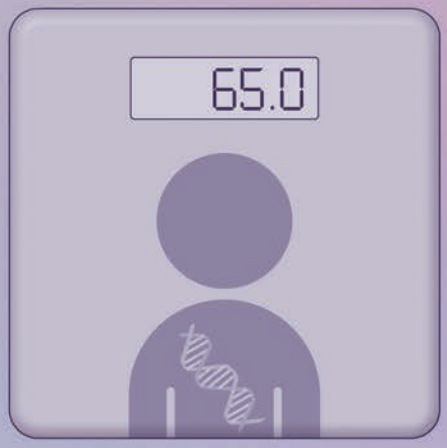
Diet and overweight

Epidemiological studies on intake, environment and genetics



Saskia van den Berg

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(met een samenvatting in het Nederlands)

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geboren op 17 april 1979
te Amersfoort

Promotor: Prof. dr. H.A. Smit

Copromotor: Dr. ir. J.M.A. Boer

Contents

Chapter 1

General introduction

Overweight is still an important public health problem

Four decades ago the number of people having an excessive bodyweight started to rise. Nowadays overweight (body mass index (BMI) ≥25 kg/m^2) has become more prevalent and is one of the most important global public health problems [1, 2].

Overweight not only is very common but also severe forms are seen more frequently. In 2013, worldwide, more than two billion (around 30%) children and adults were overweight [3]. In 2009-2010, approximately 50% of the Dutch adults were overweight and 14% were obese (BMI≥30 kg/m^2) [4]. Especially the increase of overweight among children is of great concern [5]. In 2009, the prevalence of overweight was 13-15% among Dutch children and adolescents [6]. A total of 2% of the children were obese, which represents a 4 to 6 fold increase compared to the 1980 obesity prevalence in children.

A high BMI is an established risk factor for various health problems including cardiovascular diseases, many types of cancer, musculoskeletal disorders and type 2 diabetes [7]. The prevalence of type 2 diabetes has reached epidemic proportions and globally, 44% of diabetes burden is attributable to overweight and obesity [8]. Consequently, overweight and its consequences are an important cause of disability and premature death [8].

Although overweight is a preventable condition, to date no country has reversed its obesity epidemic [9]. This thesis aims to contribute to the knowledge on causes of overweight with a main focus on diet. Figure 1.1 illustrates the dietary aspects that are dealt with in this thesis (colored blocks/arrow). These will be further addressed below.

Positive energy balance is the primary cause

Central to the etiology of overweight is a disturbance of the energy balance [10-12]. Key components of the energy balance are energy intake, energy expenditure and energy storage. When energy intake exceeds energy expenditure (positive energy balance), the excess energy is stored in the body primarily as fat mass [13-15]. In textbox 1 the components of the energy balance are described.

In 2003, Hill introduced the concept of the energy gap [16]. The energy gap reflects the excess daily energy intake over the daily energy expenditure that produces weight gain at the population level over a certain period of time. The estimation of the energy gap is often a theoretical approach based on retrospective assessment, whereby the amount of excess energy storage is estimated from the distribution of the rate of weight gain within a population [16, 17]. For many adult populations including the Dutch population, the energy

gap has now been estimated [18, 19]. The results suggest that small daily changes in energy balance (< 100 kcal per day) would be sufficient to prevent further weight gain in most of the adult population. A small daily energy gap will in the long run lead to excessive body weight gain. An increase in body weight results in an increase in energy expenditure and therefore energy requirement. The difference in the level of energy requirement before and after a period of weight gain, i.e. the maintenance energy gap, is often relatively large [11, 17]. This emphasizes the importance of preventing overweight rather than treating overweight.

A few studies estimated the energy gap that is responsible for overweight in children from the US and Germany [20, 21]. To estimate the energy gap in children it is important to take into account that part of the positive energy balance is essential for normal growth. We quantified the energy gap responsible for the development or maintenance of overweight in Dutch children.

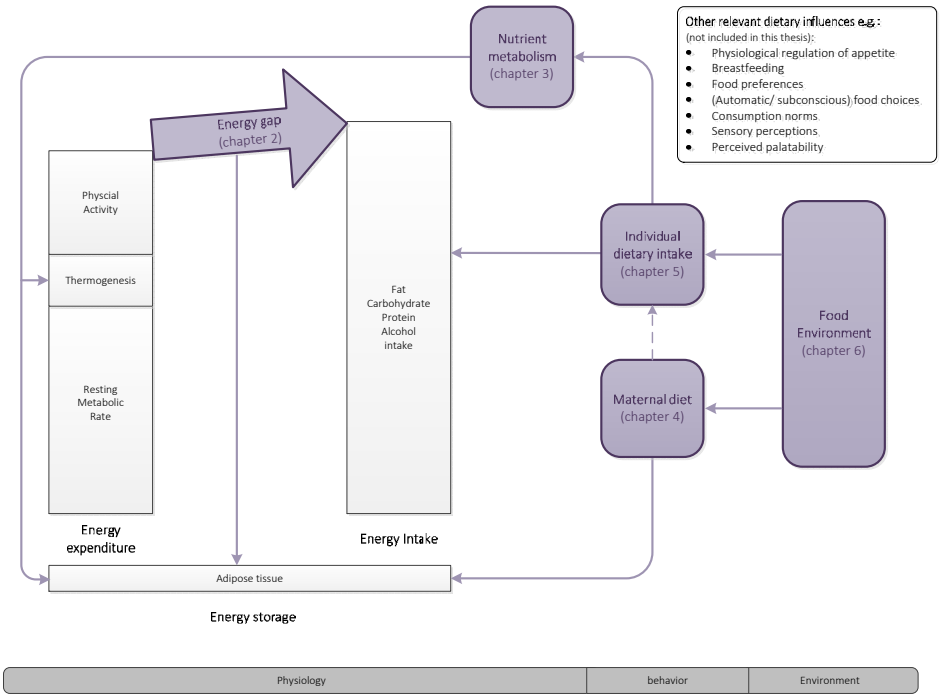

Figure 1.1 Schematic overview of a positive energy balance, its underlying components and related dietary aspects from different domains (physiology, behavior and the environment) that are dealt with in this thesis (colored blocks/arrow).

Textbox 1. Components of the energy balance

Energy intake is determined by the intake of macronutrients and alcohol. One gram of fat contains 9 kcal, which is more than two times the amount of energy provided by one gram of carbohydrate (4 kcal) or protein (4 kcal). Alcohol provides 7 kcal per gram and can therefore be a relevant source of energy. In the Dutch population, carbohydrates provide around 50%, fat around 35%, and protein around 15% of the daily energy intake [22].

Energy expenditure of humans can be divided in three main components [13, 15]. Resting metabolic rate (RMR) can be defined as the energy expended to maintain basic physiological functions of the body. It increases with higher body weight. Adaptive thermogenesis is the production of heat induced by food intake or cold. The surplus of energy is expended to digest, metabolize and store ingested macronutrients or to maintain body temperature. Physical activity is the third and normally the most variable component of total energy expenditure. The amount of energy expended is determined by the type, frequency, duration and intensity of the activity. For an inactive person, RMR accounts for 75-80%, adaptive thermogenesis for approximately 10%, and physical activity for 15-20% of total energy expenditure [23, 24].

Energy storage occurs predominantly as fat in white adipose tissue. One kilogram of fat mass corresponds approximately with an energy storage of 9000 kcal whereas one kilogram fat free mass corresponds with an energy content of 760 kcal [20, 25].

Dietary factors affect the energy balance

While the simple principle of a positive energy balance is the basic explanatory model, the etiology of overweight is multifaceted [10]. Over hundred factors related to physiology, behavior and the environment directly or indirectly affect the underlying components of the energy balance [26, 27]. Diet plays a key role in the energy balance (figure 1.1). Energy intake is completely determined by the dietary intake of an individual [13]. Individual dietary intake, in turn, is the consequence of dietary behavior, which itself is influenced by the availability and variety of foods in the environment, amongst others. Diet also partly affects energy expenditure, because metabolism of the consumed foods and macronutrients requires energy [28]. Furthermore, maternal diet during pregnancy is thought to affect energy storage of the child both in utero and later, by influencing fetal nutrition [29]. Fetal nutrition probably influences early adipose tissue development which may affect energy storage capacity of individuals later in life [30]. Thus, dietary factors influence the energy balance in many ways. Identifying relevant determinants of overweight related to nutrient metabolism, maternal diet, individual dietary intake and food environment may provide clues for prevention.

Nutrient metabolism

People differ in their efficiency to metabolize and store ingested macronutrients. Genetic factors are important determinants that can explain differences in nutrient metabolism and individual susceptibility to becoming overweight.

To date, genetic variation is the determinant that has been studied most [27]. The total variance in BMI explained by genetic variation lies between 40% and 70% [31, 32]. For waist circumference, the heritability estimates range from 40% to 80% [33]. Since the identification of the *ob* (*leptin*) gene in 1994, many overweight susceptibility loci have been identified [27, 34, 35]. Studies on rare forms of obesity, caused by a mutation in a single gene, revealed primarily genes that play a role in energy intake regulation (leptin-melanocortin pathway) [31]. Results from candidate gene studies on common obesity also showed evidence for overweight susceptibility genes involved in energy expenditure (e.g. *ADRB*, *UCP*) and energy storage (e.g. *PPARG*, *GNB3*) [32]. Most of the overweight susceptibility loci identified so far were obtained by large genome-wide association studies (GWAS) on common variants (mainly Single Nucleotide Polymorphisms (SNPs)) [34, 35]. The strongest and most robust association has been observed for a variant in the fat mass and obesity-associated (*FTO*) gene with an increase in BMI of 0.39 kg/m^2 for each additional risk allele (equivalent to an increase of ≈1.1 kg for someone 170 cm tall) [36, 37].

So far, biological pathways that underlie the observed associations between the susceptibility loci and overweight are still unclear and not uncovered by the hypothesis free approach of GWAS. From this point of view, hypothesis driven pathway-based approaches, studying genetic variants across genes from the same pathway, could be of additional value. However, such studies are seldom conducted. Nuclear receptors, such as PPAR, LXR and FXR, the SREBP transcription factors and the insulin receptor, have an important regulatory function in fatty acid and glucose homeostasis. Genetic variation in these key regulators and their pathways may be involved in the etiology of overweight and are studied in this thesis in relation to BMI and waist circumference.

Maternal diet

Overweight mostly results from an interaction between genes and the environment, including the in utero environment [38-40]. There are strong indications that the risk of overweight is already programmed prenatally by the in utero nutrient environment [29, 30, 41]. Among the suggested mechanisms are permanent changes in adipogenesis, adipocyte metabolism and the hypothalamic appetite control system [30]. Epigenetic alterations (e.g. different DNA methylation) causing changes in gene expression are thought to play a role in exerting the above mentioned mechanisms [40]. DNA methylation patterns can be affected by maternal diet and an inadequate maternal diet during pregnancy might therefore be an important in utero determinant of overweight later in life [29, 42].

Some of the earliest evidence for a role of maternal diet in the etiology of overweight came from the Dutch Famine Cohort in 1976 [43]. Infants of mothers who suffered from famine in the first or second trimester of their pregnancy, had a higher risk of obesity later in life than infants from mothers not exposed to famine. It has been postulated that the opposite situation, -i.e. in utero exposure to excess fuels as glucose and lipid in case of maternal diabetes, maternal overweight or an inadequate maternal diet-, can also program later risk of overweight [29, 30, 41]. Currently, much research has focused on this so called fetal overnutrition hypothesis. Supportive evidence comes from epidemiological studies that show that children of overweight or diabetic mothers are at higher risk of overweight later in life [44, 45]. Fewer studies have been conducted on the effect of maternal diet during pregnancy. For example, there is evidence that maternal fish consumption during pregnancy may have a beneficial effect on obesity risk at 3 years of age [46]. Fish fatty acids (EPA and DHA) are thought to have an anti-obesogenic effect by blocking the maturation of adipocytes [46]. So far, it is unknown whether the observed association persists throughout childhood. In addition, it is unclear whether such an association, if real, actually indicates in utero programming. The associations could also reflect similar dietary habits between mother and child [30]. Therefore, we investigated the longitudinal association between maternal fish consumption during pregnancy and BMI in children from birth to the age of 14 years, taking into account important maternal and child characteristics.

Individual dietary intake

There is no doubt that increased food consumption and thus energy intake, has contributed to the obesity epidemic [47, 48]. During the past several decades, our diet has shifted from a more traditional pattern to a so-called Western pattern characterized by, among others, processed and energy dense foods, sugar-sweetened beverages and bigger portion sizes [49, 50]. A large number of epidemiological studies have been conducted on dietary determinants in relation to overweight, including the type of food consumed (e.g fruit and vegetables) and intake of specific nutrients (e.g. fat) [51-53].

There is strong evidence for an association between overweight and several dietary determinants, including low intake of dietary fibre, high consumption of sugar-sweetened beverages and large portion sizes [51, 54-57]. These factors exert their effect by encouraging passive overconsumption of energy by influencing hunger and satiety regulation [51, 54].

As people do not consume only single foods or nutrients, studies have also been performed on the composition of the total diet. One important measure of total diet relevant for overweight is its energy density. The energy density of daily diets reflects the amount of available energy (kcal or kJ) per unit weight of foods or meals and is mainly determined by fat, water and fibre content [58]. Energy dense, ultra-processed foods contain high levels of fat and sugars which

make them hyper-palatable [59]. There is convincing evidence that the consumption of diets with a high energy density is associated with weight gain and overweight [60, 61]. In addition, the energy density of diets has been associated with type 2 diabetes, overweight's major co-morbidity, but studies are limited and were conducted in relatively small study populations [62]. So far, it is unknown whether this association reflects a direct (causal) relationship, or whether it is mediated by overweight. We therefore studied the association between dietary energy density and risk of type 2 diabetes in a large population and investigated whether an effect, if present, is mediated by BMI.

Food environment

Changes in our food environment over the last decades are important drivers of the overweight epidemic [16, 27, 47]. Our current food environment has often been referred to as obesogenic, i.e. an environment that promotes overconsumption of energy [63].

Determinants of the obesogenic environment can be categorized into four types of influences: physical, economic, political, and sociocultural [64]. The physical environment includes access to food and food availability. Affordability and prices of food are part of the economic environment. The political environment includes nutrition policies and regulations. The sociocultural environment includes attitudes, beliefs and values regarding diet.

The food environment can also be divided into micro (e.g. workplaces, schools and neighborhoods) and macro food environments (e.g. food production, manufacturing, distribution and marketing) [64]. Most studies are performed in micro settings, and many focused on schools [65]. Schools are considered as one of the most important settings for wide-reaching interventions to prevent or reduce overweight, because children have an intensive and prolonged contact with schools through childhood [66, 67]. In addition, schools create an environment that may stimulate or, on the contrary, discourage healthy diets and physical activity. Influence can be achieved, for example, by controlling the availability of foods and drinks in canteens or via vending machines, and by having a nutrition policy [68, 69]. The quality of food supply at schools (physical environment) and the availability and content of school policy on healthy nutrition (political environment) have been frequently studied. For example, a study conducted in 2006/2007 among Dutch secondary schools showed that unhealthy drinks and foods were widely available and only a small proportion of schools had a policy on overweight prevention [70]. Other studies showed that availability of unhealthy drinks and foods adversely affected dietary behavior and BMI [67, 71, 72]. In addition, evidence suggests that nutrition guidelines are effective in improving the school food environment and students' dietary intake [73].

Recently, in the Netherlands several national policies were launched or intensified to promote changes in the school food environment [74, 75]. However, little scientific evidence is available

as to whether such policies are effective. Therefore, we investigated changes in the food environment, i.e. -food supply, sociocultural aspects and nutrition policy,- at Dutch secondary schools between 2006/2007 and 2010/2011.

In summary, nutrient metabolism, maternal diet, individual dietary intake and the food environment play a major role in the development of a positive energy balance and overweight. Some of the knowledge on these associations has already been applied in interventions to prevent overweight. For example, since 2002 the Netherlands Nutrition Center coordinates the healthy school canteen programme which is an intervention that encourages secondary schools to set up their canteen in a way that promotes healthy food choices [75]. Started in 2010, the JOGG "Jongeren op Gezond Gewicht" intervention is a community approach involving all relevant sectors in preventing childhood overweight by changing the environment, behaviors and social norms step by step [76, 77]. Nevertheless, countries including the Netherlands are still relatively ineffective in preventing excessive weight gain among the population [9]. More insight in relevant determinants of overweight that are related to diet, their effect size and modifiability, can contribute to restoring the energy balance at the population level.

Aim of this thesis

The overall aim of this thesis was to study the role of a wide range of dietary factors on the development of overweight from a population perspective. This aim has been further specified into the following objectives:

1. To quantify the energy gap responsible for excess weight gain in Dutch children.
2. To investigate associations between determinants in the field of nutrient metabolism, maternal diet and individual dietary intake with overweight, and with diabetes as an important consequence of overweight.
3. To assess changes in the Dutch school food environment.

The determinants studied in this thesis are genetic variations in regulatory pathways of fatty acid and glucose metabolism (related to nutrient metabolism), maternal fish consumption during pregnancy (maternal diet), energy density of consumed diets (individual dietary intake) and food supply, nutrition policy and awareness regarding overweight among schools (food environment). Outcomes that are considered in this thesis are BMI, waist circumference, overweight and type 2 diabetes. Studies were conducted in mother-child pairs, adults, and children, as well as schools, using cohort and survey data.

Outline of this thesis

The quantification of the energy gap responsible for the development or maintenance of overweight during 4-years of follow-up in 2-year old Dutch children from the PIAMA birth cohort study is reported in **chapter 2** (objective 1).

The results of our studies on overweight determinants within the field of nutrient metabolism, maternal diet and individual dietary intake are presented in chapters 3, 4 and 5 (objective 2). **Chapter 3** describes the findings of our explorative study on 327 genetic variations in candidate genes from regulatory pathways that are involved in fatty acid and glucose metabolism and repeated measurements of BMI and waist circumference during 11-years in Dutch adults from the Doetinchem cohort study. **Chapter 4** presents the association between maternal fish consumption during pregnancy and BMI in children and the development of this association from birth to the age of 14 years, taking into account important maternal and child characteristics, based on data of the PIAMA birth cohort study.

Chapter 5 describes the association between dietary energy density and risk of type 2 diabetes in a large European population (EPIC-InterAct) and whether any effect is mediated by BMI.

Four year changes in the obesogenity of the school environment, the awareness of schools regarding overweight, school health policy, and actions taken by schools to prevent overweight based on data from two Dutch nation-wide surveys among secondary schools (2006/2007 and 2010/2011) are presented in **chapter 6** (objective 3).

This thesis is finalized with a discussion of the findings in a public health perspective (**chapter 7**).

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Chapter 2

Quantification of the energy gap in young overweight children. The PIAMA birth cohort study

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Abstract

Background: Overweight develops gradually as a result of a long term surplus on the balance between energy intake and energy expenditure. Aim of this study was to quantify the positive energy balance responsible for excess body weight gain (energy gap) in young overweight children.

Methods: Reported data on weight and height were used of 2190 Dutch children participating in the PIAMA birth cohort study. Accumulated body energy was estimated from the weight gain observed between age 2 and age 5-7. Energy gap was calculated as the difference in positive energy balance between children with and without overweight assuming an energy efficiency of 50%.

Results: Ten percent of the children were overweight at the age of 5-7 years. For these children, median weight gain during 4-years follow-up was 13.3 kg, as compared to 8.5 kg in the group of children who had a normal weight at the end of the study. A daily energy gap of 289-320 kJ (69-77 kcal) was responsible for the excess weight gain or weight maintenance in the majority of the children who were overweight at the age of 5-7 years. The increase in daily energy requirement to maintain the 4.8 kilograms excess weight gain among overweight children at the end of the study was approximately 1371 kJ.

Conclusions: An energy gap of about 289-320 kJ per day over a number of years can make the difference between normal weight and overweight in young children. Closing the energy gap in overweight children can be achieved by relatively small behavior changes. However, much more effort is required to lose the excess weight gained.

Background

Worldwide, the prevalence of overweight continues to rise. Especially, the increase in overweight among children is of great concern. In 2000, 10% of all children aged 5-17 were overweight, a total of 155 million [1]. A further 22 million children below 5 years of age were also affected [1].

Overweight is the consequence of a long term positive energy balance where daily energy intake exceeds daily energy expenditure [2]. Therefore, overweight can theoretically be prevented by measures that restore or maintain energy balance. In 2003, Hill et al. introduced the term "energy gap", which was defined as the excess daily energy intake over daily energy expenditure that produces weight gain at the *population* level [3]. They reported that a deficit of 418 kJ (100 kcal) per day would be sufficient to prevent further weight gain in most of the adult population. According to this study, closing the energy gap in adults can be achieved by small behaviour changes, like by walking an extra mile each day or taking a few less bites of food at each meal. The findings reported by Hill et al. provide a quantitative target how much behaviour change is required to restore energy balance in the adult population.

As childhood overweight is increasing at a disturbing rate, it is important to have quantitative targets for the prevention of excess weight gain among children. Several studies attempted to quantify the energy gap in children [4-8]. Of those, three studies calculated the energy gap for *overweight* children particularly [6-8]. However, there were large differences in the estimated energy gap between those three studies, ranging from 418-586 kJ (100-140 kcal) per day [8] to 2837-4255 kJ (678-1017 kcal) per day [7]. Explanations for these differences are the use of other assumptions and different methods. Butte and Ellis [6] quantified the energy gap among Hispanic children living in the US based on 1-year changes in body composition and different assumptions on energetic efficiency, but did not take into account the energy necessary for growth. In 2006, Wang et al. [7] estimated the energy gap in US children, but used a cross-sectional design. They estimated excess weight from children aged 2-7 years in the period 1988-1994 and adolescents aged 12-17 years in the period 1999-2002 based on the normal weight distributions in 1988-1994.

A more accurate estimation of the energy gap in overweight children may come from prospective studies with a long-term follow up, which takes into account the limitations of previously conducted studies on this topic. Recently, Plachta-Danielzik et al. used a long-term longitudinal approach to calculate the energy gap in children aged 6-10 [8]. So far, energy gap estimates for pre-school children are still lacking. Aim of this longitudinal study was to quantify the energy gap responsible for the excess weight gain between age 2 and 5-7 years in overweight children of the PIAMA cohort. Hereby, it is taken into account that part of the positive energy balance is essential for normal growth.

Methods

Study design and study population

The study population consisted of young children who participated in the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. In this study, mothers were recruited from the general population during pregnancy (*n* = 4,146) and their children were born in 1996-1997. Nearly all women (97%) had a Western ethnic background. The study protocol was approved by the independent medical ethics committee of TNO and all parents gave written informed consent. A detailed description of the study design has been published previously [9]. Data were collected mainly by annual postal questionnaires, which included questions on the child's weight and height. The number of parents who completed the questionnaires ranged from 3,746 (90% of the baseline study population) when the children were 1 year old to 3,373 (81% of the baseline study population) when the children were 7 years old. In the questionnaires parents were asked for the child's body weight (in kg) and height (in cm) the last time he or she was measured by a medical professional. When this measurement was not carried out in the last three months, parents were asked to weigh (without heavy clothes and shoes) and measure height of their child themselves. Also, date of the measurement and the person who carried out the measurements (parents or medical professional) were reported. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Overweight was defined according to age and sex specific international BMI cut-off points for children from two till eighteen years, which are based on the adult overweight cut-off point of ≥ 25 kg/m^2 [10]. For this study, weight and height at the age of 2 years were used as baseline data, which were available for 2,489 children. As thirteen children were excluded because there were more than 8 weeks between the date of body weight measurement and the date of height measurement, BMI was calculated for 2,476 2-year-old children. Sex specific standard deviation scores of BMI (SDS or z-scores) for age were calculated using the reference growth curves of the Dutch Fourth Nationwide growth study carried out in 1997 [11]. To study changes in weight, height and BMI z-scores in these children, information at 5-7 years of age was used for comparison (end point). Finally, our study population consisted of 2,190 children for which BMI was available at the age of 2 and at the age of 5-7 years.

Calculation and statistics

Changes in BMI-z scores, height and weight were calculated by subtracting the values at age 2 from the values at age 5-7. Based on overweight status at 2 and 5-7 years of age, children were divided into four groups. The first group consisted of children with a normal weight at both ages and is designated is this paper as "NN". The second group included children with a normal weight at the age of 2 and overweight at the age of 5-7 ("NO"). The third

group consisted of children with overweight at both ages ("OO"). The fourth group included children with overweight at the age of 2 and normal weight at the age of 5-7 ("ON"). Differences in characteristics between the four groups were evaluated by one-way ANOVA for normally distributed variables. However the change in weight showed a skewed distribution and therefore were evaluated by Kruskal-Wallis test.

Pathway to calculate the energy gap:

1. Calculate the *weight gain* and the *excess weight gain*; part of the weight gain that is not necessary for normal growth.
2. Estimate the gain in *fat free mass* and *fat mass* based on body fat percentages reported in literature.
3. Estimate the *energy storage*; the amount of kilojoules stored in the body as fat mass or fat free mass assuming that one kilogram corresponds with respectively 37656 kJ (9000 kcal) and 3180 kJ (760 kcal).
4. Estimate the *positive energy balance*; amount of kilojoules consumed to achieve the estimated energy storage assuming an energy efficiency of 50% for a mixed diet.
5. Quantify the *energy gap*; part of the positive energy balance responsible for the gain or maintenance of excess body weight among overweight children.

Figure 2.1 Schematic overview of the different steps that have been conducted to calculate the energy gap among young overweight children.

Excess weight gain was defined as part of the weight gain in children that is not necessary for healthy growth (figure 2.1). Excess weight gain was estimated 1) by subtracting the weight gain of children in the NN group from the weight gain of children in the NO group and 2) by subtracting the weight gain of children in the ON from the weight gain of children in the OO group.

Weight gain in children consists of a *gain in fat mass* and *a gain in fat free mass*. The absolute change in fat mass (kg) was estimated, based on the observed gain in body weight and body fat percentages for normal weight boys and girls at the age of 2 years and 5-7 years [12]. For example, a normal weight 2 year old boy has a body fat percentage of approximately 19.5%. For overweight children we assumed a body fat percentage of 22.5% and 27.5% for respectively boys and girls [13]. The change in fat free mass was estimated by subtracting the estimated change in fat mass from the change in body weight. For example, a two year old boy weighs 14 kilograms, which is a normal weight for his age. His estimated fat mass is 2.7 kilograms (14 kg *19.5%) and fat free mass is 11.3 kilograms (14 kg - 2.7 kg). At the age of six, the same boy has developed overweight and has a body weight of 27.5 kilograms. His body fat percentage is approximately 22.5%, his estimated fat mass is 6.2 kilograms (27.5 kg*22.5%)

and fat free mass is 21.3 kilograms (27.5 kg - 6.2 kg). In four years this boy gained 3.5 kilograms fat mass (6.2 kg - 2.7 kg) and 10 kilogram fat free mass (21.3 kg - 11.3 kg).

Energy storage was defined as the amount of kilojoules stored in the body as fat mass or fat free mass. Assuming that one kilogram fat mass and one kilogram fat free mass correspond with respectively an energy storage of 37656 kJ (9000 kcal) [14] and 3180 kJ (760 kcal) (19% protein; 16.7 kJ (4 kcal) per gram protein) [6], the energy storage was calculated. Since, follow up time differs per child, we calculated the energy storage per day by dividing the energy storage by the exact follow up time in days.

The *positive energy balance* was defined as the amount of kilojoules consumed to achieve the calculated energy storage. Hereby, it should be taken into account that energy is not stored at 100% efficiency, owing in part to the metabolic costs of digesting and storing various ingested nutrients. We used the assumption made by Hill that energy derived from a mixed diet is stored with an efficiency of at least 50% [3]. This means that for every 50 kJ stored at most 100 kJ have been consumed extra.

