	1	2	3	4	5	6
Blue cheese	1	2	3	4	5	6
Other cheese (examples)	1	2	3	4	5	6
Herring	1	2	3	4	5	6
Salmon, rainbow trout	1	2	3	4	5	6
Tuna	1	2	3	4	5	6
Other fish (in foods or cold cuts)	1	2	3	4	5	6
Chicken	1	2	3	4	5	6
Vegetable dishes	1	2	3	4	5	6
Meat dishes	1	2	3	4	5	6

Sausage dishes (Frankfurter style)	1	2	3	4	5	6
Cold cuts (ham, turkey)	1	2	3	4	5	6
Salami type sausage	1	2	3	4	5	6
Sausage, lauantai type	1	2	3	4	5	6
Eggs (boiled, fried, in omelets)	1	2	3	4	5	6
Fresh vegetables	1	2	3	4	5	6
Cooked vegetables	1	2	3	4	5	6
Fruits	1	2	3	4	5	6
Berries	1	2	3	4	5	6
Ice cream	1	2	3	4	5	6
Sweet pastries	1	2	3	4	5	6
Sweet desserts	1	2	3	4	5	6
Chocolate	1	2	3	4	5	6
Liquorice	1	2	3	4	5	6
Other candy	1	2	3	4	5	6
Salty snacks (chips, popcorn, nuts)	1	2	3	4	5	6
Pizza	1	2	3	4	5	6
Hamburgers	1	2	3	4	5	6
Fried food	1	2	3	4	5	6
Creamy food	1	2	3	4	5	6
Salad dressing	1	2	3	4	5	6

How often do you usually use the following drinks (think about the past 12 months)?

	Never	A few times per year or rarely	A few times per month	A few times per week	Once a day	Several times a day
Skimmed milk or sour milk	1	2	3	4	5	6
1-1.5% milk or sour milk (examples)	1	2	3	4	5	6
Whole milk or sour milk (examples)	1	2	3	4	5	6
Juice	1	2	3	4	5	6
Regular soft drinks	1	2	3	4	5	6
Diet soft drinks	1	2	3	4	5	6
Coffee	1	2	3	4	5	6
Tea	1	2	3	4	5	6

How often do you usually use the following dietary fats (on bread, in cooking, in baking)?

	Never	A few times per year or rarely	A few times per month	A few times per week	Once a day	Several times a day
Butter	1	2	3	4	5	6
Butter-vegetable oil-mixture (examples)	1	2	3	4	5	6
Margarine, fat content under 65% (examples)	1	2	3	4	5	6
Margarine, fat content, 70-80% (examples)	1	2	3	4	5	6
Vegetable-stanol spread (Benecol)	1	2	3	4	5	6
Vegetable oil	1	2	3	4	5	6