The *energy gap* was defined as part of the positive energy balance responsible for the gain or maintenance of excess body weight. The energy gap has been quantified 1) by subtracting the positive energy balance of children who had normal weight both at baseline and at the end of the study period (NN) from the positive energy balance of children who became overweight (NO) and 2) by subtracting the positive energy balance of children who were overweight at baseline and reached a normal weight (ON) from the positive energy balance of children who remained overweight (OO). For the energy gap calculation, the median positive energy balance and the corresponding 90th percentile was used.

Under the assumption of a linear accumulation of excess weight, the magnitude of the daily energy gap remains constant over time. Closing the energy gap will prevent further excess weight gain. However, to loose the excess weight previously obtained, a greater deficit on the energy balance is required [15]. This is due to the fact that excess weight leads to an increase in energy expenditure and therefore an increase energy requirement [16]. In children, the average additional daily energy expenditure associated with maintaining each extra kilogram of excess body weight assuming an average physical activity level has been estimated to be 142.8 kJ (34.14 kcal) [7]. We calculated the increase in energy requirement caused by the excess weight by multiplying the excess weight in overweight children by 142.8 kJ. In addition, to explore the accuracy of the energy gap estimate, alternative assumptions regarding the body fat percentages and energy efficiencies were used to calculate the energy gap. Firstly, age and sex specific values for body fat percentages in overweight children were applied instead of assuming only sex specific values [17]. Secondly, fat and protein specific energy efficiencies of respectively 87% and 42% were used instead of assuming an energy efficiency of 50% for a mixed diet [18]. SAS software version 9.1 (SAS Institute, Inc., Cary, NC) was used for the calculations.

Results

The percentages of children with normal weight and overweight at baseline and at the end of the study are presented in table 2.1. At the age of two, 7.4% of the children were overweight, which increased up to 10% when the children reached the age of 5-7 years. At both ages, more girls than boys were overweight. The majority of the children (85%) had a normal weight at both ages (NN). Five percent of the children were overweight at the age of two and achieved a normal weight at the age of 5-7 (ON). During the study, 7% of the children developed overweight (NO) and 3% of the children remained overweight (OO).
Table 2.2 presents BMI z-scores, height and weight at baseline and the changes in these variables between 2 and 5-7 years of age for the four groups based on BMI status. Children in the NO group already had a higher body weight ($p < 0.0001$) and BMI z-score ($p < 0.0001$) at the age of two compared with children in the NN group.

Table 2.1 Classification of 2190 children in normal weight and overweight at 2 and 5-7 years of age.

	Age (years)					
	2			5-7		
BMI- categories*	Total (*n*=2190)	Girls (*n*=1061)	Boys (*n*=1129)	Total (*n*=2190)	Girls (*n*=1061)	Boys (*n*=1129)
Normal weight (%)	92.6	91.1	94.1	90.0	87.2	92.7
Overweight (%)	7.4	8.9	6.0	10.0	12.5	7.4

BMI, body mass index.
** Classification is based on age and sex specific international BMI cut off points for children [10].*

Children in the ON group were shorter at the age of two than children in the other groups ($p < 0.008$). The median follow up time was 207.4 weeks and did not significantly differ between the four groups. For children in the NN group, the median gain in body weight was 8.5 kilograms and the mean increase in height was 28.8 centimetres during the follow-up period. Furthermore, a small decrease in mean BMI z-score of -0.19 was observed in those children. Children in the OO and ON group grew faster in height than children in the NN and NO group. The excess weight gain was 4.9 kilograms (13.4 kg-8.5 kg) in children who developed overweight during the study (NO) and 4.4 kilograms (13.1 kg-8.7 kg) in children who remained overweight during the study (OO). For children in the OO and the ON group a decline in mean BMI z-score of respectively -0.34 and -1.50 was observed. Among children who developed overweight during the study (NO), the mean BMI z-score increased with 1.12. Table 2.3 shows the daily gain in fat mass, fat free mass and body weight and also the daily energy storage and the positive energy balance assuming an energy efficiency of 50%. In the NN group, the weight gain consisted for 6% of an increase in fat mass. For children in the

NO and OO group, the increases in fat mass expressed as part of the total weight gain were approximately five times higher and were respectively 34% and 28%. In the ON group, nearly the total body weight gain consisted of an increase in fat free mass. For children in the NN group, the median energy storage was 32.7 kJ per day and the 90th percentile was 52.5 kJ per day (table 2.3). Children in the NO or OO group stored respectively 137.7 and 112.8 kJ per day. The lowest median energy storage was found among children of the ON group (19.5 kJ per day). As an energy efficiency of 50% was assumed, estimates of the positive energy balance are twice as high as the estimates of the energy storage. A visual presentation of the positive energy balance for 5-7 year old children with and without overweight separately is shown in figure 2.2. The median energy gap in the children who developed overweight (NO) was 209.2 kJ per day and the 90th percentile was 288.5 kJ per day. Among children who remained overweight (OO), the median energy gap was 186.6 kJ per day and the 90th percentile was 320.2 kJ per day.

Table 2.2 BMI z-scores, height and weight at baseline (age of 2) and change during the study (until the age of 5-7) of 2190 children divided in four groups based on BMI status.

	BMI status at 2 and 5-7 years of age*								
	Normal/ Normal (*n*=1868)		Normal/ Overweight (*n*=160)		Overweight/ Overweight (*n*=59)		Overweight/ Normal (*n*=103)		*P* value***
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age of 2:									
BMI z-score	-0.25^{bcd}	-0.91	0.43^{acd}	0.79	2.00^{ab}	0.50	1.85^{ab}	0.43	<0.0001
Height (cm)	93.6^{d}	5.2	94.0^{d}	5.0	93.5^{d}	6.0	91.2^{abc}	5.0	<0.0001
Weight (kg)	13.9^{bcd}	1.7	14.7^{acd}	1.7	16.6^{abd}	2.2	15.6^{abc}	1.6	<0.0001
Change:									
BMI z-score	-0.19^{bd}	0.95	1.12^{acd}	0.92	-0.34^{bd}	0.67	-1.50^{abc}	0.55	<0.0001
Height (cm)	28.8^{cd}	5.8	28.9^{cd}	7.9	30.7^{ab}	7.3	31.5^{ab}	6.0	<0.0001
Weight (kg)**	8.5^{bc}	7.0;10.3	13.4^{ad}	10.5;16.0	13.1^{ad}	10.5;15.2	8.7^{bc}	7.5;10.0	<0.0001

BMI, body mass index. SD, standard deviation.
[a] Significantly different (p<0.05) from group Normal/Normal.
[b] Significantly different from group (p<0.05) Normal/Overweight.
[c] Significantly different from group (p<0.05) Overweight / Overweight.
[d] Significantly different from group (p<0.05) Overweight / Normal.
**Classification is based on age and sex specific international BMI cut off points for children [10].*
*** Skewed distribution, median and 25th and 75th percentile presented.*
****ANOVA for normally distributed variables, Kruskal Wallis for variables with a skewed distribution.*

The analyses were repeated using different assumptions. Using the macronutrient specific energy efficiencies resulted in an energy gap of about 188 kJ per day instead of 289-320 kJ per day. Applying age-specific values for body fat percentages in overweight children resulted in an energy gap of 230 kJ per day instead of 289-320 kJ per day. We estimated the size of the daily energy gap responsible for the development of or maintenance of overweight in this population.

This gap would have to be closed to avoid further excess weight gain. However, excess weight leads to an increase in energy expenditure and therefore an increase in energy requirement. In overweight children, the increase in daily energy requirement to maintain the 4.8 kilograms excess weight gain at the end of the study was approximately 1371 kJ per day. Therefore, a much larger deficit on the energy balance has to be created in order to restore normal weight.

Table 2.3 Daily increase in body weight, energy storage, positive energy balance and energy gap of 2190 children divided in four groups based on BMI status.

	BMI status at 2 and 5-7 years of age*							
	Normal/ Normal (*n*=1868)		Normal/ Overweight (*n*=160)		Overweight/ Overweight (*n*=59)		Overweight/ Normal (*n*=103)	
	Median	P90	Median	P90	Median	P90	Median	P90
Body weight gain (g/day)	6.2	8.1	9.4	12.4	9.0	12.5	6.1	7.4
Excess weight gain (g/day)			3.2		2.9			
Fat mass (%gained)	6.3	18.5	34.1	41.4	27.5	34.9	0	2.3
Fat free mass (%gained)	93.7	99.7	65.9	74.3	72.5	77.5	100	100
Energy storage (kJ/day)	32.7	52.5	137.3	196.7	112.8	187.4	19.5	27.2
Positive energy balance** (kJ/day)[3]	65.5	104.9	274.7	393.4	225.6	374.6	39.0	54.4
Energy gap (kJ/day)			209.2	288.5	186.6	320.2		

BMI, body mass index. P90, 90th percentile.
** Classification is based on age and sex specific international BMI cut off points for children [10].*
*** Assuming an energetic efficiency of 50% [3].*

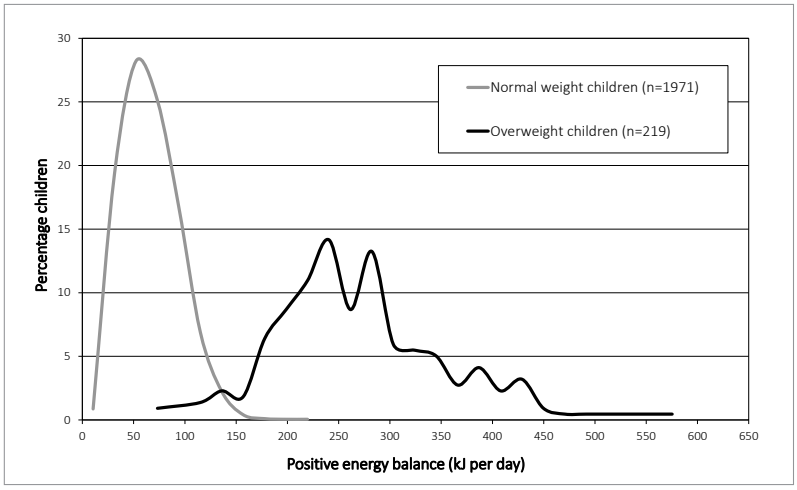

Figure 2.2 Distribution of the estimated positive energy balance (kilojoules per day) during the study (median follow up 207.4 weeks) among normal weight and overweight children at the age of 5-7, The PIAMA study, *n*=2190.

Discussion

An energy gap of about 289-320 kJ (69-77 kcal) per day was responsible for the observed excess weight gain or maintenance in the majority of the children who where overweight at the end of the study. This amount of energy corresponds with for example one glass (approximately 200 ml) of soft drink per day. To burn 289-320 kJ children have to walk for about half an hour.

Strength of this study was its longitudinal character. The same group of children was used to calculate the change in body weight over a period of 4 years. Furthermore, in this study the energy necessary for normal growth was taken into account in estimating the energy gap among overweight children. Although loss-to-follow-up and non-response on specific questionnaires were relatively low in the PIAMA study, a considerable proportion of the children had missing values on BMI. In the first year of life, when children are regularly weighed and measured in underfive clinics, response on the weight and height questions was high, but it decreased in the second year of life and even further in later years. In some cases parents reported only weight or height, but not both measurements, or they failed to report the dates of the two measurements, so that the age of the child at the time of measurement could not be calculated. Children with missing BMI data at age 2 more often had a lower educated mother than children with known BMI data at age 2 (data not shown). As obesity is more common among people with lower SES, we may have underestimated the percentages of overweight and obesity at age 2 However, there were no differences in BMI z-scores at age 1 between these groups. Furthermore, mothers of children with known BMI at age 2 and missing BMI data at age 5-7 were also more often lower educated than mothers of children with known BMI data at age 2 and age 5-7. However, there were no differences in BMI at age 2 between those groups. In addition, we observed no difference in weight gain during the study between children grouped by educational level of their mother. Therefore, we think that missing values in BMI has not materially influenced our energy gap estimates.

In this study, parental reported data on height and weight was used to estimate the energy gap. A study among 4 year old children who participated in the PIAMA cohort concluded that overweight prevalence rates are underestimated when based on reported weight and height [19]. Parents of children with a high BMI tend to underreport, whereas parents of children with a low BMI tend to over report their child's body weight, both with 0.5 kilograms on average. Therefore, the self reported data on weight may have resulted in an underestimation of weight gain of at most one kilogram in children who became overweight. At an energy efficiency of 50%, the calculated energy gap may therefore have been underestimated by at most 52 kJ per day in overweight children.

Fat mass percentages can differ substantially between normal weight individuals as well as overweight individuals [20]. Unfortunately, information about the body composition of the children was not available in this study. Therefore, assumptions about body fat percentages were made based on literature [12,13]. We also had to make assumptions about the energy efficiency. A commonly used assumption that a mixed diet is stored with an efficiency of 50% was used to facilitate the comparison with other studies that quantified the energy gap. In a sensitivity analyses, we checked the validity of these assumptions. Firstly, we applied age and sex specific body fat percentages for normal weight and overweight children specific [17]. Secondly, we used protein and fat specific assumptions regarding energy efficiency. Both adjustments resulted in a lower estimate of the energy gap. Therefore, it seems that our energy gap estimate of 289-320 kJ per day is more likely to be an overestimate than an underestimate of the actual energy gap.

This study reported an excess weight gain of about 1.1 kilograms per year and a daily energy gap of 289-320 kJ among the majority of the Dutch overweight children aged 5-7. This finding is generally in line with the results recently published by Plachta-Danielzik et al. who estimated the energy gap in older children aged 6-10 years [8]. They reported a yearly excess weight gain of 2.5 kilograms. The higher energy gap reported (527 kJ (126 kcal) per day) in children who developed overweight during the study period can largely be explained by the higher excess weight gain. They calculated excess weight gain from changes in fat mass and fat free mass z-scores which were based on measured body composition. As mentioned before we had no measured data on fat mass and fat free mass. However, when applying their approach using body weight z-scores, resulted in an only slightly lower median excess body weight gain (1 kg/year vs 1.2 kg per year) among NO children and subsequently a slightly lower energy gap. The similarity between the findings shows that our estimate of the energy gap is rather robust.

Our estimation of the energy gap is much lower than the other two studies that estimated the energy gap among overweight children. Butte and Ellis calculated an energy gap of 1431-2100 kJ (342-502 kcal) per day among US overweight children aged 5-19, at an energy efficiency of 50% [6]. This larger energy gap can be explained by the fact that the energy required for healthy growth among children was not taken into account and by the higher excess weight gain of about 2.7 kilograms per year among US overweight children. Wang et al. [7] reported the highest energy gap of 2837-4255 kJ (678-1017 kcal) per day among overweight US children aged 12-17. The yearly excess weight gain was similar to that reported by Butte and Ellis and therefore can not explain the higher energy gap. However, Wang et al. added the increase in daily energy requirement to maintain the previously gained excess body weight to their daily energy gap estimate, whereas we (and the other authors) estimated the daily energy gap that would have to be closed to avoid further excess weight gain. As,

over a study period of 10 years, excess weight gain increased up to 26.5 kilograms, this has substantially influenced the magnitude of the energy gap estimated by Wang et al. In our study, the increase in daily energy requirement among overweight children at the end of the study was 1371 kJ due to a body weight gain of 4.8 kilograms. Further excess weight gain among overweight children can be prevented by closing the energy gap. However, much more effort is required to lose the excess weight gained.

From the above it becomes clear that the different energy gaps reported in the literature might result from differences in the methodological approaches used to calculate the energy gap. However, it cannot be excluded that the observed differences could also in part reflect real life-course specific differences in energy balance. This could imply that specific recommendations for specific life periods are needed. Therefore it's worthwhile to study this issue further. Interestingly, we found a relatively high remission rate in overweight in children between age 2 and age 5-7 (64%). This should also be taken into account in future recommendations on the prevention of childhood overweight. Important to notice is that the estimated energy gap is a mean value for young Dutch children with overweight. Translation to individuals can not directly be made. For example, genetic variation may results in differences in energy efficiency between persons and therefore probably also in energy gap between individuals [21]. Furthermore, the estimated energy gap of 289-320 kJ is not applicable to more severe forms of overweight. For example, among children who became obese during the study, the calculated excess on the energy balance was 377 kJ (90 kcal) per day (data not shown).

Conclusions

An energy gap of about 289-320 kJ (69-77 kcal) per day over a number of years can make the difference between normal weight and overweight in young children. Closing the energy gap in overweight children can be achieved by relatively small changes in diet or physical activity, like consuming 1 glass of soft drink per day less or increase walking for about half an hour per day. However, larger changes in energy balance are required to lose the excess weight gained.

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Competing interests The authors declare that they have no competing interests.

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Chapter 3

Genetic variations in regulatory pathways of fatty acid and glucose metabolism are associated with obesity phenotypes: a population-based cohort study

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Abstract

Background: As nuclear receptors and transcription factors have an important regulatory function in adipocyte differentiation and fat storage, genetic variation in these key regulators and downstream pathways may be involved in the onset of obesity.

Objective: To explore associations between single nucleotide polymorphisms (SNPs) in candidate genes from regulatory pathways that control fatty acid and glucose metabolism, and repeated measurements of body mass index (BMI) and waist circumference in a large Dutch study population.

Methods: Data of 327 SNPs across 239 genes were analyzed for 3575 participants of the Doetinchem cohort, who were examined three times during 11 years, using the Illumina Golden Gate assay. Adjusted random coefficient models were used to analyze the relationship between SNPs and obesity phenotypes. False discovery rate q-values were calculated to account for multiple testing. Significance of the associations was defined as a q-value ≤0.20.

Results: Two SNPs (in *NR1H4* and *SMARCA2* in women only) were significantly associated with both BMI and waist circumference. In addition, two SNPs (in *SIRT1* and *SCAP* in women only) were associated with BMI alone. A functional SNP, in *IL6*, was strongly associated with waist.

Conclusion: In this explorative study among participants of a large population-based cohort, five SNPs, mainly located in transcription mediator genes, were strongly associated with obesity phenotypes. The results from whole genome and candidate gene studies support the potential role of *NR1H4*, *SIRT1*, *SMARCA2* and *IL6* in obesity. Although replication of our findings and further research on the functionality of these SNPs and underlying mechanism is necessary, our data indirectly suggest a role of GATA transcription factors in weight control.

Introduction

Overweight and obesity are defined by WHO as an excessive accumulation of body fat [1]. Worldwide, about 1.6 billion people are overweight (body mass index: BMI≥25 kg/m^2), of which about 400 million suffer from obesity (BMI≥30 kg/m^2) [2]. Consequences of the obesity epidemic are an increase in the occurrence of cardiovascular diseases, diabetes, musculo-skeletal disorders, such as osteoarthritis of knee and hip, and certain types of cancers [3,4]. Cardiovascular diseases are the main cause of death worldwide and diabetes has rapidly grown into a global epidemic [5]. Therefore, overweight is a serious threat to public health.

BMI is the most frequently used measure to define overweight and obesity at the population level [6]. At similar BMI levels, however, the amount of abdominal fat and associated health risk can differ significantly. A more sensitive measure is waist circumference, which gives information about the central fat distribution and is used to define abdominal obesity [7]. Although environmental factors have an important function, there is significant evidence for a heritable component in obesity [8,9]. In general, the total variance in BMI explained by genetic factors varies between 20 and 90%, with a most probable value around 40% [10,11]. For waist, the heritability estimates range from 40 to 80% [6].

The 2005 update of the human obesity gene map reported 426 associations between candidate genes and obesity phenotypes [8]. Despite the long list of positive findings, meta-analyses showed sufficient evidence for an association with BMI or obesity for five genes only (*PPARY2*, *FTO*, *MC4R*, *TNFa*, *PCSK1*) [12–16]. The inconsistencies among individual studies can partly be explained by a lack of power to replicate the typically moderate-to-small effects of a single locus on polygenic obesity [6,17]. Currently, a limited number of cohort studies evaluated associations between genetic variation in candidate genes and waist [18–20]. In the last decade, it became feasible and affordable to analyze a large number of single nucleotide polymorphisms (SNPs) simultaneously. Genome-wide association studies, a *hypothesis-free* approach to find genetic areas associated with a trait, identified associations between genetic variations in the *INSIG2*, *FTO*, *CTNNBL1* and *MC4R* gene and obesity [15,21–23]. So far, very few studies used a *hypothesis-driven* pathway-based approach to study many genes from the same pathway in relation to obesity [24].

Nuclear receptors are transcription factors that regulate the expression of target genes through binding to a specific DNA sequence in the target gene, the so-called hormone response element. Genetic variation in a DNA-binding domain of the nuclear receptor or the target gene, in the genes coding for their co-activators, co-repressors and other involved proteins, may lead to differential expression of the target genes. The nuclear receptors PPAR, LXR and FXR (encoded by *NR1H4*), the SREBP transcription factors and the insulin receptor have an important regulatory function in adipocyte differentiation, fatty acid synthesis, fatty

acid oxidation, fat storage in adipose tissue and glucose homeostasis [25]. Therefore, genetic variation in these key regulators and their pathways may be involved in the onset of obesity. The aim of this study was to explore the associations between a large number of SNPs across candidate genes from regulatory pathways that control fatty acid and glucose metabolism, and repeated measurements of BMI and waist circumference in a representative and large population-based Dutch cohort (*n*=3575).

Materials and methods

Study population

The Doetinchem Study is a cohort study on lifestyle, biological risk factors and chronic diseases [26]. Inhabitants of Doetinchem, a town in a rural area in the east of the Netherlands, were invited to take part in the study. Between 1987 and 1991, random samples were taken from the municipal population register. A total of 12 404 inhabitants aged 20–59 years were willing to participate and underwent a first measurement (response 62%). A subsample of the baseline cohort (63%) was re-invited for a second measurement between 1993 and 1997. A total of 6100 participants were re-examined (response 79%). Between 1998 and 2002, a third measurement took place and 4917 participants were reassessed (response 75%). In total, 4662 persons had three repeated measurements. All participants gave written informed consent and approval was obtained from local Medical–Ethical Committees. Pregnancy and alteration in smoking behavior are factors that influence body weight. Therefore, participants of the Doetinchem Cohort who changed their smoking habits (*n*=750), who had missing data on smoking status (*n*=11) or who were pregnant at the time of measurement (*n*=122) were excluded. Finally, 3779 participants met the inclusion criteria of this study.

Measures

Information about demographic characteristics, pregnancy and lifestyle factors, including smoking status, were collected at each measurement round using standardized questionnaires. Smoking status was defined as non-smoking (i.e. smoking less than one cigarette per month), smoking and ex-smoking. Anthropometric data were collected by four trained research assistants in a similar manner at each visit to the municipal health center. The research assistants were audited once a year on compliance to protocol procedures. All measurements were performed on subjects wearing light indoor clothing with emptied pockets and without shoes. Body weight (kg) was measured to the nearest 0.5 kg and height (cm) to the nearest 0.5 cm. To adjust for light indoor clothing, 1 kg was subtracted from the measured body weight. BMI was calculated as weight (kg) divided by the square of height (m). Overweight and obesity

were defined as, respectively, a BMI ≥25 and 30 kg/m^2. At the second and third visit, waist circumference was measured according to written instructions based on WHO criteria for waist measurement (1989) [27]. Waist circumference was determined to the nearest 0.5 cm, at midway between the lowest rib and the iliac crest, with subjects in standing position and after breathing out gently. Waist circumference was measured in duplicate and the mean of the two measurements was taken. Abdominal obesity was defined as a waist circumference ≥88cm for women and ≥102cm for men. Non-fasting blood samples were taken from all participants, which were fractionated into blood serum, buffy coat and erythrocytes and subsequently stored at-30 °C until further use. DNA was extracted using a salting out method as described by Hoebee et al.[28].

Selection of candidate genes and SNPs

Candidate genes were selected by a pathway-driven approach based on literature, with emphasis on regulatory pathways that control fatty acid, glucose, cholesterol and bile salt homeostasis [25]. The selection procedure started from the master regulator genes encoding nuclear receptors (PPARs, LXR, FXR (*NR1H4*)) and transcription factors (SREBPs) and continued by selecting their co-activators, corepressors and target genes. In addition, hormonal receptors (insulin receptor) and their downstream signaling proteins were selected. Furthermore, several candidate genes, described in literature, to be associated with blood lipids or blood pressure, were added. The selection resulted in 251 candidate genes. When available, eligible SNPs for these genes were selected based on published associations with any disorder or functional parameter, using databases NCBI (Pubmed, Gene and SNP), Genetic Association Database, [29] CDC and SNPper [30]. Subsequently, we used the web-based program SNPselector [31] to query all the genes for potential candidate SNPs. The default ranking settings of the performed 'SNPs by gene' search, including 5 kb 5' and 1 kb 3' flanking sequence, were slightly modified. First, SNPs located in repeat regions were excluded (Repeat_score>0), to avoid potential genotyping difficulties. Second, Caucasian minor allele frequencies (MAFs) higher than 0 had to be available (MAF_Caucasian>0). Third, SNPs in predicted transcription factor-binding sites (TFBS) were preferred (Regulatory=*TFBS*). Finally, subsequent ranking was based on the highest function score (Function_score), followed by the highest regulatory score (Regulatory_score), discriminating between SNPs that might affect gene transcript structure or protein product, and the regulatory potential of the SNP, respectively. For the majority of the genes, one or two SNPs that were most likely to be functional based on observed associations or the criteria from SNP selector were selected. For only a few genes, mainly the master regulator genes, which served as a starting point in our pathway approach, up to seven SNPs were selected. MAF of the selected SNPs in Caucasians had to be larger than or near to 5% based on available information (literature

and Hapmap). Four of the 253 selected candidate genes remained without eligible SNPs in the SNP search described above. For each of these genes (*SCAP*, *ACSL1*, *CEBPA*, *E2F4*), a single SNP was handpicked based on Caucasian allele frequency, SNP location and validation information in NCBI SNP. In part limited by various constrains of the Golden Gate genotyping assay, our final SNP set consisted of 384 SNPs across 251 candidate genes, including one Y chromosome marker to serve as a gender control (see Supplementary table 3.3). A total of 153 SNPs across 91 genes were selected by literature and 226 SNPs across 178 genes were selected by SNPselector. Type 2 Failure_Codes (SNP in duplicated/repetitive region) of the Illumina Assay Design Tool were ignored when occurring in Golden Gate validated SNPs (SNP_Score=1.1). All SNP_Scores were >0.4.

Gene subclassification

Genes that exercise transcriptional control of lipid and glucose metabolism were termed as transcription mediators, whereas their targets and signal transducers were termed as effectors. When both classifications were applicable; for example, several nuclear receptors and co-activators exert transcriptional control over one another, classification preference was given toward transcription mediator.

Genotyping

A total of 139 subjects were not eligible for genotyping, mainly because of failure to extract DNA or because buffy coats were not available. For 3640 subjects, high throughput SNP genotyping was performed with the Illumina Golden Gate assay using the Sentrix Array Matrix platform (Illumina Inc, San Diego, CA, USA) [32]. Illumina GenCall software, (version 6.1.3.28) was used for automated genotype clustering and calling. Two independent researchers each visually inspected all cluster plots to validate and/or correct the automated genotype calling. Genotyping failed for 43 subjects because of an overall absence of any signal. In addition, 22 subjects were excluded: 21 because of discordance for the gender control and 1 male subject because of heterozygous genotyping of several SNPs on the X chromosome. For 28 SNPs, the genotype calling did not succeed because of low signal (n=11) overlap between the genotype clusters (n=13), multiple genotype clusters (n=3) or scattering of clusters (n=1). Furthermore, genotype calling was not completely convincing for 42 SNPs. Finally, for 3575 participants (1710 men and 1865 women), genotype data was available.

Quality control

To examine the credibility of the genotyping data, a subsample of SNPs for which genotype calling was not completely convincing (*n*=10; 24%) was verified in a random sample (n=96), using Taqman or Pyrosequencing. As verification showed no disagreement with the original results, genotyping of the remaining 32 SNPs was only verified when an association with BMI or waist was found for the particular SNP and all 42 SNPs were included in the analysis. In addition, 33 SNPs were not in Hardy Weinberg equilibrium (HWE), of which three still deviated from HWE after adjustment for multiple testing (q<0.05; see statistical analysis). Verification was carried out on a random sample (*n*=96) for the eight SNPs (24%) that deviated most strongly from HWE. All yielded the same results, except for an SNP in the *SORBS1* (rs2274490) and *CETP* (rs1800775) gene, which were, therefore, excluded from further analysis.

Power calculation

On the basis of the sample size, it was estimated that for a variant with 20% prevalence, there was >80% power to detect a 0.5-kg/m^2 difference in BMI and a 1.4-cm difference in waist between genotypes. For a variant with 5% prevalence, there was >80% power to detect a 0.9-kg/m^2 difference in BMI and a 2.7-cm difference in waist.

Statistical analyses

Four SNPs were excluded because their MAF was below 0.02 (rs1805348, rs11569620, rs17634758, rs5352), resulting in too few heterozygous subjects to do a meaningful analyses. Another 22 SNPs were excluded because they were highly correlated (r^2>0.8) with another SNP in the dataset (see supplementary table 3.3). As highly correlated SNPs will show similar associations with BMI or waist, including both SNPs in the dataset will result in an unnecessary more stringent adjustment for multiple testing. Finally, data on 327 SNPs in 239 genes were available for statistical analysis. Distributions of genotypes were tested for deviation from HWE by χ^2 analysis (PROC ALLELE; SAS version 9.1; SAS Institute, Inc, Cary, NC, USA). As BMI distribution showed a positive skew, BMI values were log-transformed before analyses. BMI and waist circumference values at the second and third measurements were available for nearly all participants (>99.5%). There were no missing BMI values at the first measurement. For this study, we used repeated measurement of BMI and waist circumference to estimate a mean level of BMI or waist. To account for correlation between repeated measures within subjects, random coefficient models (multi-level modeling) were used to study the relationship between SNPs and repeated measurements of BMI and waist (PROC MIXED). For each SNP, we assumed a similar change in BMI or waist over time between the genotypes. A total of 14 SNPs deviated from this assumption for BMI and 22 SNPs for waist and were, therefore, excluded from BMI or waist analysis. As random coefficient models allow for missing outcome

data as long as any omission can be assumed to be completely random, all 3575 participants were included in the analysis [33]. Model composition started with a model comprising BMI or waist, a random intercept and time of each measurement entered as exact follow-up time in years with the first measurement coded as zero. Second, a random slope was added to the model and the model fit was evaluated by the likelihood ratio test. Third, non-linear development of BMI over time was considered by adding quadratic and cubic follow-up time to the model and model fit was evaluated. Fourth, predictor variables as age and gender were added to the model as fixed effect and statistical significance was evaluated. Fifth, model improvements were considered by adding potential interactions terms between significant predictor variables. Finally, the basic model for BMI included sex, age, age-by-time and age-by-sex interaction as fixed effects and intercept and time as random effects. Compared with the BMI model, two modifications were made with respect to the basic model for waist circumference. First, the interaction between time and sex was statistically significant and, therefore, included in the waist model. Second, the variable time defined as a random effect was not statistically significant and, therefore, time was added as a fixed effect. Differences in BMI and waist circumference level between genotypes were analyzed by adding the SNPs by turns to the basic models. Associations with BMI or waist level below *P*-value 0.05 were tested for interaction between the relevant SNPs and gender. In case of a significant interaction, the results are presented for men and women separately. All analyses regarding SNPs located on the X chromosome (n=5) were performed for men and women separately. The false discovery rate, which is a commonly accepted method in high-throughput genomic studies, was applied to take into account the multiple tests performed [34,35]. False discovery rate *q*-values were calculated by multiplying the *P*-values by the number of tests performed and then dividing them by the rank order of each *P*-value. Rank order 1 was assigned to the smallest *P*-value. To ensure that the *q*-values have the same ordering as the *P*-values, *q*-values were defined as the minimum of *q*-values observed for that *P*-value or higher *P*-values. However, there is no conventional *q*-value threshold to evaluate statistical significance. Therefore, similar to other publications, a *q*-value threshold of 0.20 was applied to indicate those SNPs with most significant associations [36,37]. The Fisher's exact test was used to evaluate the frequency of TFBS-related SNPs among the SNPs associated with overweight.

Results

The study population consisted of slightly more women than men (table 3.1). Approximately, 25% of the participants were current smokers and about 40% were non-smokers. At the start of the study, the median age was 41 years, about 40% of the participants were overweight and approximately 6% were obese. Between the first and third measurements, the prevalence of overweight increased one and a half times, whereas the prevalence of obesity more than doubled. A total of 31.3% of the participants had abdominal obesity at the second measurement, which increased to 40.2% at the last measurement.

Table 3.1 Characteristics of the study population at each measurement (n=3575).

	Measurement (median [Q1-Q3] or %)		
Characteristic	First	Second	Third
Women (%)	52.2	52.2	52.2
Age	41.0 [33.1-48.1]	47.0 [39.0-54.1]	52.0 [44.1-59.0]
Smoking (%)			
Current	25.6	25.6	25.6
Ex	35.0	35.0	35.0
Never	39.4	39.4	39.4
BMI (kg/m^2)	24.2 [22.3- 26.4]	25.1 [23.0-27.3]	25.7 [23.5-28.1]
Overweight (%)	39.7	50.6	58.0
Obesity (%)	5.7	9.3	13.4
Waist circumference (cm)			
Men	-	95.0 [89.0-101.0]	97.7 [91.3-103.0]
Women	-	85.9 [78.0-92.5]	88.5 [80.0-95.5]
Abdominal obesity (%)	-	31.3	40.2

Overweight, BMI ≥ 25 kg/m^2; Obesity, BMI ≥ 30 kg/m^2; Abdominal obesity, ≥ 88 cm for women and ≥ 102 cm for men.

Two intronic SNPs were significantly associated (q≤0.20) with BMI as well as with waist circumference (waist). Subjects with the AA or AG genotype for rs10860603 in the *NR1H4* gene had a significant lower BMI (0.8 and 0.4 kg/m^2, respectively) and waist (2.3 and 1.1 cm, respectively) than subjects with the GG genotype (figures 3.1a and b). The other SNP, located in the *SMARCA2* gene (rs17712152), showed a statistically significant interaction with gender for both BMI (P=0.006) and waist (P=0.005). Women with the GG genotype had a 0.7-kg/m^2 higher BMI and a borderline significant higher waist (1.5 cm; P=0.09) than women with the GA genotype. Women with the AA genotype had the highest BMI and waist; however, this group was very small (n=4). For men, no associations were observed. Two other polymorphisms, that is in the *SCAP* and *SIRT1* gene, were significantly related to BMI only with q-values below

0.20. For the *SCAP* intron 3 AT polymorphism (rs6800271), an interaction with gender was observed (*P*=0.005). Women with the AT genotype had a 0.7-kg/m^2 higher BMI compared with women with AA genotype. For men, no difference in BMI between the *SCAP* genotypes was found. Subjects heterozygous for the *SIRT1* 1047 TC polymorphism (rs2273773) had a 0.5-kg/m^2 higher BMI than TT subjects. Finally, a significant association (*q*=0.03) with waist was observed for the *IL6* -573 GC polymorphism (rs1800796). Subjects with the CC genotype had a 7.6- and 8.7-cm lower waist than, respectively, GC and GG subjects (figure 3.2). The associations between waist and rs1800796 (*IL6*) did not change after adjustment for BMI (data not shown). In addition, for another 17 SNPs, an association with BMI was observed with *P*-values <0.05, but *q*-values >0.20 (supplementary table 3.1). For another 10 SNPs, an association with waist was detected with *P*-values <0.05, but *q*-values >0.20 (supplementary table 3.2). An overview of the associations with BMI and waist for each SNP separately is briefly reported in supplementary table 3.3. Twenty-two SNPs highly correlating (r^2>0.80) with another SNP were excluded to achieve a less stringent multiple testing correction (see Materials and methods). None of these SNP-sets were associated with BMI or waist (*P*<0.05). To analyze whether our genetic screen implicated a particular functional property of the selected pathways, a division by functional classification into transcription mediators and effectors was made. With the exception of rs1800796 (*IL6*), the significant associated SNPs (q≤0.20) are all among the transcription mediators (tables 3.2 and 3.3). When selecting SNPs using SNPselector, preference was given to SNPs located in predicted TFBS (see Materials and methods). Remarkably, all TFBS-associated SNPs with a *q*-value ≤0.20 (3 for BMI and 2 for waist; tables 3.2 and 3.3) had conserved GATA motifs, whereas only 19 (17%) SNPs of all 110 successfully genotyped TFBS-associated SNPs had GATA motifs. Fisher's exact test indicates that this is a significant enrichment of GATA motifs among TFBS-associated SNPs for BMI (*P*=0.0045) and waist (*P*=0.029). The GATA motifs remained significantly enriched when extended to all TFBS-associated SNPs with a *P*-value below 0.05: 5 out of 12 for BMI (*P*=0.033) and 3 of 4 for waist (*P*=0.016) (supplementary tables 3.1 and 3.2), suggesting a potential role of GATA transcription factors in weight regulation.

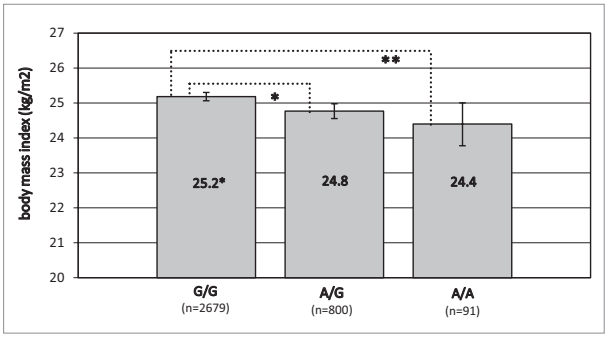

Figure 3.1a Geometric mean BMI (95% CI) according to *NR1H4* (Farnesoid X receptor) genotype (rs10860603).* *P*=0.0007; ** *P*=0.02.

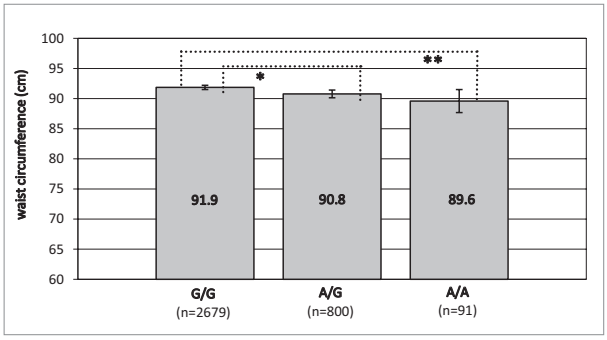

Figure 3.1b Mean waist circumference (95% CI) according to *NR1H4* (Farnesoid X receptor genotype (rs10860603). * *P*=0.004; ** *P*=0.02.

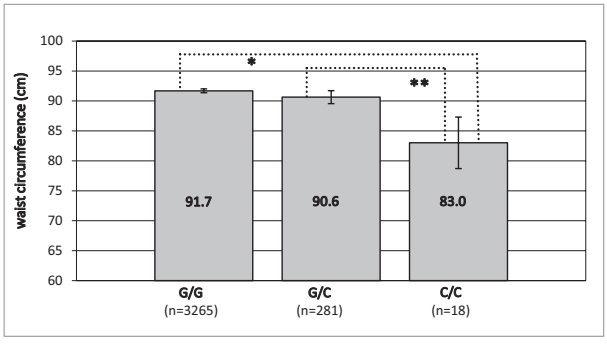

Figure 3.2 Mean waist circumference (95% CI) according to *IL6* (Interleukin 6) genotype (rs1800796).**P*<0.0001; ***P*=0.0008.

Table 3.2 Overview of single nucleotide polymorphisms (SNPs) associated with body mass index (BMI) with FDR q-values below 0.20.

				Geometric mean BMI						
Gene Name	rs number	SNP	MAF	MM	Mm	mm	P-value	FDR q- value	Conserved TFBS motif	Gene subclassification
NR1H4	rs10860603	Intron 9 G→A	A:0.14	25.2	24.8	24.4	0.0003[ab]	0.10	V$GATA_Q6[c]	Transcription mediator
SCAP[d]	rs6800271	Intron 3 A→T	T:0.43	24.3	25.0	24.6	0.0006[b]	0.10		Transcription mediator
SMARCA2[d]	rs17712152	Intron 20 G→A	A:0.045	24.8	24.1	30.0	0.001[abe]	0.10	V$GATA1_03[c]	Transcription mediator
SIRT1	rs2273773	c.1047 T→C	C:0.09	25.0	25.5	24.5	0.001[b]	0.10	V$GATA1_04[c]	Transcription mediator

Abbreviations: BMI=body mass index; MAF=measured minor allele frequency in this study; MM =homozygous for major allele; Mm=heterozygous; mm=homozygous for minor allele; FDR=false discovery rate; SNP=single nucleotide polymorphism.
[a]*Statistically significant difference in BMI between MM and mm.*
[b]*Statistically significant difference in BMI between MM and Mm.*
[c]*Different GATA motifs carrying the consensus site (A/T)GATA(A/G).*
[d]*Statistically significant interaction between the SNP and gender; association between the SNP and BMI is only significant in women.*
[e]*Statistically significant difference in BMI between Mm and mm.*

Table 3.3 Overview of single nucleotide polymorphisms (SNPs) associated with waist circumference (WC) with FDR q-values below 0.20.

				Mean WC						
Gene Name	rs number	SNP	MAF	MM	Mm	mm	P-value	FDR q- value	Conserved TFBS motif	Gene subclassification
IL6	rs1800796	p. -573 G→C	C:0.044	91.7	90.6	83.0	0.0001[ab]	0,03		Effector
SMARCA2[c]	rs17712152	Intron 20 G→A	A:0.045	87.3	85.8	105.8	0.0002[ab]	0,03	V$GATA1_03[d]	Transcription mediator
NR1H4	rs10860603	Intron 9 G→A	A:0.14	91.9	90.8	89.6	0.002[ae]	0,20	V$GATA_Q6[d]	Transcription mediator

Abbreviations: WC=waist circumference; MAF=measured minor allele frequency in this study; MM =homozygous for major allele; Mm=heterozygous; mm=homozygous for minor allele; FDR=false discovery rate; SNP=single nucleotide polymorphism.
[a]*Statistically significant difference in WC between MM and mm.*
[b]*Statistically significant difference in WC between MM and Mm.*
[c]*Statistically significant interaction between the SNP and gender; association between the SNP and WC is only significant in women.*
[d]*Different GATA motifs carrying the consensus site (A/T)GATA(A/G).*
[e]*Statistically significant difference in WC between Mm and mm.*

Discussion

In this explorative study among 3575 participants of the Doetinchem Cohort, significant associations ($q \leq 0.20$) were detected between adiposity and five SNPs across genes from regulatory pathways controlling fatty acid and glucose metabolism (*NR1H4*, *SMARCA2*, *SIRT1*, *SCAP*, *IL6*). Strengths of this pathway-driven candidate gene study were the relatively large sample size and the availability of repeated obesity-phenotype measurements, which improve study power and precision. Another strength was that we calculated false discovery rate *q*-values to reduce the chance of false positive findings. As there is no conventional threshold, an arbitrary cutoff point of 0.20 was used, as in similar research [36,37]. A more stringent threshold is often used in genome-wide association studies, which do not use prior information to select candidate genes. As an enrichment for genes in pathways influencing the studied phenotypes based on molecular studies prospects a higher frequency of true associated variations, a cutoff point of 0.20 seems to be justified. This study has limitations regarding the replication of the associations, functionalities of the associated SNPs and the underlying mechanism by which the SNPs may contribute to the phenotype of interest. Below, these aspects will be discussed for the significant ($q \leq 0.20$) individual associations.

There is *in vitro* evidence that the *IL6* -573 variant (rs1800796) is a functional polymorphism. The -573C allele has been associated with higher *IL6* gene expression levels [38]. In this study, we found a lower waist among subjects with the *IL6* -573 CC genotype. How higher IL6 gene expression levels may induce a lower waist remains to be elucidated. There is evidence for a regulatory role of IL6 in long-term energy balance by acting as an adiposity signal [39]. In human beings, IL-6 serum levels correlate with adipose tissue to the same extent as leptin. In addition, in the brain, IL6 favors a negative energy balance, as it decreases food intake. We are the first who report a lower waist among *IL6* -573 CC carriers. Therefore, replication of this finding is necessary. Our finding seems to be in contrast with a study of Slattery et al.[40] who reported a slightly higher waist-to-hip ratio (0.01) among 1330 non-Hispanic white women carrying the -573 C-allele. However, no separate data for waist circumference were given. Hamid et al. [41] found no association between the *IL6* -573 variant and BMI among 7500 Caucasian Danes. They reported a similar allele frequency and genotype distribution to our study and also observed deviation from HWE for this *IL6* variant. We verified our genotyping data, which yielded the same results. Therefore, it seems to be unlikely that errors in the genotype measurement can explain the atypical genotype distribution. This study shows that the *IL6* -573 polymorphism is strongly associated with waist, also after adjustment for BMI ($P<0.0001$). Together, this makes *IL6* an important candidate gene for abdominal obesity. We also included the most investigated *IL6* polymorphism, the -174GC variant (rs1800795). A recent meta-analysis does not support a role for this polymorphism in adiposity, [42] which is

in concordance with our study (BMI; *P*=0.11 and waist; *P*=0.92).
So far, there is no information about the functionality of the associated SNPs in *SIRT1*, *NR1H4*, *SCAP* and *SMARCA2*. In addition, no genetic association studies have been conducted on these SNP in relation to obesity phenotypes yet. However, there is some supportive evidence from genome-wide association studies for a role of these genes in obesity. Furthermore, there is some knowledge about the underlying mechanisms. In this study, subjects with the *SIRT1* rs2273773 TC genotype had a higher BMI than TT subjects. The results from a genome-wide scan show a strong association between a marker in *SIRT1* and body weight among Scandinavian subjects with diabetes [43]. SIRT1 acts as a nutrient sensor and is thought to be a molecular switch relaying the beneficial effects of caloric restriction on a healthy phenotype and longevity [44]. Hence, involvement of SIRT1 in human body weight regulation seems likely. In 2006, very strong associations between SNPs located in and near the *NR1H4* gene and waist were reported by the genome-wide SNP scan of the Framingham Heart study (*P*-values 0.006, 0.0007, 0.0008, 0.00003) [45]. In our study, for two independent SNPs both located in the *NR1H4* gene (r^2=0.23; *D'*prime=0.22), associations with BMI as well as waist were observed (rs10860603, rs35724; see Supplementary ables 3.1 and 3.2). Our results in combination with those from the Framingham Heart study designate the *NR1H4* gene as a very interesting candidate gene for obesity. As bile acids were shown to increase the metabolic rate in mice, it can be speculated that FXR (*NR1H4*), which is activated by bile acids, may influence body weight through an effect on energy expenditure [46]. This study showed a very high BMI and waist in the four women with the *SMARCA2* intron 9 AA genotype. All of them were overweight and three were obese. However, because of the small numbers, the results for this group should be interpreted with caution. Our results are supported by the findings from a genome-wide association study among Scandinavian subjects with diabetes, which showed a strong association between a marker near to the *SMARCA2* gene and body weight and waist circumference [43]. SREBF chaperone (SCAP) is the sterol sensing receptor directly interacting with and controlling SREBP transcription factor activation [47]. The few genetic association studies on this gene mainly focused on the *SCAP* Ile796Val variant (not included in our study). No associations were found between the Ile796Val variant and interindividual variation in drug-induced weight gain [48] and metabolic changes during weight gain [49]. In this study, the association between SCAP intron 3 AT polymorphism and BMI was only observed among women. The mechanism that can explain this gender difference is unknown. However, a change finding cannot be excluded either.
In summary, for all significantly associated SNPs ($q \leq 0.20$) replication of the reported association is necessary. In addition, we tried to select those SNPs most likely to be functional, but we have no certainty about the functionality, except for the *IL6* -573 variant (rs1800796). Therefore, more research is needed to investigate the functionality of the SNPs. Furthermore,

only a small part of the genetic variation for each gene was taken into account in this study. Hence, we cannot exclude the possibility that genes for which we found no association are in fact associated with obesity phenotypes.

A specific aim of this study was to highlight, by association, (sub)pathways in which inter-individual genetic variation leads to differences in body weight. As the majority of the significant associated SNPs ($q \leq 0.20$) are located in transcription mediator genes (tables 3.2 and 3.3), the potential involvement of this subpathway was further explored. Below, known protein–protein interactions for the transcription mediators genes ($P<0.05$) are briefly described (supplementary tables 3.1 and 3.2). Nuclear receptors such as the farnesoid X receptor (*NR1H4*) and the thyroid hormone receptor heterodimerize with retinoid X receptors (RXR), of which RXRG is one of three family members, to bind to DNA response elements [50]. Depending on ligand availability, the nuclear receptor heterodimer associates with repressors or co-activators, [51] influencing chromatin structure by helicase-like domain carrying proteins (SMARCA2 and SMARCA4 [52]), by mediation of histone deacetylation (NCOR2 [53]), by p160 mediated histone acetylation (NCOA2 [54]) and by bridging histone acetylation activity with the transcription mediator complex (TGS1 [55]). The latter, that is bridging of histone acetylation and transcription mediation, is combined with tissue specificity in PPAR gamma co-activators, with PPARGC1B as one of the three family members [56]. Gluconeogenic gene expression in liver driven by the best-studied PPAR gamma co-activator family member, PPAR gamma co-activator-1a, is enhanced after deacetylation by sirtuin 1 (SIRT1), a sensor for nutritional status [44]. In a recent publication, KLF5 was identified as a ligand dependant mediator of PPAR repressor activity, by attracting NCOR1 and 2 and co-activation activity through (de)SUMOylation, regulating lipid metabolism in muscle and adipocyte differentiation [57]. Hence, it is tempting to speculate that genetic variations in interacting transcription mediators influence body weight phenotypes through differential expression of nuclear receptor target genes.

Furthermore, a large fraction of the SNPs that were associated with BMI or waist ($P<0.05$), and in particular among the subclass of transcription mediators, are associated with conserved TBFS as identified by SNPselector (supplementary tables 3.1 and 3.2). Given the study design of selecting transcription factors and their targets, one might have expected most TFBS-SNPs to be present among the effectors. However, the TFBS-SNPs were not selected based on any specific transcription factor motifs, but simply ranked by Function_score and Regulatory_score as described in the Materials and methods section, resulting in only five genotyped SNPs associated with conserved motives for transcription factors and co-regulators that formed the basis of this study. None of these five SNPs reached significance levels. Instead, a combination of GATAmotifs was found to be significantly enriched, suggesting a role of GATA transcription factors in weight regulation, by controlling the transcription mediators themselves. Indeed,

at least two members of the GATA family, GATA2 and 3, have been functionally implicated as gate keepers at the onset of adiposite differentiation, requiring GATA expression to be down-regulated [58].

In conclusion, in our explorative study among 3575 participants of a large population-based cohort, we detected five SNPs strongly associated with obesity phenotypes. Findings from genome-wide association or candidate gene studies support the potential role of the *NR1H4*, *SIRT1*, *SMARCA2* and *IL6* gene in obesity. Although, replication of these findings in independent study populations is needed and further research on the functionality of most of these SNPs is necessary, our data indirectly suggests a role of GATA transcription factors in weight regulation.

Conflict of interest The authors declare no conflict of interest.

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Supplementary table 3.1 Overview of single nucleotide polymorphisms (SNPs) associated with body mass index (BMI) with a *P*-value ≤ 0.05.

Gene Name	rs number	SNP	MAF	Geometric mean BMI			*P*-value	FDR *q*-value	Conserved TFBS motif
				MM	Mm	Mm			
Transcription mediators									
NR1H4	rs10860603	Intron 9 G→A	A:0.14	25.2	24.8	24.4	0.0003[ab]	0,10	V$GATA_Q6[d]
SCAP[e]	rs6800271	Intron 3 A→T	T:0.43	24.3	25.0	24.6	0.0006[b]	0,10	
SMARCA2[e]	rs17712152	Intron 20 G→A	A:0.045	24.8	24.1	30.0	0.001[abc]	0,10	V$GATA1_03[d]
SIRT1	rs2273773	c.1047 T→C	C:0.09	25.0	25.5	24.5	0.001[b]	0,10	V$GATA1_04[d]
THRB[e]	rs1394761	Intron 1 G→T	T:0.37	24.9	24.8	24.1	0.006[ac]	0,28	V$HFH2_01
NCOA2	rs10112498	Intron 1 G→T	T:0.39	25.2	25.1	24.7	0.007[ac]	0,28	V$NKX25_01
RXRG	rs3818569[f]	c. G→A	A:0.38	24.9	25.2	25.0	0.024[b]	0,64	V$SRY_01
PPARGC1B	rs7732671	Ala203Pro	Pro:0.08	25.1	24.8	25.3	0.036[b]	0,73	
NR1H4	rs35724	Intron 9 C→G	G:0.38	25.2	25.1	24.8	0.040[a]	0,73	IMD:M000109[d]
NCOR2	rs2229840	Thr1699Ala	Thr:0.17	25.0	25.3	25.3	0.048[b]	0,77	V$MYOD_Q6
Effectors									
ABCG4[e]	rs3802885	3'UTR 3150A→C	C:0.07	24.7	25.3	23.2	0.003[bc]	0,23	
IGF1R	rs2229765	GAG1013GAA	GAA:0.45	25.3	25.1	24.8	0.005[ac]	0,28	
EDNRA	rs5333	c.1443 T→C	C:0.25	25.0	25.1	25.6	0.011[ac]	0,42	V$NKX25_02
ABCG5	rs6720173	Gln604Glu	Gln:0.16	25.0	25.3	25.5	0.017[c]	0,58	
FBP1	rs1754435	Intron 6 G→A	A:0.36	24.9	25.2	25.0	0.022[b]	0,64	V$CP2_01
APOA2	rs5082	p. -265 T→C	C:0.40	25.2	25.2	24.9	0.025[ab]	0,64	
CETP	rs1800777	Arg451Gln	Gln:0.034	25.1	25.1	20.9	0.031[ac]	0,73	
SLC8A1	rs10187203	Intron 1 A→C	C:0.48	25.3	25.1	24.9	0.033[a]	0,73	V$SRY_01
UCP3	rs1800849	p. -55 C→T	T:0.24	25.1	24.9	25.4	0.042[bc]	0,73	
ELOVL5	rs209512	Intron 1 G→A	A:0.23	25.2	24.9	25.2	0.042[b]	0,73	V$GATA1_04[d]
SOCS3	rs4969170	-5362G→A	A:0.34	24.9	25.2	25.3	0.049[b]	0,77	

BMI=body mass index; MAF=measured minor allele frequency in this study; MM =homozygous for major allele; Mm=heterozygous; mm=homozygous for minor allele; FDR=false discovery rate; SNP=single nucleotide polymorphism.

[a] *Statistically significant difference in BMI between MM and mm.*

[b] *Statistically significant difference in BMI between MM and Mm.*

[c] *Statistically significant difference in BMI between Mm and mm.*

[d] *Different GATA motifs carrying the consensus site (A/T)GATA(A/G).*

[e] *Statistically significant interaction between the SNP and gender; association between the SNP and BMI is only significant in women.*

[f] *rs3818569 has been replaced by rs1128977.*

Supplementary table 3.2 Overview of single nucleotide polymorphisms (SNPs) associated with waist circumference (WC) with a *P*-value ≤ 0.05.

Gene Name	rs number	SNP	MAF	Mean WC			*P*-value	FDR *q*-value	Conserved TFBS motif
				MM	Mm	mm			
Transcription mediators									
SMARCA2[e]	rs17712152	Intron 20 G→A	A:0.045	87.3	85.8	105.8	0.0002[ac]	0,03	V$GATA1_03[d]
NR1H4	rs10860603	Intron 9 G→A	A:0.14	91.9	90.8	89.6	0.002[ab]	0,20	V$GATA_Q6[d]
KLF5	rs4885062	Intron 3 T→G	G:0.34	91.1	92.1	91.4	0.012[b]	0,54	
TGS1	rs7823773	Cys754Phe	Cys:0.12	91.6	91.1	94.8	0.020[ac]	0,68	
SMARCA4	rs11085756[f]	c.5163 T→C	C: 0.31	91.4	92.0	90.7	0.032[c]	0,82	
NR1H4	rs35724	Intron 9 C→G	G:0.38	91.9	91.6	90.7	0.035[a]	0,82	IMD:M000109[d]
Effectors									
IL6	rs1800796	p. -573 G→C	C:0.044	91.7	90.6	83.0	0.0001[ac]	0,03	
SQLE	rs966946	Intron 8 T→C	C:0.34	91.6	91.2	92.8	0.006[ac]	0,45	
FBP1	rs1754435	Intron 6 G→A	A:0.36	91.1	92.1	91.2	0.009[b]	0,54	V$CP2_01
REL	rs10203477	Intron T→A	A:0.39	92.0	91.6	90.6	0.011[ac]	0,54	
ABCG5	rs6720173	Gln604Glu	Gln:0.16	91.4	92.1	89.7	0.018[c]	0,68	
LIPE	rs1206034	Intron 2 C→T	T:0.36	91.2	91.6	92.6	0.023[ac]	0,69	
CETP	rs1800777	Arg451Gln	Gln:0.034	91.5	92.0	78.2	0.034[ac]	0,82	

WC=waist circumference; MAF=measured minor allele frequency in this study; MM =homozygous for major allele; Mm=heterozygous; mm=homozygous for minor allele; FDR=false discovery rate; SNP=single nucleotide polymorphis.

[a] *Statistically significant difference in WC between MM and mm.*

[b] *Statistically significant difference in WC between MM and Mm.*

[c] *Statistically significant difference in WC between Mm and mm.*

[d] *Different GATA motifs carrying the consensus site (A/T)GATA(A/G).*

[e] *Statistically significant interaction between the SNP and gender; association between the SNP and WC is only significant in women.*

[f] *rs11085756 has been replaced by rs7275.*

Supplementary table 3.3 Results obtained from all performed tests on associations between SNPs and obesity phenotypes (BMI and waist circumference).

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
AACS	rs900410	0.48	NA	0.67
ABCA1	rs1800977	0.33	0.11	0.40
ABCA1	rs2066716	0.07	0.62	0.54
ABCA1	rs2230806	0.26	0.51	0.81
ABCA1	rs2275543	0.09	0.80	1.00
ABCB11	rs2287622	0.38	0.99	0.61
ABCB4	rs2109505	0.18	0.16	0.21
ABCB4	rs31653	0.12	0.07	0.45
ABCB4	rs8187799	0.08	0.78	NA

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
ABCD2	rs11172661	0.21	0.56	0.97
ABCD3	rs16946	0.48	0.63	0.42
ABCG1	rs2839482	0.18	0.62	0.35
ABCG1	rs915843	0.15	0.78	0.81
ABCG4[i]	rs3802885[m]	0.07	0.61	0.08
ABCG4[i]	rs3802885[v]	0.07	0.003	0.08
ABCG5	rs6720173	0.16	0.02	0.02
ABCG8	rs4148211	0.41	0.49	0.23
ABCG8	rs4148217	0.15	0.65	0.63
ACACA	rs10512477	0.20	HC	HC
ACACA	rs2252757	0.19	HC	HC
ACACA	rs8071315	0.33	0.14	0.32
ACACA	rs9330250	0.20	0.40	0.37
ACADL	rs2286963	0.36	0.74	0.93
ACADM	rs11549022	0.30	0.78	0.73
ACADVL	rs507506	0.39	0.67	0.53
ACAS2	rs17309872	0.08	NA	0.06
ACAT2	rs25683	CALL	CALL	CALL
ACAT2	rs3464	0.17	0.99	0.64
ACE	rs4343	0.46	0.15	0.49
ACLY	rs2304497	0.13	0.88	0.76
ACOX1	rs1137582	0.36	0.50	0.48
ACSL1	rs1803898	0.33	0.22	0.59
ADAMTS4	rs4233367	0.42	0.10	0.53
ADD1	rs4961	0.20	0.99	0.99
ADIPOQ	rs17300539	0.07	0.37	0.23
ADIPOQ	rs17366743	0.04	0.07	0.49
ADIPOR1	rs2275737	0.42	0.95	0.76
ADIPOR2	rs1029629	0.35	0.19	0.28
ADIPOR2	rs1044471	0.45	0.50	0.37
AGER	rs1062070	0.16	0.63	0.84
AGER	rs1800624	0.27	0.96	0.23
AGER	rs2070600	CALL	CALL	CALL
AGT	rs5050	0.16	0.28	0.11
AGT	rs699	0.40	0.66	0.38
AGTR1	rs5186	0.31	0.78	NA
AGTR2[x]	rs1403543[m]	0.49	0.67	0.65
AGTR2[x]	rs1403543[v]	0.49	0.36	0.50
AKT1	rs3001371	0.30	0.78	0.15
AKT2	rs748236	0.30	0.58	0.71
APOA1	rs11216158	0.15	0.20	0.31
APOA1	rs670	CALL	CALL	CALL
APOA2	rs3813628	0.36	0.52	0.69
APOA2	rs5082	0.40	0.03	0.27

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
APOA4	rs675	0.19	0.54	0.85
APOA5	rs3135506	0.08	0.41	0.87
APOA5	rs662799	0.07	0.72	0.80
APOB	rs1042031	0.17	0.85	NA
APOB	rs1367117	0.31	0.32	0.72
APOC2	rs1132899	0.49	NA	0.55
APOC3	rs2854116	0.36	0.83	0.29
APOC3	rs4520	0.27	0.49	0.84
APOE	rs429358	CALL	CALL	CALL
APOE	rs7412	CALL	CALL	CALL
BCL6	CCCAGACCGC[G/C] GAGAGGACGC‡	CALL	CALL	CALL
CAMK4	rs1469442	0.47	0.97	0.20
CARM1	rs12460421	CALL	CALL	CALL
CBL	rs4938638	0.47	0.84	0.81
CCL2	rs1024611	CALL	CALL	CALL
CCL2	rs4586	0.37	0.86	0.77
CCND3	rs3218108	0.27	0.58	0.34
CD36	rs1049654	0.45	0.42	0.84
CD36	rs1049673	0.45	0.33	0.59
CD5L	rs2261295	0.30	0.48	NA
CDKN2A	rs3088440	0.08	0.76	0.58
CDKN2A	rs3731249	0.03	0.59	0.84
CEBPA	rs12691	0.14	0.38	0.70
CEBPB	rs6020348	0.41	0.17	0.29
CEBPD	rs10088698	0.09	0.16	0.39
CETP	rs1800775	0.48	NOHWE	NOHWE
CETP	rs1800777	0.03	0.03	0.03
CETP	rs5882	0.31	0.43	0.08
CPT1B	rs131758	0.39	0.75	0.75
CPT1B	rs140515	0.38	0.80	0.62
CPT1B	rs470117	0.47	0.79	0.97
CREB1	rs2551640	0.32	0.54	0.75
CREBBP	rs1296720	0.21	0.73	0.51
CREBBP	rs130003	0.02	0.09	NA
CREBBP	rs130005	0.10	0.42	0.84
CREBBP	rs2239316	0.28	0.34	0.73
CRK	rs16946807	0.37	0.14	0.21
CRSP3	rs2781667	0.32	0.94	0.65
CYP11B2	rs1799998	0.42	0.82	0.49
CYP4A11	rs3890011	0.21	0.30	0.61
CYP4B1	rs2297809	0.10	0.20	0.28
CYP51A1	rs7797834	0.40	0.17	0.12
CYP7A1	rs3808607	0.38	0.86	NA

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
CYP8B1	rs735320	0.18	0.36	NA
DHCR24	rs7373	CALL	CALL	CALL
DHCR7	rs1044482	0.27	0.78	0.37
E2F4	rs11700	0.07	0.52	0.55
EBP[x]	rs3048[m]	0.37	0.28	0.68
EBP[x]	rs3048[v]	0.37	0.21	0.19
ECE1	rs213045	0.29	0.68	0.32
ECE1	rs213046	0.10	0.54	0.12
EDN1	rs1794849	0.27	0.55	0.44
EDNRA	rs1801708	0.33	0.23	0.46
EDNRA	rs5333	0.25	0.01	0.13
EDNRB	rs5352	0.015	MAF	MAF
EHHADH	rs11919970	0.07	0.53	0.89
EIF4EBP1	rs6605631	0.22	0.68	0.95
ELOVL2	rs4532436	0.42	0.21	0.07
ELOVL5	rs209512	0.23	0.04	NA
ELOVL6	rs11098071	0.50	0.34	0.35
ELOVL6	rs4146696	0.22	0.42	0.42
EP300	rs4822012	0.38	0.06	0.18
ESR1	rs12204714	0.36	0.86	0.75
ESR1	rs17081749	0.14	0.63	0.76
ESR1	rs2234693	0.48	0.36	0.09
ESRRA	rs2276014	0.16	0.08	0.16
FABP1	rs2241883	0.33	0.60	0.29
FABP2	rs1799883	0.26	0.70	0.68
FABP3	rs17495262	0.15	0.34	0.23
FABP4	rs16909196	0.16	0.32	0.34
FADS1	rs174546	0.33	0.76	0.22
FADS2	rs482548	0.09	0.69	0.42
FASN	rs2228309	0.44	0.95	0.79
FBP1	rs1754435	0.36	0.02	0.009
FBXW7	rs2676330	0.30	0.73	0.71
FDFT1	rs10098874	0.15	0.17	0.13
FDFT1	rs1047643	0.15	HC	HC
FDFT1	rs9205	0.23	0.23	0.40
FDPS	rs2297480	0.24	0.68	0.21
FLOT1	rs8512	0.13	0.06	0.17
FOXC2	AGCAAGAAGA[C/T] TTTTGAAACT[‡]	0.40	0.91	0.91
FOXO1A	rs2721044	0.33	0.12	0.25
FRAP1	rs2076657	0.31	0.40	0.50
G6PC	CAGACTCATA[G/A] CAGAGCAATC[‡]	CALL	CALL	CALL
G6PD	rs1050757	CALL	CALL	CALL

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
GABPA	rs2829887	0.42	0.61	0.43
GABPB2	rs1050571	0.09	0.80	0.83
GK	rs12512319	0.12	0.79	0.54
GNB3	rs5443	0.31	0.29	0.12
GPAM	rs2254537	0.28	0.65	0.60
GRB2	rs4788891	0.17	NA	0.98
GSK3B	rs17810676	0.37	0.17	0.27
GSK3B	rs334558	0.34	HC	HC
GSK3B	rs3755557	0.14	0.65	0.91
HMGCR	CAAACTCTTT[T/G] GTCATATCAG	0.03	0.44	NA
HMGCS1	rs1548097	0.50	0.22	0.15
HMGCS2	rs536662	0.48	0.38	0.78
HNF4A	rs1800961	0.04	0.34	0.41
HNF4A	rs745975	0.24	0.41	0.96
HPGD	rs8752	0.40	0.57	NA
HRAS	rs4963176	0.34	NA	0.49
HSD17B7	rs10917598	0.35	0.44	0.58
IDI1	rs7075141	0.30	0.39	0.91
IFNG	rs2069718	0.40	0.28	0.32
IGF1R	rs2229765	0.45	0.005	0.12
IL1B	rs1143634	0.24	0.30	0.92
IL1B	rs16944	CALL	CALL	CALL
IL6	rs1800795	0.39	0.11	0.92
IL6	rs1800796	0.04	0.06	0.0001
ILK	rs2288283	0.13	0.46	0.72
INPPL1	rs2276048	0.20	NA	0.55
INPPL1	rs9886	0.06	0.57	0.31
INS	rs3842748	0.21	0.70	0.69
INS	rs689	CALL	CALL	CALL
INSIG1	rs9769506	0.43	0.81	0.28
INSIG2	rs9308762	0.17	0.20	0.19
INSR	rs2059806	0.25	0.85	0.87
INSR	rs2860172	0.18	NA	0.13
INSR	rs2963	0.08	0.87	0.75
INSRR	rs10908521	0.28	0.28	0.20
IRS1	rs1801278	0.08	0.19	0.24
IRS2	rs4773092	0.40	0.85	0.94
IRS4[x]	rs2073115[m]	0.14	1.00	0.97
IRS4[x]	rs2073115[v]	0.14	0.63	0.45
KCNMB1	rs827778	0.12	0.10	0.31
KLF5	rs4885062	0.34	0.22	0.01
LDLR	rs5925	0.43	HC	HC
LDLR	rs688	0.43	0.80	0.78

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
LEP	rs2167270	0.35	0.73	0.78
LEPR	rs1137100	0.27	0.13	0.09
LEPR	rs1137101	0.46	NA	0.15
LEPR	rs8179183	0.18	0.88	0.92
LIPC	rs1800588	0.20	0.70	0.78
LIPC	rs6082	0.09	0.55	0.19
LIPC	rs690	0.40	0.80	0.65
LIPE	rs1206034	0.36	0.11	0.02
LPA	rs1853021	CALL	CALL	CALL
LPL	rs1059507	0.16	0.28	0.70
LPL	rs1801177	CALL	CALL	CALL
LPL	rs268	0.02	NA	0.31
LPL	rs3208305	0.30	0.44	NA
LPL	rs328	0.11	0.16	0.28
LSS	rs2254524	CALL	CALL	CALL
MAP2K1	rs17586159	0.02	NA	0.68
MAPK14	rs6457878	0.10	0.62	0.74
MAPK3	rs7698	CALL	CALL	CALL
MBTPS1	rs3759972	0.30	0.12	0.29
MBTPS2[x]	rs3213451[m]	0.34	0.26	0.76
MBTPS2[x]	rs3213451[v]	0.34	0.14	0.12
ME1	rs1144181	0.31	0.47	0.47
MEF2C	rs12521662	0.13	0.33	0.24
MEF2C	rs244760	0.23	0.61	0.88
MEF2C	rs2457979	0.39	0.38	0.70
MEF2D	rs1925950	0.32	0.83	0.87
MLYCD	rs11649200	0.17	0.48	0.96
MMP9	rs2664538	0.37	0.91	0.90
MSR1	rs3747531	0.04	0.96	0.78
MSR1	rs433235	CALL	CALL	CALL
MTP	rs1800591	0.25	HC	HC
MTP	rs3816873	0.24	0.47	0.32
MVD	rs8854	0.10	0.79	0.66
MVK	rs7957619	0.09	0.12	0.22
MYBBP1A	rs751670	0.16	NA	NA
NCOA1	rs11125744	0.10	0.99	0.71
NCOA1	rs17737058	0.24	0.65	0.63
NCOA1	rs3731628	0.05	0.97	0.87
NCOA2	rs10112498	0.39	0.007	0.36
NCOA2	rs3088092	0.08	0.08	0.51
NCOA3	rs2076546	0.08	0.27	0.21
NCOA3	rs2230782	0.08	0.93	0.95
NCOA4	rs10761618	0.28	0.60	0.84
NCOA6	rs3787220	0.18	0.92	0.25

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
NCOA6	rs6088618	0.48	NA	NA
NCOR1	rs178802	0.47	0.09	0.16
NCOR2	rs2229840	0.17	0.05	0.17
NCOR2	rs2272368	0.14	0.38	0.37
NDN	rs850791	0.09	0.65	0.72
NFKB1	rs170731	0.33	HC	HC
NFKB1	rs1801	0.36	0.74	0.94
NFKB2	rs1056890	0.36	0.54	0.14
NFKBIA	rs11569620	0.001	MAF	MAF
NFKBIA	rs2233409	0.25	0.57	0.64
NOS2A	rs1060826	0.42	0.84	0.67
NOS2A	rs2297518	0.19	0.13	0.55
NOS3	rs1799983	0.30	NA	0.37
NOS3	rs2070744	0.38	NA	0.80
NPPA	rs5063	0.04	0.72	0.46
NPPB	rs198389	0.43	0.82	0.31
NR0B2	rs6659176	0.08	0.92	0.76
NR1H2	rs1405655	0.32	0.71	NA
NR1H2	rs17373080	0.30	HC	HC
NR1H2	rs2248949	CALL	CALL	CALL
NR1H2	rs4802703	CALL	CALL	CALL
NR1H3	rs12221497	0.17	0.58	0.21
NR1H3	rs1449627	0.36	0.13	0.90
NR1H3	rs2279238	CALL	CALL	CALL
NR1H4	rs10860603	0.14	0.0003	0.002
NR1H4	rs35724	0.38	0.04	0.04
NR1H4	rs4764980	0.48	0.46	0.92
NR3C1	rs6190	CALL	CALL	CALL
NR3C1	rs6191	0.47	0.87	0.46
NR3C1	rs6196	0.14	0.53	NA
NRF1	rs1882094	0.29	0.27	0.29
NRIP1	rs2229741	0.41	0.19	0.74
NRIP1	rs2229742	0.12	NA	0.46
NSDHL[x]	rs5969919[m]	0.15	0.19	0.16
NSDHL[x]	rs5969919[v]	0.15	0.52	0.09
PBEF1	rs9770242	0.25	0.14	0.50
PCAF	rs3021408	0.42	0.43	0.89
PCK1	rs2071023	0.45	0.49	0.10
PCK1	rs707555	0.15	0.48	0.26
PCK2	rs2759409	0.22	0.74	0.89
PDK4	rs6931	0.41	0.15	0.33
PDPK1	rs1005273	0.43	0.80	0.66
PIAS1	rs1489599	0.43	0.30	0.22
PIK3CA	rs7614305	0.18	0.56	0.87

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
PIK3R1	rs706713	0.23	0.54	0.40
PIK3R2	rs613339	0.05	0.57	0.34
PLA2G7	rs1051931	0.19	0.92	0.61
PLTP	rs394643	0.45	0.92	0.60
PLTP	rs553359	0.36	0.51	0.59
PMVK	rs1891805	0.07	0.79	0.32
PPARA	rs1055659	0.19	HC	HC
PPARA	rs135539	0.43	0.71	0.79
PPARA	rs1800206	0.07	0.97	0.91
PPARA	rs4253655	0.18	0.55	NA
PPARA	rs4253778	0.19	HC	HC
PPARA	rs6008259	0.20	0.91	1.00
PPARBP	rs17634758	0.01	MAF	MAF
PPARBP	rs4795367	0.16	0.43	0.23
PPARBP	rs7501488	0.26	0.92	0.74
PPARD	TCCAGTGGAC[C/T] TAGCACTGGG‡	0.06	0.67	0.23
PPARD	rs1053049	0.24	HC	HC
PPARD	rs2016520	0.19	0.18	0.63
PPARD	rs2076167	0.24	0.60	0.62
PPARG	rs10865710	0.25	0.49	0.28
PPARG	rs1801282	0.12	0.59	0.82
PPARG	rs3856806	0.12	0.46	0.53
PPARG	rs709158	0.33	0.13	0.16
PPARG	rs880663	0.25	0.50	0.24
PPARGC1A	rs2932976	0.25	0.40	0.55
PPARGC1A	rs2970847	0.22	0.94	0.98
PPARGC1A	rs2970869	0.20	0.12	NA
PPARGC1A	rs2970870	0.39	0.64	0.15
PPARGC1A	rs3755863	0.39	0.35	0.82
PPARGC1A	rs3796407	0.25	0.23	0.24
PPARGC1A	rs8192678	0.33	HC	HC
PPARGC1B	rs1076064	0.37	0.71	NA
PPARGC1B	rs11959820	0.03	0.38	0.65
PPARGC1B	rs32588	0.14	0.79	0.99
PPARGC1B	rs7732671	0.08	0.04	0.01
PPP3CA	rs3804404	0.24	0.08	0.08
PPRC1	rs2815402	0.41	0.61	0.59
PRKACA	rs729372	0.11	0.49	0.61
PRKCA	rs17633437	0.37	HC	HC
PRKCA	rs7210446	0.40	0.23	0.09
PTEN	rs2735343	0.36	0.60	0.97
PTGS2	rs20417	0.14	0.66	0.49
PTGS2	rs5272	CALL	CALL	CALL

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
PTGS2	rs5275	0.31	0.21	0.16
PTGS2	rs5277	0.17	0.19	0.58
PTPN1	rs718630	0.41	0.94	0.73
PTPN11	rs11066322	0.19	0.85	0.55
RAF1	rs6795441	0.47	0.60	0.83
RAPGEF1	rs7854489	0.33	0.08	0.74
RARA	rs7217852	0.13	0.40	0.35
REL	rs10203477	0.39	0.12	0.01
RELA	rs7101916	0.13	0.37	0.20
RELB	rs10856	0.07	0.41	0.37
RETN	rs1862513	0.31	HC	HC
RETN	rs3745367	0.24	0.33	0.42
RHOQ	rs1451152	0.35	0.08	0.25
RPS6KB1	rs1051424	0.21	0.35	0.14
RXRA	rs1805348	0.018	MAF	MAF
RXRA	rs1805352	0.31	HC	HC
RXRA	rs3118571	0.34	0.61	0.63
RXRA	rs3132300	0.18	0.47	0.51
RXRB	rs1547387	0.11	0.82	0.61
RXRB	rs6531	0.31	0.40	0.17
RXRG	rs10918169	0.18	HC	HC
RXRG	rs10918172	0.17	0.21	0.35
RXRG	rs3818569	0.38	0.02	0.09
SAH	rs5716	0.08	0.13	0.81
SC4MOL	rs17585739	0.06	0.37	0.98
SC5DL	rs1061332	0.29	0.81	0.97
SCAND1	rs6060717	0.20	0.86	0.64
SCAP[i]	rs6800271[m]	0.43	0.75	0.76
SCAP[i]	rs6800271[v]	0.43	0.0006	0.76
SCARB1	AGGGTGGGCC[C/T] GGCCATGGCT[‡]	0.08	0.31	NA
SCARB1	rs5888	0.47	0.83	NA
SCD	rs11598233	0.40	0.57	0.59
SCNN1A	rs2228576	CALL	CALL	CALL
SCNN1A	rs3759324	0.25	0.47	0.90
SHC1	rs4845401	0.40	0.33	0.73
SIRT1	rs2273773	0.09	0.001	NA
SIRT1	rs7069102	0.36	0.63	0.65
SLC27A1	rs3746318	CALL	CALL	CALL
SLC2A4	rs5435	CALL	CALL	CALL
SLC8A1	rs10187203	0.48	0.03	0.14
SMARCA2[i]	rs17712152[m]	0.05	0.76	0.87
SMARCA2[i]	rs17712152[v]	0.05	0.001	0.0002
SMARCA4	rs11085756	0.31	0.19	0.03

Gene	SNP	Minor allele frequency	*P*-value	
			BMI	Waist
SNX26	rs231228	0.20	0.27	0.08
SOCS3	rs4969170	0.34	0.05	0.49
SORBS1	rs2274490	0.37	NOHWE	NOHWE
SORBS1	rs2281939	0.04	0.42	0.84
SOS1	rs1059310	0.37	0.54	0.20
SP1	rs3741651	0.19	0.99	0.58
SPP1	rs1126616	0.27	0.55	0.35
SPP1	rs1126772	0.22	HC	HC
SQLE	rs966946	0.34	0.06	0.006
SREBF1	rs11868035	0.30	HC	HC
SREBF1	rs4925119	0.35	HC	HC
SREBF1	rs8066560	0.35	0.42	0.45
SREBF2	rs2229442	0.08	0.20	0.75
SREBF2	rs2269657	0.23	HC	HC
SREBF2	rs4822063	0.24	0.90	0.37
SREBF2	rs6002527	0.08	HC	HC
STAT1	rs2066802	0.05	0.35	0.44
STAT5A	rs2293158	0.27	1.00	0.98
STAT5B	rs6503691	0.10	0.96	0.56
SUMO1	rs6755690	0.37	0.63	0.66
TFAM	rs1937	CALL	CALL	CALL
TGS1	rs7823773	0.12	0.08	0.02
THRB[i]	rs1394761[m]	0.37	0.91	0.19
THRB[i]	rs1394761[v]	0.37	0.006	0.19
TM7SF2	rs1546532	0.07	0.70	0.49
TNF	rs1799724	0.09	0.36	0.52
TNF	rs1800629	0.18	0.87	0.64
TRIB3	rs6115830	0.42	0.09	0.08
UBE2I	rs8063	0.29	0.62	NA
UCP1	AACAATCACC[G/A] CTGTGGTAAA[‡]	0.09	0.88	0.66
UCP1	rs1800592	0.26	0.55	0.35
UCP1	rs2270565	CALL	CALL	CALL
UCP2	rs659366	0.38	0.73	0.72
UCP2	rs660339	0.42	HC	HC
UCP3	rs1800849	0.24	0.04	0.13
UCP3	rs2075577	0.45	0.87	NA
USF1	rs3737787	0.27	0.12	0.76
Y-marker	rs3899	Y marker	Y marker	Y marker

NA = SNP did not fulfil the assumption of similar change in BMI or waist over time between the genotypes and is therefore excluded from this analyses; CALL = unsuccessful genotype calling; HC = SNP is highly correlated (r^2 >0.8) with another SNP in the same gene, which is also included in this analysis; NOHWE = SNP is out of HWE and verification of genotyping showed different results; MAF = SNP is excluded from analysis because minor allele frequency < 0.02; x = gene is located on X chromosome, results are presented for men and women separately; i = interaction between gender and SNP for BMI and/or waist, results are presented for men and women separately; m = results among men; v = results among women; ‡ rs number does not exist or is no longer available in NCBI, SNP coordinate is given.

Chapter 4

Maternal fish consumption during pregnancy and BMI in children from birth up to age 14 years: the PIAMA cohort study

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Abstract

Purpose: This study aimed to investigate the association between maternal fish consumption during pregnancy and BMI in children and the development of this association between birth and 14 years of age, taking into account relevant mother and child covariates.

Methods: The study population consisted of 3684 Dutch children born in 1996–1997 who participated in the PIAMA birth cohort study. Maternal fish consumption during pregnancy and the child's body weight and height (up to 11 times) were reported by questionnaire. Generalized estimating equations were used to investigate whether BMI of children differed according to maternal fish consumption during pregnancy.

Results: The crude overall association between maternal fish consumption during pregnancy and BMI in children was non-significant (P = 0.17), but differed by the child's age (P interaction = 0.03). Children of mothers who consumed fish ≥1×/week during pregnancy (n = 909) had statistically significant lower mean BMI z scores than children of mothers who never consumed fish (n = 1025) at the ages 4, 7, 8.5, and 11.5 years. Adjustment for maternal covariates (particularly pre-pregnancy BMI) attenuated the differences, which remained statistically significant at the age of 7 years only (mean difference in BMI z score: –0.14 95% CI –0.25; –0.03). Additional adjustment for child covariates hardly affected the results.

Conclusions: In a population with relatively low fish consumption, higher fish consumption by pregnant women seems rather an indicator for more healthy maternal characteristics in general than a causal factor for the lower BMI in their children.

Introduction

There is evidence that in utero exposure to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) may have a beneficial effect on early adipose tissue development [1, 2]. These n-3 long-chain polyunsaturated fatty acids (LCPUFA) are thought to have an anti-obesogenic effect by blocking the maturation of adipocytes and so may lower risk of obesity in later life [3]. Fish is an important dietary source of EPA and DHA [4]. This suggests that fish consumption during pregnancy may beneficially affect children's body mass index (BMI) later in life. If this turns out to be true, this may influence dietary guidelines to prevent obesity in children already during pregnancy [5]. However, several issues need to be further investigated.

To the best of our knowledge, so far, only Donahue et al. [6] investigated the association of maternal fish consumption during pregnancy and adiposity in children. They found that higher consumption of fish (expressed in servings per week) by pregnant women (n = 1120) at 29 week of gestation was associated with a 23% (95% CI 5–38) lower risk of obesity among their children at 3 years of age [6]. The association with BMI z score as continuous outcome was non-significant [mean SDS –0.03 (95% CI –0.09; 0.03)]. Further studies are needed to elucidate this finding. In addition, insight is needed whether maternal fish consumption during pregnancy only affects the child's body weight in the first years of life or whether the association persists throughout childhood. Prospective studies including repeated BMI measures at different ages could provide more insight into the development of the association over time [7]. In addition, such a study design reduces the risk of chance findings as compared to studies, which include outcome data observed at one specific time point only. Another issue that needs to be clarified is whether maternal fish consumption during pregnancy may be an indicator for other healthy characteristics rather than a causal factor in decreasing the risk of obesity. Fish consumption during pregnancy may reflect a healthy lifestyle of the mother and also of her child later in life [8]. Adjustment for relevant maternal and child covariates can give more insight into the impact of those factors on the association. In the study of Donahue et al. [6], which included many maternal and child characteristics, adjustment hardly affected the association. This suggests that a higher consumption of fish by pregnant women was responsible for the observed lower obesity risk in early childhood.

The aim of the current study is to investigate the association between maternal fish consumption during pregnancy and BMI in children measured 11 times in the PIAMA birth cohort study, including 3684 mother–child pairs. We additionally aimed to investigate the development of the association by the age of the child between birth and 14 years of age. Finally, we aimed to investigate the impact of other relevant maternal and child characteristics on the observed association, if any.

Materials and methods

Study design and study population

The Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study started with 3963 children born in 1996–1997. In this study, mothers were recruited from the general population during pregnancy (*n* = 4146). A total of 183 (4.5%) women were lost to follow-up before any data on the child had been collected. The study protocol was approved by the medical ethics committees of the participating institutes, and all parents gave written informed consent. A detailed description of the study design has been published previously [9]. Questionnaires were completed by the participating parents during pregnancy (95% in third trimester), 3 months after the child was born, yearly from 1 to 8 years of age and at 11 and 14 years of age. The number of parents who completed the questionnaires ranged from 3934 when the children were 3 months old (95% of the baseline study population) to 2337 when the children were 14 years old (56% of the baseline study population). From each multiple birth (*n* = 18 in this study), only one child was randomly selected to be part of PIAMA birth cohort. For the current study, we excluded children who were born preterm (<37 weeks of gestation, *n* = 190) or who had no information on maternal fish consumption during pregnancy (*n* = 40) or had missing information on all 11 BMI measurements (*n* = 49). This resulted in a study population of 3684 pairs eligible for analysis.

Data collection

We obtained questionnaire information during 11 waves of follow-up. Maternal diet during pregnancy was ascertained through a short food frequency questionnaire (FFQ) consisting of eight food items. Expectant mothers were asked how often they consumed vegetables, fresh fruit, fish, eggs, milk, milk products, nuts, and nut products during the last month. Answering options were (1) never, (2) 1–3 times a month, (3) once a week, (4) 2–4 times a week, (5) more than 4 times a week, (6) once a day, and (7) several times per day. Furthermore, information on maternal demographic characteristics (education, employment, household composition), anthropometric characteristics (body weight before pregnancy, height), and lifestyle factors [smoking during pregnancy, breast-feeding duration (weeks)], sex of the child, and date of delivery was obtained. Data on the child's weight and height were reported at each wave of follow-up. Parents were asked to copy their child's weight and height data and the date of measurement from their child's medical record. When data from medical records were not available, parents were asked to measure their child's weight and height without shoes and heavy clothing themselves and report those together with the date of measurement.

The questionnaire at 7 years of age contained a FFQ asking the parents how often in the previous month their child consumed a certain food or drink (47 food items queried such as

soft drinks, candy bars, fried snacks or chips, crisps or salty snacks, vegetables, fruit, fish). The questionnaire at 11 years of age contained more detailed questions on the use of sugar-containing beverages (soft drinks, fruit juices, milk drinks, energy drinks, and sport drinks) and snacks, asking the child how many times per week and how much a day he or she consumed such foods (18 food items, but not fish). In addition, the child was asked to report the number of hours a day he or she watched television, watched videos, or used the computer and how many times per week he or she practiced an active sport.

Definition of exposure

Maternal fish consumption was regrouped into three categories: never (n = 1025), 1–3 times a month (n = 1750), and once a week or more (n = 909). As only 132 women reported to consume fish more than once a week, it was not possible to make additional categories of maternal fish consumption. Because the short FFQ was not validated, we assessed the quality of the reported fish consumption data in a subsample of 276 women. We compared the reported fish consumption with measured EPA and DHA concentrations [10] in breast milk. Women who reported to eat fish once a week or more had the highest sum of EPA and DHA concentrations in their breast milk, while women who consumed no fish at all had the lowest concentrations ($P < 0.0001$).

Definition of outcome

Although each questionnaire was sent around a child's date of birth, the exact ages of the children at the time of height and weight measurement differed. To facilitate the longitudinal data analysis and interpretation of the results, all reported weight and height data were therefore combined and regrouped by the age of the child at the time of measurement (range 0.1 months–16.4 years). One-year age categories were constructed with round years as midpoint and 6-month boundaries before and after the midpoint (except the first category, which ranged from 0 to 0.5 years). For the analysis, ages 8 and 9, 11 and 12, and 14 and 15 years were combined to retain sufficient numbers per age category ($n \approx 2000$). This resulted in the following 11 age categories: (1) 0–0.5 years, (2) 0.5–1.5 years, (3) 1.5–2.5 years, (4) 2.5–3.5 years, (5) 3.5–4.5 years, (6) 4.5–5.5 years, (7) 5.5–6.5 years, (8) 6.5–7.5 years, (9) 7.5–9.5 years, (10) 10.5–12.5 years, and (11) 13.5–15.5 years. A total of 891 and 6 children, respectively, had two and three BMI measurements within one age category. The measurement that was closest to the midpoint of the age category was included in the present analysis. Furthermore, we deleted some BMI measurements which were outside the predefined age categories (n = 126; 9.5–10.5 and 12.5–13.5 years). The number of available BMI measurement per child ranged from 1 to 11, with a median of 8. For 90% of the children, four or more BMI measurements were available. BMI z scores were calculated

using reference data from the 1997 Dutch Growth Study [11]. BMI *z* scores represent the deviation in measured BMI from the mean BMI of the general population of children of the same sex and age (months) in units of the population standard deviation.

Table 4.1 Maternal and child characteristics according to maternal fish consumption during pregnancy.

	Maternal fish consumption during pregnancy				
	Never (n=1025)	1-3 times a month (n=1750)	Once a week or more (n=909)	*P* value[a]	# Missing values
Maternal characteristics					
Demographic					
Age at child birth (yrs)	29.6±3.7	30.5±3.9	31.0±4.0	<0.0001	25
Number of older children in household (%)				0.0008	0
0	46.8	52.2	44.8		
1-2	48.4	44.8	50.8		
>3	4.8	3.0	4.4		
Education (%)				<0.0001	112
low	31.5	19.2	20.7		
medium	43.1	42.7	38.6		
high	25.5	38.1	40.7		
Employment (% yes)	59.3	67.7	66.9	<0.0001	199
Anthropometry before pregnancy					
BMI (kg/m^2)[b]	23.2±3.9	22.8±3.2	22.7±3.2	0.17	430
BMI classification (%)[c]				0.03	430
Normal weight	77.1	81.1	82.6		
Overweight	17.6	15.4	13.8		
Obese	5.3	3.5	3.6		
Height (cm)	170.4±6.3	170.9±6.4	170.0±6.3	0.008	324
Lifestyle					
Fruit consumption during pregnancy (%)				<0.0001	6
less than once a day	31.7	20.4	17.9		
once a day	25.8	26.3	25.3		
more than once a day	42.5	53.3	56.7		
Vegetables consumption during pregnancy (%)				<0.0001	3
less than 5 times a week	21.6	11.9	9.5		
5-6 times per week	32.9	31.7	22.4		
once a day or more	45.6	56.4	68.1		
Smoking during pregnancy (% yes)	22.7	15.6	16.2	<0.0001	28
Breastfeeding duration (wks)[b]	13.0±14.5	15.6±14.9	16.7±15.6	<0.0001	49

	Maternal fish consumption during pregnancy				
	Never (n=1025)	1-3 times a month (n=1750)	Once a week or more (n=909)	*P* value[a]	# Missing values
Child characteristics					
Birth					
% girls	46.5	48.3	50.3	0.26	0
Birth weight (g)	3545±510	3578±482	3535±470	0.06	0
Gestational age (weeks)	40.1±1.2	40.1±1.2	40.0±1.2	0.63	19
At 7-years of age (n=3173)					
Fish consumption (range 1-5)[b,d]	2.0±0.7	2.3±0.7	2.6±0.7	<0.0001	546
Fruit and vegetable consumption (range 3-15)[b,d,e]	10.8±1.9	11.4±1.7	11.6±1.9	<0.0001	534
Snacks consumption (range 4-20)[b,d,f]	10.6±2.3	10.3±2.3	10.2±2.4	0.0007	520
At 11-years of age (n=2504)					
Sugar containing drinks (glasses/ week)[b,g]	19.2±8.5	17.9±7.6	18.7±9.6	0.03	1248
Screen time (hrs/wk)[b,h]	13.2±8.5	12.8±7.8	13.1±8.2	0.82	1205
Practices active sport (%)					
Once a week or less	28.0	22.9	18.8	0.003	1199
2 times a week	22.4	24.8	24.3		
3 times a week	49.6	52.3	56.9		

The PIAMA birth cohort study (n = 3684).
Values are mean±sd, unless otherwise stated.
[a] ANOVA for normally distributed variables; Kruskal-Wallis test for variables with skewed distributions; Chi-square test for discrete variables.
[b] Variable has skewed distribution.
[c] Normal weight is defined as a BMI < 25 kg/m², overweight as a BMI ≥25 and <30 kg/m², and obesity as a BMI ≥30 kg/m².
[d] Answer options ranged from 1 to 5 and were 1) never, 2) less than once a week, 3) 1 to 2 days a week, 4) 3 to 5 days a week and 5) 6 to 7 days per week.
[e] Represents the sum of three variables: consumption of 1) uncooked vegetables, 2) cooked vegetables, and 3) fruit. The combined score ranged from 3 – 15.
[f] Represents the sum of four variables: candy bars, fried snacks or chips, crisps or salty snacks, The combined score ranged from 4 to 20.
[g] Includes consumption of soft drinks, fruit juices, milk drinks, energy drinks and sport drinks.
[h] Includes computer screen time as well as TV screen time.

Covariates

All maternal and child demographic and lifestyle factors that have been associated with obesity based on literature and for which information was collected in this study (see table 4.1) were considered as covariates. Only covariates that were also associated with maternal fish consumption during pregnancy were considered as confounder.

Data analysis

To identify factors that could explain a possible association between maternal fish consumption during pregnancy and BMI in children, we evaluated differences in the selected maternal and child characteristics (*see covariates*) between maternal fish consumption groups. This was done by oneway ANOVA for normally distributed covariates, by the Kruskal–Wallis test for covariates with skewed distributions and by the Chi-square test for discrete covariates. Generalized estimating equations (GEEs) were used to study the association between maternal fish consumption and BMI in children, taking into account the correlation between the repeated measures in the same child. A ten-dependent working correlation structure fitted the data best. BMI *z* scores showed a normal distribution and were used as a continuous dependent variable. Maternal fish consumption during pregnancy (three groups, never consumers as reference) was used as independent categorical variable. Mean BMI *z* scores of the two groups of children whose mothers consumed fish during pregnancy were compared with the mean BMI *z* scores of children of mothers who never consumed fish during pregnancy (reference group). Crude models are presented as model 1. Characteristics of the mother that differed by maternal fish consumption groups based on univariate analysis (see table 4.1) were added to model 2 to investigate whether those factors could explain a possible association. Model 3 additionally included child factors that were associated with maternal fish consumption. Adjustment for covariates obtained at 11 years of age did not alter the magnitude of the effect size (data not shown), but substantially lowered the numbers available for the analysis (see table 4.1). Therefore, these covariates were not added to model 3. To investigate whether the association between BMI in children and maternal fish consumption during pregnancy differed by the child's age, an interaction term between maternal fish consumption and age of the child at the time of measurement (11 groups) was added to the model. Mean BMI *z* scores of the two groups with mothers that consumed fish were compared to the reference group in each age category.

We performed several sensitivity analyses to evaluate the robustness of our results. First, the analysis was restricted to children with complete information on covariates only (n = 3179) to investigate the effect of missing covariate information on the association. Second, the analysis was restricted to children with more than seven BMI measurements (n = 2321) to get more insight in the effect of incomplete follow-up. Data were analyzed using SAS software version 9.3 (SAS Institute, Inc., Cary, NC). P values of less than 0.05 were considered statistically significant.

Results

Women who never consumed fish during pregnancy were younger and more often overweight or obese before pregnancy compared to the other maternal fish consumption groups (table 4.1). In addition, women who never consumed fish were less educated, less employed, smoked more often during pregnancy and consumed less fruit and vegetables during pregnancy. Furthermore, children of mothers who never consumed fish during pregnancy were breast-fed for a shorter period of time. In addition, like their mothers, those children had a less healthy lifestyle. They consumed less fruit and vegetables, more snacks and soft drinks, and they practiced active sports less often than the other children.

The crude overall association between maternal fish consumption during pregnancy and repeated BMI *z* scores in children was non-significant (*P* = 0.17, table 4.2). However, the crude association between BMI *z* scores in children and maternal fish consumption during pregnancy differed by the child's age (*P* interaction: 0.03 Fig. 4.1). Children of mothers who consumed fish once a week or more during pregnancy had a statistically significant lower mean BMI *z* score (0.14–0.17) at the ages of 4, 7, 8.5, and 11.5 years compared to the reference group. No statistically significant differences in BMI were found between children of mothers who consumed fish 1–3 times a month during pregnancy and the reference group (except at the age of 4 years) in crude analyses.

Table 4.2 Overall association between maternal fish consumption during pregnancy and BMI z-scores of children between birth and age 14 years.

Model	N	Maternal fish consumption during pregnancy					P value[c]
		Never	1-3 times a month		Once a week or more		
			Δ mean BMI z score	95% CI	Δ mean BMI z score	95% CI	
1: Crude	27678	*Ref*	-0.013	-0.073; 0.047	-0.063	-0.134; 0.008	0.17
2: Maternal factors[a]	24928	*Ref*	-0.000	-0.062; 0.062	-0.029	-0.104; 0.047	0.66
3: Maternal+child factors[b]	23008	*Ref*	0.010	-0.058; 0.077	-0.027	-0.112; 0.057	0.59

Results are obtained by Generalised Estimating Equations analyses with a 10 dependent working correlation structure, taking into account the correlation between up to 11 repeated measures in the same child.
[a] *Model adjusted for maternal highest attained education, age, pre-pregnancy BMI and height, fruit and vegetables consumption during pregnancy, number of older children in the household and duration of breastfeeding (weeks).*
[b] *Model 2 additionally adjusted for child's consumption of fruit, vegetables, fish and snacks.*
[c] *P-value for overall difference in BMI z-score between maternal fish consumption groups.*

After adjustment for maternal covariates, the overall association became weaker (*P* = 0.66, table 4.2). Additional adjustment for child covariates hardly affected the association. The interaction term between maternal fish consumption and age of the child at the time of measurement, however, remained statistically significant (*P* = 0.01). Nevertheless, the observed difference in children's mean BMI *z* scores became smaller. This was mainly due to adjustment for maternal pre-pregnancy BMI (Fig. 4.2). Only at the age of 7 years, children of mothers who consumed fish once a week or more still had a statistically significant lower BMI *z* score (–0.14 95% CI –0.25; –0.03) compared to the reference group. Additional adjustment for child covariates did not considerably affect the results (Fig. 4.3).

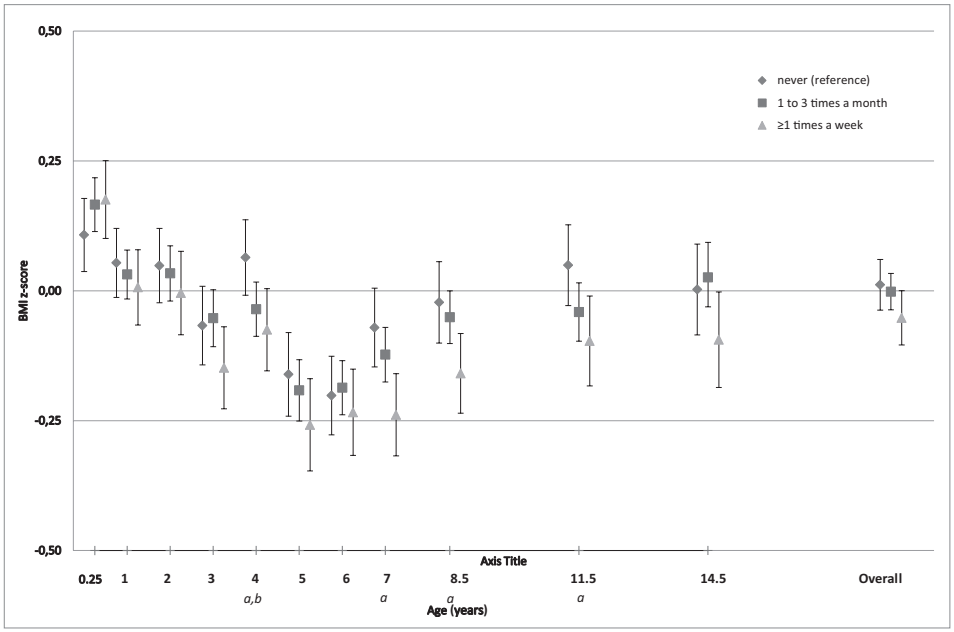

Figure 4.1 Crude association between maternal fish consumption during pregnancy and BMI z scores in children, overall and by age, the PIAMA birth cohort study.

P=0.03 for interaction between maternal fish consumption and age category.
a: P<0.05 for difference in mean BMI z score between children of mothers who consumed fish once a week or more compared to reference children.
b: P<0.05 for difference in mean BMI z score between children of mothers who consumed fish 1-3 times a month compared to reference children.

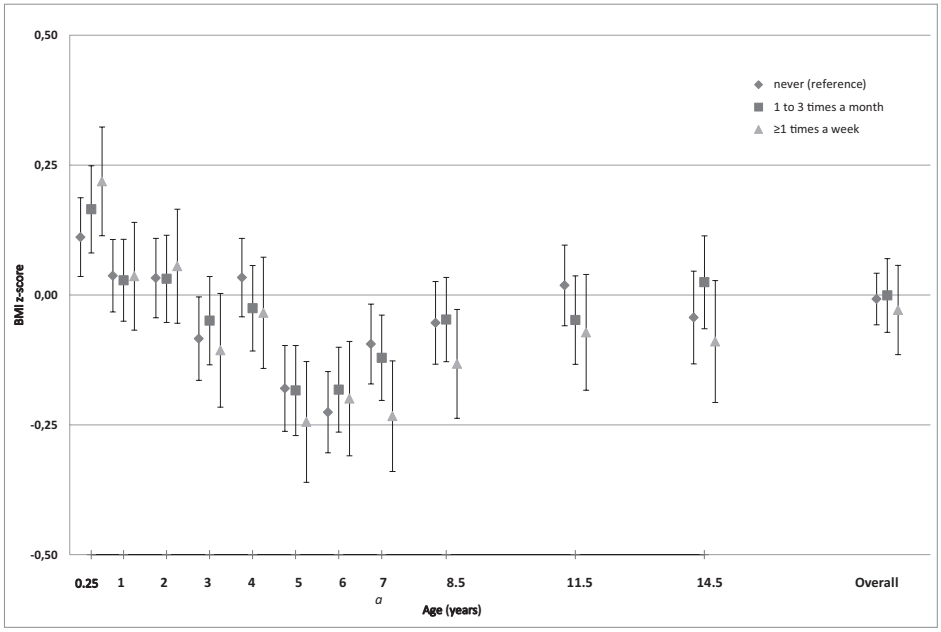

Figure 4.2 Adjusted association between maternal fish consumption during pregnancy and BMI z scores in children overall and by age, the PIAMA birth cohort study.

Adjusted for maternal pre-pregnancy BMI, P=0.04 for interaction between maternal fish consumption and age category.
a: P<0.05 for difference in mean BMI z-score between children of mothers who consumed fish once a week or more compared to reference children.

Mothers of children with incomplete follow-up more often smoked during pregnancy, were lower educated, less employed, and were more often coming from the lowest and highest maternal fish consumption category. In the analysis among children with complete information on covariates, differences in the crude mean BMI *z* scores between children of mothers who consumed fish once a week or more and reference children became somewhat smaller and borderline statistical significant at the ages of 4, 8.5, and 11.5 years (data not shown). However, at the age of 7 years, crude differences in mean BMI *z* scores were similar for the complete and incomplete case analysis. Restricting the adjusted analysis to children with more than seven BMI measurements resulted in similar or somewhat larger differences in mean BMI *z* scores. Children of mothers who consumed fish once a week or more had statistically significantly lower BMI *z* scores at the age of 4 and 7 years ($P = 0.04$ and $P < 0.01$, respectively) compared to the reference group.

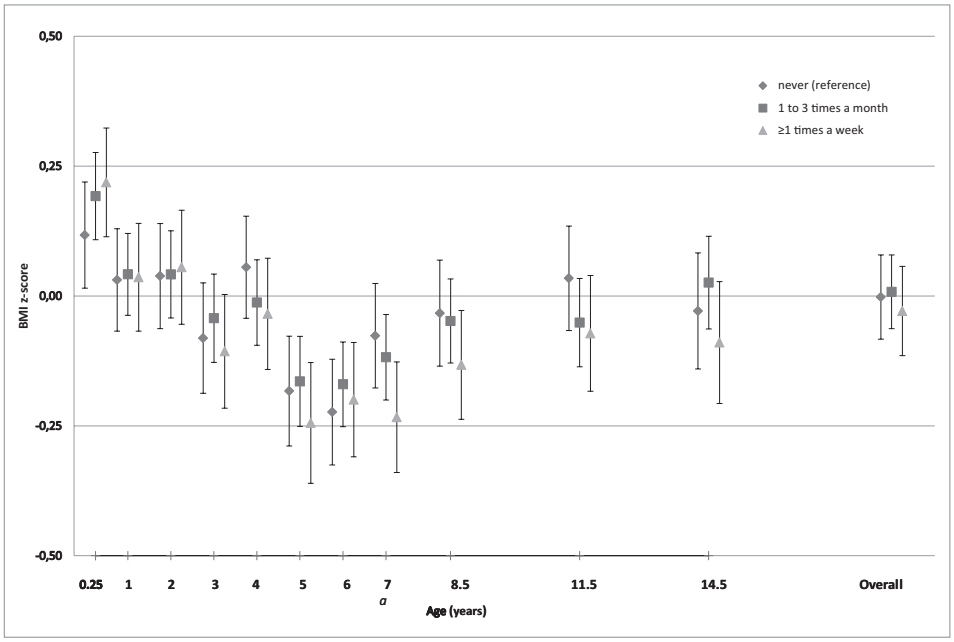

Figure 4.3 Adjusted association between maternal fish consumption during pregnancy and BMI z scores in children overall and by age, the PIAMA birth cohort study.

Adjusted for maternal highest attained education, age and height, pre-pregnancy BMI, fruit and vegetables consumption during pregnancy, number of older children in the household, and duration of breastfeeding (weeks) and child's consumption of fruit, vegetables, fish and snacks. P=0.01 for interaction between maternal fish consumption and age category.
a: P<0.05 for difference in mean BMI z-score between children of mothers who consumed fish once a week or more compared to reference children.

Discussion

This study shows that children of mothers who consumed fish once a week or more had a lower BMI at several ages between birth and 14 years of age as compared to children of mothers who never consumed fish during pregnancy. Differences in BMI were more apparent at higher ages, but could almost completely be explained by other health characteristics of the mothers, particularly pre-pregnancy BMI. Additional adjustment for child risk factors for obesity hardly affected the results.

The main strengths of this study were the large number of mother–child pairs and repeated outcome measurements over a long follow-up period. This allowed us to study the association over time in a large study population and reduce the possibility of chance findings because

BMI was based on a considerable number of measurements instead of one, which is more common. In addition, we were able to study the independent association between maternal fish intake during pregnancy by taking into account important maternal and child covariates. The self-reported information on maternal fish consumption may have led to misclassification. In a subsample of our study, reported fish consumption by pregnant women correlated well with EPA and DHA concentrations in their breast milk and the comparison gave no indication of differential misclassification. This suggests that our exposure measure was valid. In addition, maternal fish consumption was only assessed in the third trimester of pregnancy and this might not fully reflect diet during pregnancy. However, there is evidence that maternal dietary intake does not change significantly during pregnancy [12], and therefore, it is less likely that this has affected our results. Data on height and weight of the children were reported by parents. It has been shown that in the PIAMA cohort parents of children in the highest BMI quartile underreport their child's body weight by 0.5 kg on average, whereas parents of children in the lowest BMI quartile tended to overreport their child's weight [13, 14]. Under the assumption of a beneficial effect of maternal fish consumption on children's BMI, this recall bias might have resulted in an underestimation of the differences in BMI between fish consumption groups. Generally, missing data and loss to follow-up are important issues in longitudinal studies. In the current study, 17% of the observations were excluded from the multivariate analysis because of missing covariate information. Incomplete data on covariates slightly attenuated the crude differences in mean BMI *z* scores but did not affect our overall conclusion. In addition, we had incomplete follow-up data on the child's BMI which was associated with some maternal characteristics (i.e., education, fish consumption, and smoking during pregnancy). This selective lack of completeness in follow-up data has only marginally affected our results as a sensitivity analysis among a more complete and selective group (children with more than seven BMI measurements available) showed similar or somewhat larger differences in mean BMI *z* scores.

We hypothesized that maternal fish consumption could affect BMI in children through a favorable effect of EPA and DHA on early adipose tissue development [1, 3]. Our crude results indeed showed a beneficial association between weekly fish consumption during pregnancy on the child's BMI at several ages. However, higher fish consumption during pregnancy was associated with a healthier lifestyle of both mother and child. Adjustment for maternal prepregnancy BMI almost completely explained the observed crude differences in children's BMI between maternal fish consumption groups. Therefore, our results do not provide evidence for a programming effect in utero by maternal fish consumption, but underline the importance of healthy body weight and lifestyle in general of mothers to be. In general, the consumption of fish in the Netherlands is rather low and we did not have information on maternal consumption of fatty fish during pregnancy. Therefore, we cannot rule out a

possible beneficial effect on children's BMI at higher maternal fish consumption levels or for maternal consumption of fatty fish during pregnancy.

We are aware of one previous study that investigated fish consumption by 1120 pregnant women in relation to BMI in their children [6]. This study found no association between maternal fish consumption and BMI *z* scores in children at age 3 years, crude as well as after adjustment for maternal and child characteristics. This result is in line with our results based on data at age 3 years. However, in that study [6], higher maternal fish consumption was associated with a 23% lower risk of obesity among children at 3 years of age. In our study, prevalence of obesity among children was too low to study obesity as an outcome. Therefore, we cannot exclude the possibility that maternal fish consumption during pregnancy affects excess adiposity. Several studies have been conducted on n-3 LCPUFA exposure or supplementation during the prenatal period on later adiposity in children. However, results from these studies are conflicting [6, 15–19]. Differences between the studies in, for example, study design make it hard to compare the individual results. For example, each study measured adiposity of the child at a different age, ranging from several months to several years. Only two studies used repeated measures of adiposity in children, but only two or three measurements during the study, i.e., at 1, 3, and 21 months [17], and at 4 and 6 years of age [15]. Our study included up to 11 BMI measurements from birth up to the age of 14 years. We observed that the development of BMI in children over time differed between groups of maternal fish consumption during pregnancy as shown by the significant interaction term. However, at each specific age differences in BMI were small and not statistically significant after adjustment for maternal and child characteristics (except at the age of 7 years). If we had only investigated BMI at 7 years of age as outcome variable instead of using repeated BMI measurements, our conclusion would have been different. This underlines the important value of multiple measurements in research.

Conclusion

The results of the present study do not provide evidence for programming of obesity in utero by maternal consumption of fish. Maternal pre-pregnancy BMI could almost completely explain the small beneficial effect of weekly fish consumption during pregnancy on BMI in children. This suggests that maternal fish consumption is rather an indicator for more healthy maternal characteristics in general than a causal factor in lowering the risk of childhood obesity. Although we cannot completely rule out an effect of maternal fish consumption on BMI development in children, such effects, if any, are likely to be small. Future studies conducted in a population with a wide range in maternal fish consumption during pregnancy and information on the type of fish consumed (lean vs. fatty fish) are of added value.

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Conflict of interest None of the authors had a conflict of interest.

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Chapter 5

The Association between Dietary Energy Density and Type 2 Diabetes in Europe: Results from the EPIC-InterAct Study

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Abstract

Background: Observational studies implicate higher dietary energy density (DED) as a potential risk factor for weight gain and obesity. It has been hypothesized that DED may also be associated with risk of type 2 diabetes (T2D), but limited evidence exists. Therefore, we investigated the association between DED and risk of T2D in a large prospective study with heterogeneity of dietary intake.

Methodology/Principal Findings: A case-cohort study was nested within the European Prospective Investigation into Cancer (EPIC) study of 340,234 participants contributing 3.99 million person years of follow-up, identifying 12,403 incident diabetes cases and a random subcohort of 16,835 individuals from 8 European countries. DED was calculated as energy (kcal) from foods (except beverages) divided by the weight (gram) of foods estimated from dietary questionnaires. Prentice weighted Cox proportional hazard regression models were fitted by country. Risk estimates were pooled by random effects meta-analysis and heterogeneity was evaluated. Estimated mean (sd) DED was 1.5 (0.3) kcal/g among cases and subcohort members, varying across countries (range 1.4–1.7 kcal/g). After adjustment for age, sex, smoking, physical activity, alcohol intake, energy intake from beverages and misreporting of dietary intake, no association was observed between DED and T2D (HR 1.02 (95% CI: 0.93–1.13), which was consistent across countries (I^2 = 2.9%).

Conclusions/Significance: In this large European case-cohort study no association between DED of solid and semi-solid foods and risk of T2D was observed. However, despite the fact that there currently is no conclusive evidence for an association between DED and T2DM risk, choosing low energy dense foods should be promoted as they support current WHO recommendations to prevent chronic diseases.

Introduction

Over the past decades the prevalence of type 2 diabetes (T2D) has increased dramatically, and it is estimated that the global prevalence of diabetes will continue to rise, with an estimated 489 million cases in the year 2030, increased from an estimated 285 million in 2010 [1]. Major non-genetic risk factors for T2D such as obesity, smoking, physical inactivity and diets high in (saturated) fat and low in dietary fiber are potentially modifiable [2]. Targeting those risk factors by lifestyle intervention among high risk individuals has shown to be effective in the prevention of T2D [3,4,5].

The energy density of foods or diets is defined as the amount of available energy per unit weight of foods or meals (kJ/g or kcal/g) [6]. Experimental data suggest that people tend to eat a similar volume of food to feel satiated, and accordingly consumption of energy-dense foods could cause passive over-eating in terms of energy [7]. Several observational studies have observed a positive association between dietary energy density (DED) and subsequent weight or waist circumference gain [8,9,10].

As obesity is an important and well known risk factor for T2D [2], it is likely that consumption of energy dense foods might be associated with an increased risk of T2D. Besides this indirect effect, a direct effect of DED on T2D risk can also be hypothesized. High energy dense diets are associated with low diet quality characterized by, amongst other features, a higher intake of saturated fat and a higher glycemic load [10,11]. Both of these dietary factors have been associated with the development of insulin resistance [12,13]. So far, there is limited epidemiological evidence for an association between DED and risk of T2D [14] or related metabolic traits [15,16].

Our aim was to investigate the association between DED and risk of T2D. A secondary objective was to perform stratified analysis of the association between DED and T2D risk by BMI status and energy under-reporting. We had the opportunity to do this in the EPIC-InterAct project which assembled a large case-cohort study in European populations with substantial diversity of dietary intakes.

Materials and Methods

Ethics statement

This study complied with the Declaration of Helsinki. The Internal Review Board of the International Agency for Research on Cancer and the Institutional Review board of all centers, i.e., France, Heidelberg, Potsdam, Copenhagen, Aarhus, Asturias, Granada, San Sebastian, Murcia, Navarro, Cambridge, Oxford, Imperial, Florence, Milan, Ragusa, Turin,

Naples, Bilthoven, Utrecht, Malmö, and Umeä , approved the EPIC study. Written consent was obtained from each EPIC participant at enrolment into the study.

Study design

A case-cohort study was nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) [17] as part of the InterAct Project, hereafter referred to as the EPIC-InterAct Study. With the exception of Norway and Greece, all other eight EPIC countries (France, Italy, Spain, UK, the Netherlands, Germany, Sweden and Denmark) participated in the EPIC-InterAct study. An extensive description of the study design and cohort has been published elsewhere [18]. In brief, the EPIC-InterAct case-cohort study consists of 12,403 verified incident T2D cases recruited between 1991 and 1997. In addition, a center stratified random subcohort of 16,835 individuals was selected from the baseline cohort (n= 340,234; 3.99 million person years). After exclusion of 548 individuals with prevalent diabetes and 133 with unknown diabetes status, 16,154 subcohort individuals were included, of whom 778 had developed T2D between 1991 and 2007. EPIC participants without stored blood or reported diabetes status were not eligible for inclusion in the study. For the present study, participants with missing information on diet and therefore DED (n =117) and on covariates (smoking status, physical activity, BMI, energy intake from beverages; n= 364) were excluded to allow a complete case analysis. In addition, participants who did not complete the FFQ adequately, identified as the top and bottom 1% of the ratio for energy intake to estimated basal metabolic rate (EI/BMR), were excluded (n= 619). In total, 11,734 incident T2D cases and 15,434 subcohort individuals (5,825 men and 9,609 women), of whom 733 had developed incident T2D were eligible for analysis. An overlap between the case set and the sub-cohort is a design feature of a case-cohort study [19].

Assessment of T2D

A pragmatic, high sensitivity approach was used for case ascertainment with the aim of identifying all potential incident T2D cases and excluding all individuals with prevalent diabetes [18]. Prevalent cases were identified on the basis of baseline self-report of a history of diabetes, doctor diagnosed diabetes, antidiabetic drug use or evidence of diabetes, where the date of diagnosis preceded recruitment. Ascertainment of incident diabetes involved a review of the existing EPIC datasets at each center using multiple sources of evidence including self-report (self-reported history of diabetes, doctor diagnosed diabetes, anti diabetic drug use), linkage to primary care registers, secondary care registers, medication use (drug registers), hospital admissions and mortality data. Cases in Denmark and Sweden were not ascertained by self-report, but identified via local and national diabetes and pharmaceutical registers and hence all ascertained cases were considered to be verified. To increase the specificity of the

case definition for centers other than those from Denmark and Sweden, identified cases were verified with further evidence, including reviewing individual medical records in some centers. The date of diagnosis for incident cases was set as either the date of diagnosis reported by the doctor, the earliest date that diabetes was recorded in medical records, the date of inclusion into the diabetes registry, the date reported by the participant, or the date of the questionnaire in which diabetes was first reported. If the date of diagnosis could not be ascertained from any of the sources listed above, the midpoint between recruitment and censoring was used. Follow up was censored at the date of diagnosis, 31st of December 2007 or the date of death, whichever occurred first.

Dietary assessment

The assessment of diet was undertaken using a self- or interviewer-administered dietary questionnaire which was developed and validated within each country to estimate the usual individual food intakes of study participants [17,20,21]. In a previous study, DED measured by the Dutch FFQ was validated against DED derived from the weighted average of multiple 24-hour recalls in a subset of this study population [10]. Results indicated a good validity of the DED values measured by this FFQ (Spearman correlation coefficients: 0.64 in men and 0.56 in women). DED was calculated by dividing daily energy intake (kcal) from foods (solid foods and semi-solid or liquid foods such as soups) by the reported weights (g) of these foods. It was decided *a priori* to exclude caloric and non-caloric beverages (including water, tea, coffee, juices, soft drinks, alcoholic drinks and milk) from the DED calculation. The main reason was that DED calculations based on the inclusion of beverages were associated with higher day to day variation within individuals [22]. This may diminish associations when examining health outcomes [22]. Also, beverages may add more weight than energy to diets, thereby lowering individual DED values disproportionately [22]. To be able to identify under-reporters and over-reporters of diet, the ratio of energy intake versus basal metabolic rate (EI/BMR) was calculated and compared with Goldberg cut-off values [23,24]. BMR was estimated using the Schofield equation [25]. Participants with a ratio of EI/BMR below 1.14 were classified as under-reporters, those with a ratio of EI/BMR above 2.1 were defined as over-reporters, whereas all other participants were defined as plausible reporters of diet [26].

Other measurements

At baseline, information on lifestyle was collected via self-administered questionnaires included amongst others age, sex, educational level, physical activity, smoking and menopausal status. Smoking status was divided into current, former or never smoking. Women were classified as pre-, peri-, post- or surgical postmenopausal. Education level was indicated as the highest level of school achieved and participants were classified into either primary

school or less, technical-professional school, secondary school, university or higher. Physical activity level was assessed using a brief validated questionnaire covering occupational and recreational activities and participants were classified as either inactive, moderately inactive, moderately active or active [27]. Body weight, height and waist circumference were measured at baseline by trained technicians using standard study protocols as previously described [28]. BMI was calculated as body weight in kilograms divided by the square of the height in meters. Normal weight, overweight and obesity were defined as a BMI <25 kg/m^2, between 25 kg/m^2 and 30 kg/m^2 and ≥30 kg/m^2, respectively.

Statistical analyses

Characteristics of the subcohort participants at baseline were examined by quintiles of DED which were derived from the DED distribution in the overall subcohort. Means and standard deviations were presented for describing continuous data, except when the data were not normally distributed, in which case medians and interquartile ranges were given. For categorical variables percentages were presented. The DED variable had a normal distribution. Stepwise linear regression analysis was performed (significance level entry = 0.15, significance level stay = 0.15) to investigate the contribution of food groups and nutrients to the inter-individual variation in DED in two separate models. All standard derived main food groups [21], except beverages, were entered into the regression model: potatoes, vegetables, legumes, fruits, dairy products, nuts and seeds, cereals, meat, fish, eggs, fats, sugar and confectionery, cakes and biscuits, condiments and sauces, soups, and miscellaneous. Seven macronutrients: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, polysaccharides, mono-&disaccharide carbohydrates, animal protein and plant protein were also entered in a regression model. Since we used data from a subcohort instead of the full cohort, a weighted Cox regression suitable to case-cohort designs was applied to derive the hazards ratios (HRs) for incident T2D [19]. Prentice weights were used i.e. all subcohort members (cases and non-cases) are weighted equally and cases outside the subcohort are not weighted before failure [29]. At failure these cases have the same weight as the subcohort members (also called the unweighted method). Models were fitted separately for each country with time as the underlying timescale. HRs were calculated for incident T2D per 1 kcal/g increase in DED and quintiles of DED. Random effects meta-analysis was performed to pool hazard ratios across countries and evaluate heterogeneity (I^2) between countries. In addition, forest plots were generated. Age, sex, center, educational level, smoking, physical activity, alcohol (g/day), energy intake from beverages (kcal/day), family history of diabetes and misreporting of diet were considered as potential confounding variables. These variables were added one by one to the crude model. Variables that changed the β-coefficient for DED by ten percent or more were added to the multivariable models. The first model included sex and age. Additional

adjustments were subsequently made for known risk factors of diabetes: smoking status, physical activity, consumption of alcohol, energy intake from beverages (model 2). In model 3 further adjustments were made for misreporting of diet (under-, plausible and over-reporters). Additional adjustment for menopausal status was made in women only. In case of a statistically significant association between DED and incident T2D (model 3), potential mechanisms underlying the association were explored by addition of BMI, waist circumference and several dietary factors (energy intake, fiber, total fat, fruits and vegetables) one by one. Potential interactions of DED with sex, age (2 groups based on median of 54.1 years), BMI, waist circumference, physical activity and misreporting of diet were investigated by the inclusion of interaction terms to model 3. Under-reporting of diet is a known phenomenon in epidemiological studies on diet-disease relationships, especially among obese individuals [30]. To get more insight in this potential bias, characteristics of BMI status and misreporting of diet were explored by country. In addition, regression models were stratified by status of misreporting of diet (except for over reporters, due to small numbers) for normal weight, overweight and obese individuals separately. To be able to compare our results with previous related research by Wang et al [14] within the EPIC NORFOLK cohort (which is also part of the EPIC-InterAct study) and to get more insight in methodological issues regarding DED calculation, a sensitivity analysis was performed based on DED calculations including all foods and all beverages except water. Apart from the random-effect meta-analysis, which was conducted using STATA 11.0 (Stata-Corp, Texas, USA), all analyses were performed using SAS 9.2 (SAS, Institute, Cary, NC). *P* values <0.05 were considered to be statistically significant.

Table 5.1 Baseline characteristics of the subcohort (n=15,434) across quintiles of dietary energy density: the EPIC-InterAct Study.

Characteristics	Overall *n=15,434*	Q1 *n=3,035*	Q2 *n=3,189*	Q3 *n=3,134*	Q4 *n=3,059*	Q5 *n=3,017*
Energy density[1] (kcal/g)[2]	1.5 (0.3)	1.1 (0.1)	1.4 (0.1)	1.5 (0.04)	1.7 (0.1)	2.0 (0.2)
Age, years[2]	52.4 (9.1)	53.7 (8.9)	53.4 (8.9)	52.4 (9.2)	51.7 (8.9)	51.0 (9.4)
Sex (women)[3]	62.3	80.6	70.0	62.4	54.5	43.4
Education[3]						
Primary or lower	40.9	47.8	43.1	39.5	37.1	36.9
Technical/professional	23.2	18.9	22.0	23.4	25.0	27.0
Secondary school	15.1	14.6	16.0	15.9	14.9	14.3
University degree or higher	20.7	18.7	18.9	21.3	23.0	21.8
Waist circumference, cm[2]						
Men	95.1 (10.0)	96.6 (10.4)	96.3 (9.7)	95.5 (9.5)	94.6 (9.9)	94.0 (10.3)
Women	81.2 (11.2)	82.3 (11.2)	81.6 (11.0)	80.7 (11.1)	80.8 (11.2)	79.7 (11.2)
Body mass index[2], kg/m[2]	26.0 (4.2)	26.5 (4.4)	26.2 (4.3)	25.9 (4.1)	25.9 (4.1)	25.6 (4.0)
Smoking[3]						
Never	46.9	57.5	52.0	47.6	42.0	35.3
Former	27.2	26.7	25.3	28.2	28.3	27.6
Current	25.9	15.8	22.7	24.2	29.8	37.1
Physical activity index[3]						
Inactive	23.7	27.0	24.5	22.8	20.6	23.5
Moderately inactive	33.7	33.2	34.3	33.9	34.1	32.9
Moderately active	22.7	21.1	21.5	23.1	24.7	22.9
Active	20.0	18.8	19.7	20.3	20.6	20.7
Family history diabetes, yes[3]	19.1	19.6	20.0	18.7	19.9	17.6
Hypertension, yes[3]	18.9	20.3	19.5	19.2	18.5	16.7
Hyperlipidemia, yes[3]	18.6	19.0	19.7	19.1	17.3	17.4
Menopausal status, postmenopausal[3]	47.5	52.6	51.4	47.7	40.1	40.1
Hormone replacement therapy, users[3]	14.9	14.2	16.1	14.9	14.9	14.0

Characteristics	Overall *n=15,434*	Q1 *n=3,035*	Q2 *n=3,189*	Q3 *n=3,134*	Q4 *n=3,059*	Q5 *n=3,017*
Dietary intake (per day)						
Energy, kcal[2]	2137 (634)	1783 (501)	2041 (566)	2157 (602)	2278 (615)	2431 (681)
Energy from beverages, kcal[4]	239 (143; 368)	184 (108; 289)	215 (132; 334)	246 (149; 369)	269 (167; 409)	294 (185; 440)
Total foods, g[2]	1236 (381)	1407 (412)	1309 (375)	1227 (360)	1169 (337)	1068 (326)
Fat, en%[2]	34.8 (5.9)	31.9 (6.0)	33.9 (5.2)	34.9 (5.3)	35.7 (5.3)	37.8 (5.9)
Fiber, g/1000 kcal[4]	10.6 (8.8; 12.7)	13.9 (12.0; 16.0)	11.5 (10.1; 13.1)	10.4 (9.0; 11.9)	9.6 (8.2; 11.1)	8.3 (7.0; 9.8)
Alcohol, g[4]	6.4 (0.8; 17.7)	2.7 (0.0; 11.2)	5.1 (0.6; 14.3)	6.8 (1.1; 19.4)	8.7 (1.7; 23.4)	8.4 (1.7; 23.3)
Fruit and vegetables, g[4]	376 (241;554)	628 (474; 831)	472 (346; 613)	373 (265; 509)	300 (212; 407)	203 (137; 292)
Other diet related factors						
Glycemic load[4]	126 (100;157)	106 (84;133)	122 (99;149)	127 (102;158)	134 (108;165)	142 (112;179)
Glycemic index[2]	56 (3.9)	54 (4.0)	56 (3.4)	56 (3.4)	57 (3.5)	58 (3.8)
Ratio energy intake / basal metabolic rate[2]	1.4 (0.4)	1.2 (0.3)	1.4 (0.4)	1.4 (0.4)	1.5 (0.4)	1.5 (0.4)
Misreporting of diet[3,5]						
Under-reporters	27.5	44.3	28.4	25.7	21.0	18.1
Plausible reporters	67.4	54.3	68.2	69.5	72.6	72.3
Over-reporters	5.1	1.5	3.4	4.9	6.5	9.6

[1] *DED Dietary energy density based on solid foods only.*
[2] *Values are mean (SD).*
[3] *Values are percentages.*
[4] *Values are median (Q1, Q3).*
[5] *Misreporting of diet was estimated by using the ratio of reported energy intake to the predicted basal metabolic rate (EI/BMR). Individuals with an EI/BMR <1.14 were defined as under-reporters, EI/BMR >1.14 and <2.1 as plausible reporters and EI/BMR >2.1 as over-reporters.*

Results

Estimated mean DED was 1.5 kcal/g (SD 0.3) within the subcohort as well as among incident diabetes cases. The highest mean DED was observed in Germany (1.7 kcal/g) whereas the lowest DED was found in France, Spain and the UK (1.4 kcal/g). Participants with a higher DED were more often men and current smokers with a higher educational level and had a higher ratio of EI/BMR (table 5.1). Furthermore, they were younger, had a lower BMI and waist circumference and less often reported having hypertension or hyperlipidemia. Although consuming a lower amount (total grams) of foods, participants with a higher DED had higher intake of total energy, total fat, dietary fiber and energy from beverages and a lower intake of fruit and vegetables. These differences in baseline characteristics were observed for both sexes (data not shown). Of all sixteen food groups, differences in consumption of fruits explained most of the variation between DED in individuals (30%), followed by vegetables (12%), fats (11%) and, cakes and biscuits (6%) (table 5.2). Of all macronutrients, saturated fat explained most of the variation in DED (20%), followed by mono and disaccharide carbohydrates (9%) and plant protein (8%). DED was inversely associated with consumption of fruits and vegetables and intake of mono- and disaccharide carbohydrates, animal protein and plant protein but positively associated with consumption of fats, cakes and biscuits, and intake of saturated fat.

Table 5.2 Relationships of food groups and macronutrients with dietary energy density (DED; kcal/g) in the subcohort (n=15,434): the EPIC-InterAct Study.

	Correlation with DED[3]	Linear relationship with DED[1]		
		β[4]	Partial R^2[5]	Cumulative R^2[6]
Food groups[2]				
Fruits	-0.59	-0.09	0.30	0.30
Vegetables	-0.48	-0.12	0.12	0.42
Fats	0.32	0.57	0.11	0.53
Cakes and biscuits	0.27	0.19	0.06	0.58
Meat and meat products	0.23	0.11	0.03	0.61
Soups and bouillon	-0.16	-0.08	0.03	0.64
Potatoes and other tubers	0.11	0.03	0.02	0.66
Dairy (excl milk beverages)	-0.09	-0.04	0.02	0.68
Cereal and cereal products	0.21	0.05	0.02	0.70
Nuts and seeds	0.11	0.30	0.02	0.72
Sugar and confectionary	0.34	0.13	0.01	0.73

	Correlation with DED[3]	Linear relationship with DED[1]		
		β[8]	Partial R^2[5]	Cumulative R^2[6]
Macronutrients[7]				
Saturated fat	0.42	0.04	0.20	0.20
Mono- & disaccharides	-0.34	-0.02	0.09	0.29
Plant protein	-0.23	-0.02	0.08	0.37
Animal protein	-0.31	-0.07	0.06	0.43

[1] Results obtained from stepwise linear regression analyses among the subcohort.
[2] Sixteen food group variables (grams per day) were included: potatoes, vegetables, legumes, fruits, dairy products (except milk beverages), nuts and seeds, cereals, meat, fish, eggs, fats, sugar and confectionery, cakes and biscuits, condiments and sauces, soups, and miscellaneous.
[3] Spearman's rank correlation coefficient presented which represent the correlation between DED and food groups or nutrients.
[4] β regression coefficient represents the energy density (kcal/g) difference explained by 100 g foods.
[5] Partial R^2 (explained variance) represents the inter-individual variation in DED explained by the individual food group or nutrient. Only food or nutrient items had Partial R^2 >0.01 were listed here.
[6] Cumulative R^2 represents the sum of the inter-individual variation in DED explained by the specific food group or nutrient and previously listed food groups or nutrients.
[7] Seven macronutrients (en% per day) were included: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, polysaccharides, mono-&disaccharides, animal protein and plant protein.
[8] β regression coefficient represents the energy density (kcal/g) difference explained by 1% of the energy contributed by the individual nutrient.

Pooled HRs for T2D per 1 kcal/g increase in DED are presented (table 5.3). After adjustment for sex and age (model 1) an inverse association between DED and T2D was found, which became statistically significant following adjustment for known risk factors of diabetes (model 2, HR 0.88 (95% CI 0.79–0.99, I^2= 23.0%). Thus an increase of 1 kcal/g in DED was associated with 12% lower hazard of T2D, which corresponds with a shift from the lowest to the highest quintile of DED. However, the inverse association disappeared after further adjustment for dietary misreporting (Model 3, HR 1.02 (95% CI 0.93–1.13; I^2= 2.9%). HRs were also close to unity comparing quintiles of DED (data not shown). The association between DED and incidence of type 2 diabetes by country is shown in figure 5.1. HRs are all not statistically significant, and showed weak positive associations in France, UK, the Netherlands Germany, Sweden and Denmark, whereas inverse associations were observed in Italy and Spain. There was no evidence of interaction between DED and sex (p = 0.17), age (p = 0.41), BMI (p = 0.49), waist circumference (p = 0.63), physical activity (p = 0.96) or misreporting of diet (under- vs plausible reporters; p = 0.38). The highest percentage of normal weight individuals (79.4%) and the lowest percentage of individuals classified as under-reporters of diet (9.5%) were observed in France (table 5.4). The lowest percentage of normal weight individuals was observed in Spain (22.2%), whereas the highest percentage of under-reporters was observed in Germany (36.1%). Stratified analysis by misreporting of diet affected the HRs especially in individuals with a normal weight at baseline (table 5.5). Among plausible dietary reporters with a normal body weight, a positive but not statistically significant association between DED

and incident T2D was observed (HR 1.15, 95% CI (0.84–1.58) I^2= 25.9%; figure 5.2) whereas among those defined as dietary under-reporters DED tended to be inversely associated with incident T2D (HR 0.64, 95% CI (0.41–1.02). Among overweight and obese individuals, no clear differences in HRs between under- and plausible reporters were observed. Results from sensitivity analysis showed a statistically significant positive association between DED and incident T2D (HR 1.54 95% CI: 1.13–2.10, supplementary figure 5.1) when all foods and all beverages except water were included in the DED calculation. There was a large increase in heterogeneity (from 2.9% to 71.3%) when using this DED estimate.

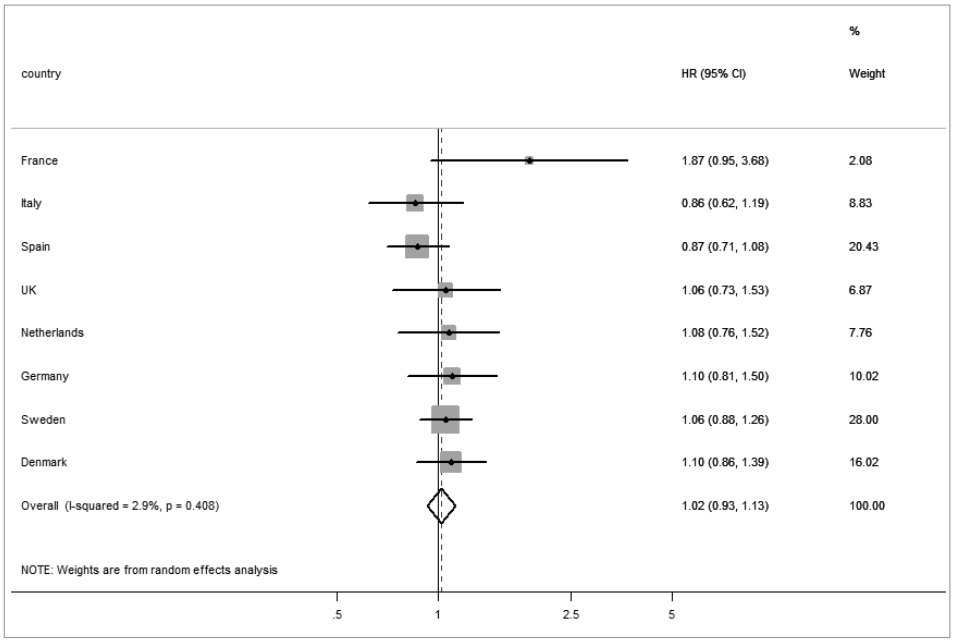

Figure 5.1 Association between dietary energy density and incident type 2 diabetes in Europe[1,2].

HR: hazard ratio per 1 kcal/g increase in energy density; 95% CI: 95% confidence interval for the HR.
[1] Dietary energy density based on solid and semi-foods only.
[2] Adjusted for age, sex, misreporting of diet (under-, plausible, over-reporter), smoking status (never, former, current), physical activity (inactive, moderate inactive, moderate active, active), alcohol (g/day), energy intake from beverages (kcal).

Table 5.3 Pooled hazard ratios[1] for the association between dietary energy density (DED; kcal/g) and incident type 2 diabetes in Europe: the EPIC-InterAct Study.

	Dietary energy density[2]	
	HR (95% CI)	$I^{2\,3}$
Model		
1: age sex	0.95 (0.86-1.06)	21.3
2: model 1 + risk factors DM[4]	0.88 (0.79-0.99)	23.0
3: model 2 + misreporting of diet[5]	1.02 (0.93-1.13)	2.9

BMR=basal metabolic rate; CI=confidence interval; DED=dietary energy density; DM=diabetes mellitus; EI=energy intake; HR=hazard ratio.
[1] Analysis stratified by country and pooled using a random effect meta-analysis; based on 11,734 T2DM cases and 15,434 subcohort members (overlap n=733).
[2] Dietary energy density based on solid foods only.
[3] I^2 represents the variation in the estimate between countries attributable to heterogeneity.
[4] Smoking status (current, never, former), physical activity (inactive, moderately inactive, moderately active, active), alcohol (g/day), energy intake from beverages (kcal).
[5] Misreporting of diet was estimated by using the ratio of reported energy intake to the predicted basal metabolic rate (EI/BMR). Individuals with an EI/BMR <1.14 were defined as under-reporters, EI/BMR >1.14 and <2.1 as plausible reporters and EI/BMR >2.1 as over-reporters.

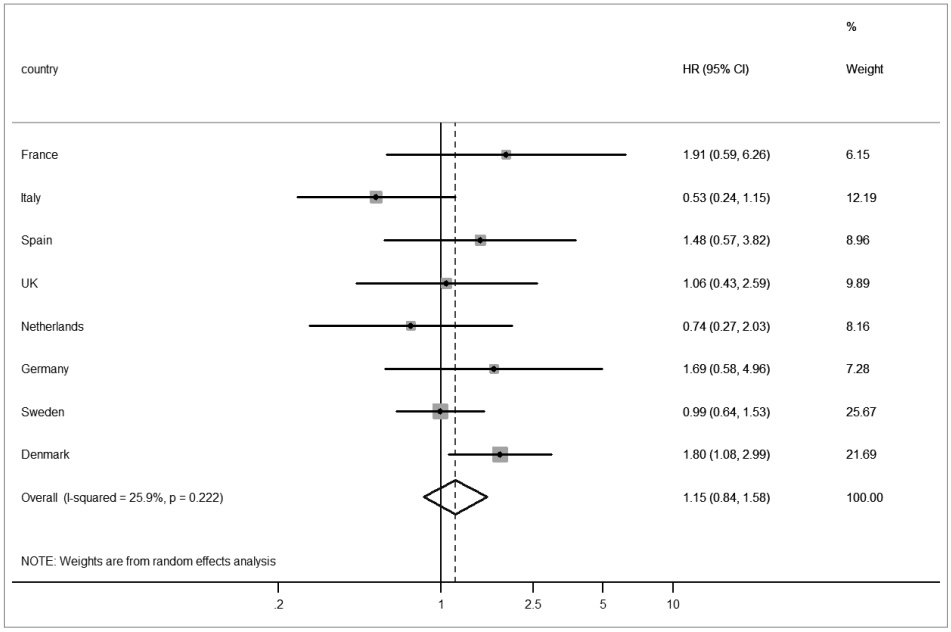

Figure 5.2 Association between dietary energy density and incident type 2 diabetes in Europe among plausible dietary reporters of energy with a normal body weight[1-3].

HR: hazard ratio per 1 kcal/g increase in energy density; 95% CI: 95% confidence interval for the HR.
[1] Dietary energy density based on solid foods only.
[2] Adjusted for age, sex, smoking status (never, former, current), physical activity (inactive, moderate inactive, moderate active, active), alcohol (g/day), energy intake from beverages (kcal).
[3] Normal weight is defined as a BMI < 25 kg/m^2 and plausible reporting of diet is defined as a ratio of energy intake versus estimated basal metabolic rate between 1.14 and 2.1.

Table 5.4 Characteristics of BMI status and misreporting of diet by country in the subcohort (n=15,434): the EPIC-InterAct Study.

Country	N	% Women	BMI status (%)[1]			Misreporting of diet (%)[2]		
			Normal weight	Over-weight	Obesity	Under-reporters	Plausible reporters	Over-reporters
France	549	100	79.4	15.7	4.9	9.5	78.3	12.2
Italy	1,921	66.9	46.8	39.6	13.7	16.9	71.9	11.2
Spain	3,457	61.7	22.2	48.8	29.0	29.1	66.4	4.5
UK	1,200	61.4	52.2	37.4	10.4	32.3	61.9	5.8
Netherlands	1,366	83.2	53.4	35.5	11.1	30.2	68.6	1.2
Germany	2,012	58.4	47.4	38.4	14.2	36.1	60.3	3.5
Sweden	2,852	57.1	53.7	35.6	10.7	29.0	67.0	4.1
Denmark	2,077	46.6	44.1	42.7	13.2	24.5	71.9	3.7

[1]Normal weight was defined as a BMI <25kg/m^2, overweight as a BMI between 25 and 30 kg/m^2 and obesity as a BMI ≥30 kg/m^2.
[2] Misreporting of diet was estimated by using the ratio of reported energy intake to the predicted basal metabolic rate (EI/BMR). Individuals with an EI/BMR <1.14 were defined as under-reporters, EI/BMR >1.14 and <2.1 as plausible reporters and EI/BMR >2.1 as over-reporters.

Table 5.5 Pooled hazard ratios[1] for the association between dietary energy density and incident type 2 diabetes in Europe stratified by BMI status and misreporting of diet: the EPIC-InterAct Study [1-3].

Misreporting of diet[4]	HR (95% CI)	BMI		
		< 25 kg/m^2	25-30 kg/m^2	≥30 kg/m^2
		HR (95% CI)	HR (95% CI)	HR (95% CI)
Under-reporters	1.00 (0.85-1.18)	0.64 (0.41-1.02)	1.03 (0.79-1.35)	1.04 (0.72-1.48)
Plausible reporters	1.02 (0.88-1.19)	1.15 (0.84-1.58)	0.96 (0.78-1.19)	0.96 (0.74-1.24)
Total[5]	1.01 (0.92-1.12)	0.98 (0.78-1.25)	0.97 (0.83-1.13)	0.93 (0.77-1.12)

BMI=body mass index; BMR=basal metabolic rate; CI=confidence interval; DED=dietary energy density; EI=energy intake; HR=hazard ratio.
[1] Analysis stratified by country and pooled using a random effect meta-analysis; based on n=11,045 T2DM cases and n=14,162 subcohort members, (overlap n=704).
Due small numbers no reliable estimates could be calculated for over-reporters (n=1,220), and under-reporters in France (n=88), which therefore are excluded from this analysis.
[2] Dietary energy density based on solid foods only.
[3] Adjusted for age, sex, smoking status (current, never, former), physical activity (inactive, moderately inactive, moderately active, active), alcohol (g/day), energy intake from beverages (kcal).
[4] Misreporting of diet was estimated by using the ratio of reported energy intake to the predicted basal metabolic rate (EI/BMR). Individuals with an EI/BMR <1.14 were defined as under-reporters, EI/BMR >1.14 and <2.1 as plausible reporters and EI/BMR >2.1 as over-reporters.
[5] Additionally adjusted for misreporting of diet.

Discussion

Overall, this large European case-cohort study among 11,734 incident T2D cases and a subcohort of 15,434 participants showed no evidence for an association between DED of solid and semi-solid foods and risk of T2D. This observation was consistent across the eight participating countries, located in different geographical areas in Northern and Southern Europe.

The results of this study should be interpreted with caution because of the difficulties involved when assessing obesity related diet-disease relationships in epidemiological studies [31]. One of the limitations of this study is the use of DED estimates based on self-report of habitual food intake, which might have caused bias due to conscious or sub-conscious under- or over-reporting of specific food items [32]. In this study we observed large differences between countries in the number of participants classified as under-reporters. This could be due to the use of country-specific questionnaires. Also, the prevalence of overweight and obesity varied between countries. Under-reporting of energy intake has been shown to be more prevalent and severe among individuals with a higher body mass index [23,33]. As obesity is a well-established risk factor for T2D the association between DED and T2D may be prone to bias due to obesity-related under-reporting. A recent analysis in EPIC showed that the BMI effect on underreporting is the same across countries and that exclusion of individuals who misreported energy according to the Goldberg cut-off removed the obesity-related bias [34]. Results from our stratified analysis showed that among overweight and obese individuals HRs were close to unity for those categorized as under-reporters and those categorized as plausible reporters. However, in normal weight individuals categorized as under-reporters an inverse association between DED and risk of T2D was seen, whereas DED tended to be positively associated with incidence of T2D among normal weight individuals classified as plausible reporters. Risk of T2D was 15% higher per 1 kcal/g increase in DED, which corresponds to a change in DED from the lowest to the highest quintile. This association was not statistically significant, despite the large sample size in this sub group analysis. Overall, our results indicate that the obesity related underreporting did not affect our overall results much. Still, we cannot exclude an overall bias in energy reporting across all BMI categories. The main strengths of this study are the large sample size, the large number of verified T2D cases, its prospective design, the variation in dietary intake across participants from eight European countries and the availability of information on important potential confounding variables such as smoking behavior, alcohol consumption, physical activity and measured waist circumference and body weight.

To our knowledge, the association between DED and risk of T2D has been investigated only once, by Wang et al. [14]. DED was calculated using FFQs and included all beverages except water. They reported a 20% higher risk of diabetes per unit (kJ) increase in DED in the EPIC-Norfolk study (n= 21,919) which included 725 incident T2D cases (HR 1.20 (95% CI 1.05–

1.37)). This positive association is in line with the result of our sensitivity analysis in which we used the same DED calculation method. So far, no clear consensus has been reached on the calculation of DED. A previous review of the literature identified eight different calculation methods which mostly differed in the inclusion or exclusion of water, other energy-free beverages and energy-containing beverages [22]. In the current study, data on consumption of bottled and tap water was not available in all centres. Because detailed water intake is generally not collected in epidemiologic studies, excluding other energy-free beverages such as coffee and tea eliminates potential bias that could be created by excluding only water [22]. However, coffee and tea often provide energy through added sugar or milk and this should be taken into account. Unfortunately, in our study (as in many others) no such information was available. Independent of the limitations mentioned above, we decided *a priori* to include only solid and semisolid foods and to exclude caloric and non-caloric beverages from the DED calculation while partially adjusting for energy intake from beverages in the models. The rationale for this choice was, amongst others (see Methods section) that inclusion of beverages into DED calculation is associated with higher day to day variance within individuals. This may lead to biased associations when examining health outcomes [22]. This is supported by the result of our sensitivity analysis in which DED was calculated including all food and beverages except water. The pooled estimates showed a large increase in heterogeneity (I^2= 71.3%) compared to our main results (I^2 = 2.9%). This means that the association between DED and risk of T2D is less consistent across countries when drinks are included into the DED calculation. To compare, an I^2 of 25%, 50% and 75% could roughly be interpreted as indicating low, medium and high heterogeneity [35]. Furthermore, when individual study results are inconsistent (i.e. heterogeneity is considerable), the obtained pooled estimate is less valid [35,36]. Together, this favors the exclusion of drinks from DED calculation and adjusting models for the energy intake from beverages. On the other hand, it could be speculated that, despite the methodological limitations, the observed higher T2D risk when drinks are included in the DED calculation is driven by a positive association between energy-containing beverages and risk of T2D as reported in literature [37,38]. This would suggest that energy density of drinks rather than the energy density of solid foods is important in determining the risk of diabetes.

It has been hypothesized that diets high in DED affect risk of T2D indirectly via an increase in body fat mass. Literature shows that foods with a higher energy density are more palatable and less satiating as compared to foods with a lower energy density and can thus lead to passive over consumption and a higher energy intake [39,40]. Moreover, prospective studies have shown that DED is positively associated with risk of (abdominal) obesity, a well-established risk factor of T2D [8,9,10]. On the other hand, it can be postulated that high DED diets have a direct effect on T2D risk. In the current study, a diet high in DED is characterized by a

lower intake of fiber, fruit and vegetables, a higher intake of energy and saturated fat and a higher glycemic index (GI) and glycemic load. This is in agreement with findings of previous studies [6,10,41]. Saturated fat has been suggested to adversely affect insulin sensitivity of muscles as well as glucose stimulated insulin secretion [12]. High GI diets can rapidly increase postprandial glucose concentrations. This may lead to pancreatic exhaustion as a result of the increased demand for insulin [42]. In addition, high GI diets can increase postprandial free fatty acid release, directly increasing insulin resistance [13].

The composition of low energy dense diets meet the dietary recommendations given by WHO to promote human health and to prevent diet-related chronic diseases [43]. In addition, high energy-dense diets have been found to predict (abdominal) obesity [8,9,10]. Therefore, despite the fact that there currently is no conclusive evidence for an association between DED and risk of T2D, choosing low energy dense foods should be promoted as they support the current dietary recommendations.

In conclusion, the results of this large European case-cohort study do not provide evidence for an association between DED of solid and semi-solid foods and the risk of T2D. However, we found some indication that misreporting of diet and BMI status may have obscured a positive association between DED and risk of T2D, and that such an association, if any, would most likely be small.

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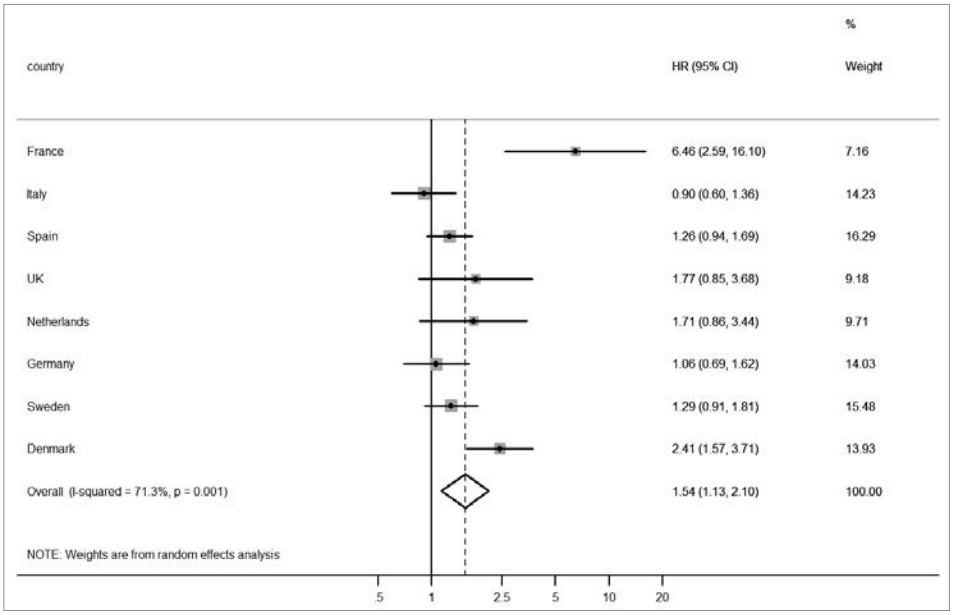

Supplementary figure 5.1 Association between dietary energy density and incident type 2 diabetes in Europe[1,2].

HR: hazard ratio per 1 kcal/g increase in energy density; 95% CI: 95% confidence interval for the HR.
[1] Dietary energy density based on all foods and beverages (except water).
[2] Adjusted for age, sex, misreporting of diet (under-, plausible, over-reporter), smoking status (never, former, current), physical activity (inactive, moderate inactive, moderate active, active), alcohol (g/day).

Chapter 6

Changes in school environment, awareness and actions regarding overweight prevention among Dutch secondary schools between 2006–2007 and 2010–2011

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Abstract

Background: Schools can be an important setting for the prevention of overweight. This nation-wide survey investigated changes in the obesogenity of the school environment, the awareness of schools regarding overweight, school health policy, and actions taken by schools to prevent overweight.

Methods: In 2006/2007 and 2010/2011, questionnaires were sent to all Dutch secondary schools, (n = 1250 and n = 1145, response rate 44% and 33% respectively, repeated data for 187 schools).

Results: The percentage of schools with vending machines for soft drinks (~90%) and sweets (~80%) remained fairly stable, whereas slightly more schools indicated to have a canteen (87%-91%). The food supply was reported to be healthier in 2010/2011 compared to 2006/2007. Canteens and/or vending machines offered more often fresh fruits (+8%), sandwiches (+11%), water (+11%) and salad (+7%) and less often sugar sweetened soft drinks (–10%). However, unfavorable changes such as an increase in the supply of pizza slices (+13%) and milk and yoghurt drinks with added sugar (+12%) were also reported. Between 2006/2007 and 2010/2011, the presence of water coolers increased (12% versus 33%) as well as facilities for physical activity (67% versus 77%). However, more schools had vending places of unhealthy foods in the vicinity (73% versus 85%). Compared to 2006/2007, a higher percentage of schools indicated that they have taken actions to stimulate healthy eating behavior (72% versus 80%) or to prevent overweight (34% versus 52%) in 2010/2011. Less schools indicated that they expect to pay more attention to overweight prevention in the near future (56% versus 43%), but none of them expected to pay less attention.

Conclusions: Several aspects of the school environment changed in a positive way. However, schools should be encouraged to contribute to the prevention of overweight, or to continue to do so.

Background

Worldwide, obesity remains one of the major public health issues [1]. In the past decades, overweight and obesity prevalence has substantially increased, also in the Netherlands. In 2009, 12.9% of the boys and 14.8% of the girls in the Netherlands aged 2–21 years were overweight and respectively 1.8% and 2.2% were obese [2]. This is a two to three fold increase of the 1980 overweight prevalence rates and a four to six times increase in the 1980 obesity prevalence [2]. Prevention is considered to be an appropriate way to counteract overweight among adolescents, though this is a major challenge [3]. An important aspect of overweight prevention is to tackle the obesogenic environment [4]. The latter refers to an environment that promotes an unhealthy diet and physical inactivity and thereby contributes to the development of an excessive fat mass.

Since schools reach many children, and children spend a lot of time at school, schools are considered to be a relevant and important setting for the prevention of overweight and obesity [5]. Several cross-sectional studies investigated school food environments and policies [6-10] and their association with children's dietary intake [11-13] and body weight [14-16]. However, longitudinal studies conducted on this topic are limited [17]. During the 2006–2007 school year, we conducted a nation-wide written survey on school environment, school policy, awareness of the schools regarding overweight and actions taken to prevent overweight at Dutch secondary schools [10]. The main findings were that unhealthy drinks and foods were widely available at secondary schools. In addition, one third of the schools indicated that overweight had increased among students and half of the schools considered themselves to be (co)responsible for the prevention of overweight. Only three percent of the schools had a policy on the prevention of overweight.

Since the 2006–2007 school year several developments in national policies took place regarding the prevention of overweight and obesity. In 2009, the Dutch Government launched a policy document on overweight. In addition, national policies were intensified through several initiatives as amongst others the Dutch Covenant on Healthy Weight [18]. This covenant is a collaboration of 27 actors from national and local governments, industry and civil society organizations who collectively committed to fight against the rising trend of overweight and obesity. A concrete example of an initiative from this covenant is promoting the so called JOGG approach (which is based on the successful French EPODE methodology [19]) to prevent overweight among young persons (See for more detailed information: http://www.epode-international-network.com/programmes/jogg). Another concrete example is the sub covenant 'school' that includes the The Healthy School Canteen programme from the Dutch Nutrition Centre [20]. The goal set by the Dutch Ministry of Public Health, Welfare and Sports of realizing 100% health school canteens by 2015 has been adopted within this

sub covenant. In 2009, also a policy document on Sports, Physical Activity and Education was launched in which local authorities and organizations acting in the field of education and sports collaborate. Main aim of this policy was to achieve that 50% of the children aged 4–17 satisfy the physical activity norm in 2012. Another relevant development since 2006–2007, is the establishment of the RIVM Centre for Healthy Living, which supports the delivery of efficient and effective local health promotion in the Netherlands for different settings including school.

Of interest is whether these developments in recent years have led to improvements in the school environment. The above mentioned survey was repeated during the 2010–2011 school year, yielding the opportunity to investigate whether improvements have occurred and allows us to assess the magnitude and the direction of the potential changes therein. Aim of the current study was to investigate changes over time in the school environment, the awareness of schools regarding overweight and changes in school policy and specific actions taken to prevent overweight.

Methods

Study design

In 2006–2007 a first national survey on nutritional and physical environment at Dutch secondary schools was conducted (in this paper referred to as 'baseline'). In 2010–2011, a second national survey was carried out (in this paper referred to as 'follow-up). Both surveys were conducted through a postal questionnaire and consisted of two mailings. The first mailing was sent to all secondary schools of the Netherlands in November 2006 (baseline) and January 2011 (follow-up) and a second mailing was performed in January 2007 (baseline) and March 2011 (follow-up) to schools that had not responded so far to increase the total response. Together with the second mailing a non-response card was sent to the schools in which the reason for non-response and some main questions from the original questionnaire (selected questions differed between baseline and follow-up) were queried. In the Netherlands, children who attend a secondary school are aged 12 to 18. In addition, the majority of the secondary schools consist of different sites. To increase the readability of this paper 'school sites' will be referred to as 'schools'. The design and the response rate (44%) of the baseline survey have been extensively described by Scholtens et al.[10]. At follow up, 375 of the 1145 approached schools completed the questionnaire, from which 202 responded on the first mailing and 173 on the second. The non-response card was returned by 117 schools. Main reason for non-response was the fact that schools are confronted with many requests for participation in a study (50% of the schools indicated that this was a reason

for non-response). For this study, no ethical approval was necessary according to the Dutch Central Committee on Research involving Human Subjects http://www.ccmo.nl because the questionnaires were not directed at children, no direct health related questions had to be answered and no medical investigations were included.

Study population

This study included schools that filled out the questionnaire at both baseline and follow-up (n = 187). The 187 schools included in this study did not differ on school size, school level (vocational, mixed or higher) and degree of urbanization compared to all 577 and 375 schools (including the 187 schools with repeated measurements) and compared to the 390 and 188 schools (excluding the 187 schools with repeated measurements) that completed the questionnaire at respectively baseline and follow-up.

Questionnaire

The baseline questionnaire consisted of 80 questions [10]. Most questions were repeated in the follow-up questionnaire. In addition, several questions were added to the follow-up questionnaire which finally consisted of 102 questions. The baseline and follow-up questionnaire were both divided in six parts, 1) general characteristics of the schools; 2) the school environment (including questions on the canteen, water coolers and vending machines for drinks, candy and fresh fruit); 3) health education; 4) participation in projects on overweight prevention; 5) school's policy on health, diet, physical activity and overweight; 6) closing questions (e.g. who completed the questionnaire). Questions with regard to health education and participation in projects on overweight prevention were not analyzed in this study, because within a school level the curriculum is fairly similar between schools and the way of inquiring differed between baseline and follow-up.

School environment

For the canteen 11 specific types of 'healthy' and 11 types of 'less healthy' foods were queried and for the drinks vending machines this was done for 4 types of 'healthy' and 4 types of 'less healthy' drinks through pre-coded questions. Respondents could mark whether a particular food item was sold at their school. In addition, school representatives were asked to value the proportion of unhealthy and healthy foods in canteen or vending machines. For example "How would you describe the proportion of high caloric and low caloric drinks in soft drink vending machines? The five predefined answer options were: 1) nearly all high caloric drinks; 2) more high caloric than low caloric/healthy drinks; 3) equal amount of high caloric and low caloric/healthy drinks; 4) more low caloric/healthy than high caloric drinks; and 5) nearly all low caloric/healthy drinks".

Awareness and responsibility of the schools towards overweight and school policy
School representatives where asked to indicate whether they observe an increase in the number of students with overweight at their school (yes; no; I do not know) and whether they think that overweight is more prevalent at their school compared to the general population of children aged 12–18 (yes; no; just as much). In addition, they were queried who they held responsible for the prevention of overweight among their students (school; parents; students themselves; government; no opinion; otherwise, namely… (possible to indicate more answers)) and whether they expect that their school will be pay more attention to overweight in the future (yes, more attention; no, just as much attention; no, less attention). Furthermore, it was asked whether the school has a policy on general health, (healthy) nutrition, sports and physical activity, and overweight. For example "Does your school have a policy on (healthy) nutrition? The five answer options were: 1) yes, lay down in writing and part of the general health policy, 2) yes, lay down in writing, but no part of the general health policy, 3) yes, verbally expressed, 4) no, and 5) I do not know." In addition, the questionnaire contained questions about the compliance to those policies and about obstacles/barriers that have been experienced by the implementation of those policies. For example, "Do you think that the policy on nutrition, physical activity and/or overweight are well complied to? Answer options were 1) very well; 2) well; 3) sufficient; 4) insufficient; 5) poor; 6) not applicable."

Actions to prevent overweight
School representatives were asked to indicate whether several predefined general measures are taken by the school to stimulate healthy eating behavior (n = 7), to discourage unhealthy eating behavior (n = 9), to stimulate physical activity (n = 7), and regarding overweight (n = 3). For this study, the questions relevant for the study question that were inquired (in a similar way) in both questionnaires were selected. This resulted in 18 questions describing the school environment (including all questions on food supply) and 14 questions on school policy (including awareness of schools and specific actions undertaken by schools to prevent overweight). In some cases, questions were combined to one outcome variable or were split into several outcome variables.

Data analysis

In total, 58 outcome variables were a priori chosen to be tested. In addition, specific actions undertaken by schools to prevent overweight were also evaluated but not tested. Changes in dichotomous outcome variables were analyzed using conditional logistic regression. Outcome variables were added as dependent variable and the point of measurement as independent variable (0 'baseline', 1 'follow-up). School was added as stratum to the regression models to ensure comparison within schools. All models were adjusted for (changes in) school level and

school size. Changes in ordinal outcome variables were analyzed by linear regression analysis. We tested whether the change over time in the outcome of interest (added as dependent variable) differs statistically significant from zero by evaluating the intercept of this model. Potential confounding variables (school level and size) were added as centered variables (deviation from the mean) to the models to maintain the possibility to interpret the intercept. At baseline, five outcome variables (i.e. content of soft drink vending machines, sweets/candy bars vending machines and the canteens, schools opinion about the prevalence of overweight among the students compared to the general population, and the action "it is forbidden to sell certain unhealthy foods at school") differed between school levels [10]. Therefore, interaction between changes in those outcome variables and school level was investigated, which however did not appear to be the case. To gain more insight in the robustness of our findings, a non-response analysis was performed, with a focus on actions undertaken by schools regarding the prevention of overweight as this was queried in the non-response cards distributed at baseline as well as the follow up. P-values below 0.05 were considered to be statistically significant. Data analysis was conducted using SAS software version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

School characteristics

Main characteristics of the schools at baseline and follow up are presented in table 6.1. Of the 187 schools, 58% (*n* = 108) was located in a rural area, whereas 42% (*n* = 78) was located in urban area. Most of the schools (92%) reported the same educational level at baseline and follow up. Approximately, 44% of the schools were vocational education schools, 44% were mixed schools and 12% were higher education schools. The median school size was about 650 students at baseline and follow up. Most questionnaires were completed by more than one person (≈55%). More than 70% was (co-) completed by the principal or (assistant) manager of the school, approximately half of the questionnaires was (co-) completed by a teacher in biology, in physical activity, or in health and hygiene, and a quarter was (co-) completed by an employee of the canteen at school at baseline as well as at follow up.

Table 6.1 Content of the questionnaire and main characteristics of the secondary schools at baseline and follow up (*n* = 187).

	Baseline 2006-2007	Follow up 2010-2011
Content of the questionnaire		
Number of questions on:		
The school environment	28	32
School policy	17	21
Characteristics		
Degree of urbanisation (%)		
Rural	58	58
Urban	42	42
School size[1,2] (median)	**670**	**654**
< 500 (%)	37	41
500-1000(%)	29	25
>1000(%)	33	34
Educational level[3] (%)		
Vocational	44	44
Mixed	45	44
Higher	10	12
Who (co-) completed the questionnaire? (%)		
Prinicipal or (assistent) manager	76	72
Teacher[4]	45	54
Employee of the canteen	24	23

[1] 36 schools (19%) moved from school size category between baseline and follow up.
[2] Vocational education schools included schools only offering 'preparatory vocational education'. Mixed schools included schools offering 'preparatory vocational education' senior general education' and/ or 'university preparatory education'. Higher education schools included schools offering 'senior general education' and or 'university preparatory education'.
[3] Of 16 schools (8.5%) the educational level changed between baseline and follow up.
[4] Teacher in biology, in physical activity or in health and hygiene.

School environment

At the majority of the schools a soft drink vending machine, a vending machine containing sweets and candy bars and/or a canteen was present (table 6.2). There was no difference between baseline and follow up regarding the presence and the number of vending machines present at schools, whereas the number of schools with a canteen was slightly higher at follow up (P = 0.03). The percentages of schools who indicated that their vending machines and/ or canteen contained a less favorable selection of food and drinks was statistically significant

lower in 2010–2011 compared to 2006–2007. Compared to baseline, school representatives indicated that their soft drink vending machines contained more often water (60% vs 71%), but also milk and yoghurt drinks with added sugar (16 vs 28%) at follow up. Canteens at schools offered more often fresh fruit (26% vs 34%), sandwiches (56% vs 67%) and salad (6% vs 13), but also pizza slices (17% vs 30%) at follow up compared to baseline. A total of 95% of the schools indicated that they offered sugar sweetened soft drinks in canteens and/or vending machines at baseline, whereas this percentages was 10% lower at follow up. At baseline as well as at follow up 83% of the schools indicate that school management can influence the food supply offered in their canteen. The presence of water coolers at schools was higher in 2010–2011 than in 2006–2007 ($P < 0.0001$). At follow up, 40% of the schools indicated to provide water for free, which was 14% at baseline. At the majority of the schools (85%) where water was not freely distributed, the price of one cup is ten eurocent. Compared to baseline, the percentage of schools that had a supermarket, fast food restaurant or gas station in the neighborhood (within 1 km of the school) was statistically significant higher at follow up (73% versus 85%). At follow up, 77% of the schools indicated to have facilities at or around the school property where students can be physically active, compared to 67% of the schools at baseline. This increase was mainly due to soccer fields on the school property.

Awareness and responsibility of the schools towards overweight and school policy

The percentage of schools that indicated that overweight was not more prevalent among the students of their school than among the 12 to 18 year old children in the general population was lower at follow up compared to baseline (P 0.04; table 6.3). There was no statistically significant difference between baseline and follow up in the opinion regarding the responsibility for the development of overweight among students (about 40% of the schools found themselves responsible) and whether the prevalence of obesity has increased among their students. The proportion of schools with a policy on (healthy) nutrition (49% versus 57%) or a policy on overweight prevention (11% versus 17%) seemed to be higher at follow up than at baseline, however differences were not statistically significant. At baseline and follow-up, approximately 90% of the schools evaluate the compliance to those policies as sufficient. However, about 60% of the schools with a policy on diet, physical and/or overweight at baseline and follow-up experience barriers (mainly lack of time and no financial resources) that hinder the implementation of those policies.

Table 6.2 The school environment.

Outcome variable[1]	Baseline (2006/2007)	Follow up (2010/2011)	P value[2]
	% (*n*)	% (*n*)[1]	
Soft drink vending machine present at school	**91 (170)**	**89 (167)**	**0.59**
Soft drink vending machines contain:			**0.02**
More unhealthy than healthy drinks	53 (83)	45 (71)	
Unhealthy and healthy drinks equally.	38 (60)	33 (52)	
Less unhealthy than healthy drinks	9 (14)	22 (34)	
Vending machine present at school that contains sweets /candy bars	**81 (151)**	**79 (146)**	**0.63**
Sweets/candy bars vending machines contain:			**0.04**
Mainly unhealthy foods	64 (80)	55 (69)	
A good balance between unhealthy and healthy foods	37 (46)	41 (52)	
Mainly healthy foods	0 (0)	4 (5)	
Water cooler present at schools	**12 (22)**	**33 (60)**	**<0.0001**
Vending machines present at school that contains fresh foods	**11 (19)**	**11 (19)**	**0.91**
Canteen present at school	**87 (161)**	**91 (170)**	**0.03**
Proportion of healthy and unhealthy foods present in the canteen:			**0.004**
Mainly less healthy foods	14 (21)	9 (14)	
More less healthy than healthy foods	34 (51)	27 (40)	
Equal distribution of healthy and less healthy foods	31 (46)	28 (42)	
More healthy than less healthy foods	14 (21)	28 (42)	
Mainly healthy foods	8 (12)	9 (13)	
Availability of specific healthy foods and/or drinks[4]			
Drinking water offered in canteen and/or vending machine	60 (109)	71 (129)	0.03
Fresh fruits offered in canteens	26 (48)	34 (63)	0.03
Sandwiches offered in canteens	56 (104)	67 (124)	0.003
Salad offered in canteens	6 (10)	13 (21)	0.06
Availability of specific less healthy foods and/or drinks[4]			
Sugar sweetened soft drinks offered in canteen and/or vending machine	95 (174)	85 (156)	0.003
Milk and yoghurt drinks with added sugar offered in vending machines	16 (29)	28 (51)	0.003
Pizza slices offered in canteens	17 (31)	30(55)	0.0006
There is a supermarket, gas station or fast food restaurant in the neighborhoods of the school.	**73 (127)**	**85 (148)**	**0.003**
There are facilities at and around the school property where the student can be physically active.	**67 (120)**	**77 (138)**	**0.04**
Indicated percentage of students who walk to school or travel by bike *(median [Q1;Q3])*[3]	**80 [70;90]**	**80 [70;90]**	**0.31**
There is sufficient space for students to park their bikes at bike parks	**85 (159)**	**95 (173)**	**0.01**

[1] % yes (n) or otherwise indicated.
[2] P for change. Changes in dichotomous outcome variables are tested using conditional logistic regression.

Changes in ordinal outcome variables are analyzed by linear regression analysis. All models are adjusted for (changes in) school level and school size. P values below 0.05 are considered to be statistically significant.
[3] Q1:first quartile, Q3: third quartile.
[4] It was also queried whether other 'less healthy foods' like: sport drinks, fruit juices with added sugar, candy bars, sweets, cakes, fried snacks, Russian salad, ice creams crisps and other 'healthy foods' like: (fresh) fruit juices without added sugar, artificially sweetened soft drinks, rice cakes, soup, salad, yoghurt were offered in canteens or vending machines but no statistically significant change was observed.

Table 6.3 Awareness and responsibility of the schools towards the overweight problem and school policy.

Outcome variable[1]	Baseline (2006/2007)	Follow up (2010/2011)	P-value[2]
	% (*n*)	% (*n*)	
Awareness and responsibility			
The prevalence of obesity has increased among students			0.27
No	40 (72)	40 (72)	
Yes	34 (61)	29 (52)	
Don't know	27 (48)	31 (55)	
Overweight is more prevalent among the students at school than in the general population			0.04
Yes	6 (10)	8 (13)	
Equal	12 (21)	20 (34)	
No	82 (140)	73 (124)	
Responsibility for the prevention of overweight among students			
Schools	40 (72)	37 (67)	0.38
Parents	98 (177)	98 (177)	1.00
Students	81 (146)	77 (139)	0.17
Government	15 (27)	17 (32)	0.49
Policy			
School has policy on:			
General health	12 (22)	17 (30)	0.11
(Healthy) nutrition	49 (85)	57 (96)	0.16
Physical activity	55 (86)	52 (82)	0.58
Overweight	11 (19)	17 (28)	0.14
Compliance to policy on diet, physical and/or overweight at schools?[3]			0.76
Very well	0 (0)	4 (2)	
Well	32 (17)	26 (14)	
Sufficient	59 (31)	64 (34)	
Insufficient	9 (5)	6 (3)	
Poor	0 (0)	0 (0)	
School experiences barriers/obstacles by implementation of their policy on diet, physical and/or overweight[3]	63 (40)	58 (43)	0.47

[1] % yes (n) or otherwise indicated.
[2] P for change. Changes in dichotomous outcome variables are tested using conditional logistic regression. Changes in ordinal outcome variables are analyzed by linear regression analysis. All models are adjusted for (changes in) school level and school size. P-values below 0.05 are considered to be statistically significant.
[3] Among schools with a policy on diet, physical and/or overweight at baseline and follow up (n = 97).

Table 6.4 Actions taken by the schools to prevent overweight.

Outcome variable[1]	Baseline (2006/2007)	Follow up (2010/2011)	P value[2]
	% (*n*)	% (*n*)	
Actions taken to stimulate healthy eating behavior:	**72 (127)**	**80 (140)**	**0.08**
Healthy products are made less expensive than unhealthy products	26 (46)	30 (52)	
Introduction of water coolers	15 (27)	26 (45)	
Participation in national project "Healthy School Canteen Programme"	11 (19)	20 (35)	
Canteen offers wide variety of healthy foods	28 (49)	30 (52)	
Vending machines offers wide variety of healthy foods	17 (29)	19 (34)	
Attempt to offer a good balance in food and beverages	34 (60)	46 (80)	
After-school meetings organized on healthy diet	7 (13)	12 (22)	
Other	16 (27)	20 (35)	
Actions taken to discourage unhealthy eating behavior:	**89 (155)**	**93 (161)**	**0.23**
It is forbidden to sell certain unhealthy foods in the canteen	38 (65)	56 (98)	
Parents are tackled about the eating behavior of their child	9 (16)	18 (32)	
It is forbidden to eat in the classroom	79 (137)	87 (151)	
Adding healthy products to vending machines	43 (75)	44 (76)	
Regulation of media that stimulate less healthy eating behavior	4 (7)	1 (1)	
Other	26 (45)	35 (60)	
Actions taken to stimulate physical activity:	**76 (135)**	**80 (142)**	**0.21**
School stimulates the students to be physically active during breaks	20 (36)	17 (31)	
Collaboration with sport associations	32 (57)	26 (46)	
School often organizes activities for the students to be physical active after school hours.	62 (110)	57 (102)	
School policy on physical activity active after school hours.	17 (31)	12 (21)	
Other	8 (14)	26 (47)[3]	
Actions taken to prevent overweight:	**34 (49)**	**52 (74)**	**0.0009**
After-school meetings organized on overweight	5 (7)	5 (7)	
There are guidelines to identify ant to help students with overweight	15 (21)	14 (20)	
Students who are overweight get more attention during physical activity classes	10 (14)	12 (17)	
Other	13 (19)	49 (70)[4]	
Regarding overweight in the future, school expects to pay			**0.03**
More attention	56 (98)	43 (76)	
Equal attention	44 (77)	57 (100)	
Less attention	1 (1)	0 (0)	

[1] % yes (n) or otherwise indicated.
[2] P for change. Changes in dichotomous outcome variables are tested using conditional logistic regression. Changes in ordinal outcome variables are analyzed by linear regression analysis. All models are adjusted for (changes in) school level and school size. To reduce the number of tests performed and the chance of false positive findings, it was a priori decided to only evaluate, but not test changes in specific actions undertaken by schools. P values below 0.05 are considered to be statistically significant.
[3] Includes 36 schools (20%) that indicated to have discussion with the local government about facilities to be physical active. This action is only queried at follow-up and not at baseline.
[4] Includes 61 schools (43%) that indicated that they bring students with overweight and their parents in contact with (health) professionals. This action is only queried at follow-up and not at baseline.

Actions taken to prevent overweight

Compared with baseline. a higher percentage of schools indicated that they have taken actions to stimulate healthy eating behavior (P = 0.08) or actions to prevent overweight (P = 0.0009) at follow up (table 6.4). With regard to specific actions, 46% attempted to offer a good balance in food and beverages, 20% participated in the national project "Healthy School Canteen Programme", 56% has banned the sale of certain unhealthy foods in the canteen and 18% are tackling the parents about the eating behavior of their child at the follow up measurement. Those percentages were 9% lower at baseline. There was no change between baseline and follow up in the percentage of schools that indicated to have taken actions to stimulate physical activity. A statistically significant lower percentage of schools indicated that they expect to pay more attention to overweight prevention in the future (56% versus 43%), but none of them expected to pay less attention.

Discussion

The results of these longitudinal analyses suggested that the environment inside and also partly outside secondary schools has become less obesogenic in 2010–2011 compared to 2006–2007. Schools reported that the supply of foods and drinks in vending machines and canteens has become healthier. This favorable change was illustrated by a decreased availability of sugar sweetened soft drinks and an increased availability of drinking water, fresh fruit, salad and sandwiches at secondary schools. However, unfavorable changes were also observed, as an increased availability of pizza slices and milk and yoghurt drinks with added sugar. With regard to physical activity, there was an increase in the presence of soccer fields at schools, and also the environment outside the school improved with respect to the facilities available for students to be physical active. However, there are also more facilities in the neighborhood of the schools for students to buy less healthy foods. More schools indicated that they have taken actions to stimulate healthy eating behavior or actions to prevent overweight. A lower percentage of school representatives indicated that they expect to pay more attention to overweight prevention, but none of them expected to pay less attention to the issue of overweight in the near future.

One of the strengths of our study is its longitudinal design. This allowed us to include a relatively large group of secondary schools in the analyses, despite the fact that overall response rates dropped between baseline (school year 2006–2007) and follow-up (school year 2010–2011). This may have led to selection bias, for example if the response was higher for school representatives with a higher interest in the prevention of overweight. Non-response analysis, including data obtained by non-response cards, indeed showed that the prevalence of schools

that had taken actions to prevent overweight was lower among non-responders compared to responders, at baseline as well as follow-up. However changes over time, which is the most important outcome indicator in this manuscript, did not differ between responders and non-responders (only possible for the schools that filled out a non-response card at baseline as well as at follow-up). In addition, school level, size and location did not differ between schools that completed both questionnaires with those that completed only one of them. Finally, our results (based on 187 schools with repeated measurements) are confirmed by the results of a cross-sectional comparison between the 375 schools that filled out the questionnaire at follow-up and the 515 schools included at baseline (results not part of this paper) [21]. So, overall we feel confident about representativeness of the reported changes over time, despite disappointing response rates.

In our study, we only examined changes in self reported indicators of the obesogenic environment at Dutch secondary schools. This may be subject to recall bias and/or social desirability bias. The reported favorable changes in food supply are reflected to some extent in the reported changes in the availability of specific healthy and less healthy products in the canteen and/or vending machines at schools (eg. increase in fresh fruits and sandwiches and a decrease in sugar sweetened soft drinks). However, less favorable trends were also observed (eg. increase in pizza slices and milk and yoghurt drinks with added sugar). More objective exposure data could be collected by visiting schools and/or using purchase data.

In this study, data on body mass index and dietary behavior of students was not available. Such measurements would provide more objective outcome measures of the potential impact of the changes in the obesogenic environment of Dutch secondary schools. Literature shows for example that attending a middle or high school without stores or snack bars was associated with a reduced sugar-sweetened beverage consumption in children [11]. In addition, consumption of sugar-sweetened beverages has been linked with an increased risk to develop overweight among children [22]. There is also evidence for a direct association between food supply at schools and risk of obesity among children. For example, a cross-sectional research among middle school children showed that the availability of unhealthy foods and drinks in vending machines was associated with a higher BMI among middle school children [15]. On the other hand, the availability of such foods in the cafeteria was associated with a lower BMI. In addition, none of these associations was found for high school students. As far as we know, one longitudinal study has been performed on this topic, which did not find an association between the sale of unhealthy foods and drinks and weight gain among middle school children [17]. More longitudinal research among middle as well as high school children is needed to elucidate this topic.

In contrary to many countries, the Netherlands does not have a tradition of providing meals at school. Most students bring their packed lunch from home, and – either in addition or

instead – they can use the opportunity to buy foods and drinks at school. Thus the impact on food supply at schools on the dietary pattern of students may be less than in countries providing school meals. For the interpretation of our results it should be kept in mind that multiple tests (n = 58) were performed. By using a P value below 0.05 as a threshold for statistically significance (58*0.05), three false positives findings can be expected [23]. As this study showed statistically significant changes in 17 outcome variables, the majority of our findings will be true positive findings. Obesity policies in the Netherlands do not involve obligatory regulations or legislation, but is rather dependent on self-regulation, the mobilization of action, projects and campaigns e.g. by national and local health promoting institutes. In recent years, developments in those national policies have been started or continued in the Netherlands (for examples see introduction). In addition, financial resources for projects that aimed to tackle overweight and obesity in the Netherlands were also increased. It is tempting to assume that the observed improvements in the obesogeneity of the environment at secondary schools are, at least in part, the results of those developments.

Conclusions

Our longitudinal analyses indicate some positive changes in the obesogenic environment at Dutch secondary schools. For example, about 25 000 more students now have access to water coolers (38 schools indicated that they have introduced a water cooler; median school size at follow-up 654), so for these students a healthy alternative has at least become available. However our results also show that there is room for further improvement with respect to the obesogenic environment, the awareness of schools, and the school policy regarding overweight. In line with recommendations of the European Commission and the WHO [24,25], schools should remain a priority setting for the prevention of overweight and obesity in national and international policies on health and education. Taking specific measures or actions that contribute to the reduction of the obesogenic environment at schools should remain an important focus of their preventive efforts.

Competing interests The authors declare that they have no competing interest.

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Chapter 7

General discussion

Summary of main results and comparison with the literature

The first objective of this thesis was to quantify the energy gap responsible for the excess weight gain in Dutch children. Our study showed that an energy gap of 75 kcal per day (e.g. 1 glass of soft drink) over a number of years makes the difference between a normal body weight and overweight in 90% of the young Dutch children who were overweight at the age of 5-7 years (**chapter 2**). Our estimate of the energy gap is lower than the energy gap estimates for overweight children in the US and Germany [1-3]. The higher estimates in other studies can be explained by a truly higher yearly excess weight gain [1], by not taken into account the energy required for healthy growth in children [2] and by adding the excess energy required to maintain the extra weight to the energy gap estimate [3]. Our estimate of the energy gap is higher than the energy gap estimate for all young US children, including those without overweight, as published by Wang and colleagues in 2012 [4]. They reported that it is necessary to eliminate an average energy gap of 17 kcal per day to halt the rising trend in mean body weight in children aged 2-5 years. One explanation for our higher estimate of the energy gap compared to Wang and colleagues' study [4] is that our estimate only applies to children who remained or became overweight and thus had a higher mean excess weight gain. Recently, a more sophisticated mathematical model to simulate the energy excess underlying overweight for children aged 5-18 years became available [5]. This model supports the idea that a small daily energy gap results in the development of overweight in the long run, and that, in turn, the maintenance of overweight requires a much higher excess energy intake because of the increased energy needed to maintain the excess body weight. Overall, it can be concluded that the change in daily energy balance required to stop the trend of increasing excess weight gain and the resulting increasing prevalence of overweight in Dutch children is small relative to the daily energy intake.

The second objective of this thesis was to investigate possible associations between on the one hand determinants of nutrient metabolism (**chapter 3**), maternal diet (**chapter 4**) and individual dietary intake (**chapter 5**), and on the other hand overweight, and its important consequence type 2 diabetes.

In **chapter 3** we studied a large number of variations in candidate genes involved in regulatory pathways controlling fatty acid and glucose metabolism. We detected strong associations between adult BMI and/or waist circumference and five genetic variants located across the *NR1H4*, *SIRT1*, *SMARCA2*, *SCAP* and *IL6* genes. Observed differences in mean BMI between genotypes ranged from 0.4 to 5.9 kg/m^2 and for mean waist circumference from 1.1 to 18.5 cm. At the time we published the findings of this study, there was some supportive evidence

from candidate gene studies and genome wide association studies (GWAS) for a role of *NR1H4*, *SIRT1*, *SMARCA2* and *IL6* in (abdominal) obesity [6-8]. Meanwhile, new evidence has become available (except for *SMARCA2*). A meta-analysis conducted in 2012 reported an association between the most investigated *IL6* polymorphism (the -174GC variant) and risk of obesity, which stands in contrast to the results of a previous meta-analysis that did not find an association between this genetic variant and BMI or waist circumference [8, 9]. We also did not observe an association between the *IL6* -174GC polymorphism and BMI or waist circumference in our study. In 2013, no association was observed between 7 tagging SNPs within the *NR1H4* gene and BMI [10]. Recently, the results were published of the largest GWAS meta-analyses for BMI and waist circumference so far [11, 12]. Among the included SNPs were three of the five genetic variants (in the *NR1H4*, *SCAP* and *IL6* genes) that were associated with obesity phenotypes in our study. They were, however, not among the top findings of the GWAS meta-analyses [11, 12]. But it should be noted that in GWAS a large number of SNPs are tested in a hypothesis free manner, and therefore strong adjustments for multiple testing have to be made in order to prevent false positive findings. The correction for multiple testing in the GWAS meta-analysis might have resulted in disappearance of some true associations. Therefore, we checked the observed associations between the three genetic variants in the *NR1H4*, *SCAP* and *IL6* and BMI and/or waist circumference in the supplementary data files to the two GWAS publications. This showed that the corresponding beta's were small and far from statistically significant. Overall, evidence for a role of *NR1H4*, *SCAP* and *IL6* in the development of overweight should be considered inconclusive. There is, however, growing evidence from candidate gene studies for a (etiological) role of sirtuin 1 (SIRT1) in the regulation of body weight [13-17]. One study included the same *SIRT1* SNP as we did but found no association with BMI in morbidly obese young adults [13]. It is likely that this is due to the different range of BMI studied. Most studies used tagging SNPs to cover most of the genetic variation within the *SIRT1* gene and reported significant associations between one or more *SIRT1* SNPs and obesity measures [14-17]. So far, the exact underlying biological mechanism for the observed associations is unclear. In our study SIRT1 was selected based on its known function in glucose and fat metabolism. A recent review postulated that *SIRT1* can prevent weight gain through modulation of leptin sensitivity in the hypothalamus [18]. Thus, the effects on BMI might also result from an influence of SIRT1 on energy intake. In conclusion, we were one of the first who contributed to the evidence for a role of the *SIRT1* gene as an overweight susceptibility locus. Potential roles for *NR1H4*, *SMARCA2, SCAP* and *IL6* in (abdominal) obesity remain unclear.

In **chapter 4** we studied maternal fish consumption during pregnancy as a potential determinant of overweight in the child. Donahue at al. [19] reported that higher maternal fish consumption during pregnancy was associated with a higher BMI in children at 3 years of age.

However, our study does not provide evidence for a long term effect of maternal fish consumption during pregnancy on body weight in children up to the age of 14 years. Using 11 measurements of BMI between birth and 14 years of age, we observed that higher maternal fish consumption during pregnancy was associated with a lower BMI in the children at several ages. But it was likely that other healthy maternal characteristics were responsible for this association, especially a lower maternal BMI before pregnancy. We cannot rule out completely an effect of maternal fish consumption on BMI development in children, because our study was conducted in a population with relatively low fish consumption. Due to the lack of other studies on long term effects, there is currently no clear evidence to support an independent association between maternal fish consumption during pregnancy and BMI in children up to 14 years of age.

In **chapter 5** we studied the consumption of diets with high energy density, which has often been positively associated with overweight, in relation to type 2 diabetes [20]. As type 2 diabetes is the major co-morbidity of overweight, we expected that dietary energy density would also be positively associated with risk of type 2 diabetes. A previously conducted small study indeed showed such an association, but our study, which was performed in a much larger population, did not confirm these results [21]. Nevertheless, we cannot exclude a positive association between dietary energy density and risk of type 2 diabetes due to methodological issues such as overweight related underreporting of dietary intake (see page 105 of this general discussion). Among normal weight individuals classified as plausible reporters of diet by using Goldberg cut-off values [22], risk of type 2 diabetes was 15% higher per each unit (1 kcal/g) increase in dietary energy density. A one unit increase corresponds to a large change in dietary energy density (from the lowest to the highest quintile). The association was not statistically significant despite the large sample size in this sub group. Since our publication, no other studies have been conducted on this topic. Therefore, we conclude that an association between dietary energy density and type 2 diabetes, if any, would most likely be small.

Overall, it can be concluded that genetic variation in glucose and fat metabolism affects overweight phenotypes in adults. Currently, there is no evidence that maternal fish consumption during pregnancy is associated with BMI in children or that consumption of high energy dense diets are associated with a higher risk of type 2 diabetes among adults.

The third and last objective of this thesis was to assess changes in the food environment at Dutch schools. This thesis showed favorable changes in food supply, attitudes and beliefs regarding overweight and policies on health and nutrition at Dutch secondary schools between 2006/2007 and 2010/2011 (**chapter 6**). For example, a higher proportion of Dutch secondary schools reported that vending machines and canteens contain a more favorable balance between healthy and unhealthy foods and drinks after four years of follow-up.

The presence of water coolers increased (+21%). More schools indicate that it is forbidden to sell certain unhealthy foods in the canteen (56% vs 38%) and that parents are informed about unhealthy dietary behavior of their child (18% vs 9%). However, less favorable developments were also observed after four years of follow-up. For example, more schools had vending places of unhealthy foods in their vicinity (+12%). Only a small, statistically non-significant, increase in the proportion of schools with a policy on (healthy) nutrition was found (57% vs 49%). There were no differences in the opinion of schools about the responsibility for overweight among students. About 40% of schools considered themselves partly responsible. Our results are in line with findings from national surveys conducted in the UK which also report that the food and drink provision during lunch time at secondary schools has become healthier between 2004 and 2011 [23]. A recent review (including 18 studies) concludes that schools, and the policy makers who influence them, can have a positive impact on the dietary behaviors of their students by improving the school food environment [24]. There is also evidence that modification of the school food environment can decrease BMI in children, but studies are still limited [25, 26]. To date, the majority of studies were conducted in the United States and the United Kingdom [24]. Most studies are mainly 'natural experiments' reporting on the effect of state or national policy changes, that impact the school food environment but not in a controlled manner. It is hard to ascribe positive changes in student's BMI observed in these studies to changes in the school food environment. Other changes in behavior or the environment occurring over the same period could also be an explanation [24].

Overall, our results suggest that the food environment at Dutch secondary schools has become less obesogenic. There is supportive evidence that such changes can result in a more favorable dietary intake and BMI among children. However, more high quality interventions are needed to provide conclusive evidence, also in other countries than the UK and the USA.

Methodological considerations related to studies on diet and overweight

A large body of research has been performed on dietary determinants to gain insights into possibilities to prevent overweight. Many statistically significant associations with measures of overweight have been found. However, even for associations that seems logical in theory (e.g. consumption of energy dense snacks and risk of overweight [27]), it turns out that scientific evidence is sometimes difficult to obtain. Possible explanations are methodological limitations that are frequently encountered in overweight research. Major methodological challenges in research on diet and overweight are the accurate measurements of the exposure (diet) and of the outcome (often BMI) [28].

In (large-scale) epidemiological research, dietary intake and body weight are often not actually measured but self-reported by the participants, which is prone to bias [29, 30]. People tend to give socially desirable answers and therefore food consumption and body weight in general tend to be underreported [31-33]. This occurs more frequently and to a larger extent among people with overweight and for foods labeled as unhealthy [34-38]. This selective underreporting of food consumption and BMI can lead to the absence of, or even an inversed association, and to incorrect conclusions in general. When studying diet-overweight relationships these are therefore issues of great concern [33, 39-41].

Underreporting of diet probably played a role in our study on dietary energy density and risk of type 2 diabetes (**chapter 5**). Foods with a high energy density are considered to be less healthy [42]. Therefore, the consumption of energy dense foods is more likely to be underreported as people may find it difficult to admit that they are engaged in unhealthy behavior [37]. Underreporting of weight, and maybe also of food consumption, will occur more frequently in people with type 2 diabetes because they are also more often overweight than people without type 2 diabetes [43]. We did not observe an association between dietary energy density and risk of type 2 diabetes. However, subgroup analysis showed that in normal weight individuals (based on measured body weight data) categorized as plausible reporters, dietary energy density tended to be positively associated with risk of type 2 diabetes. Therefore, underreporting of food consumption, particularly by people with overweight, may have obscured a positive association. Underreporting of diet was probably of less relevance in our study on maternal fish consumption during pregnancy and BMI in children (**chapter 4**). First, consumption of fish is probably less prone to (overweight associated) underreporting because people may not directly relate fish consumption to overweight risk [38]. Second, we had the opportunity to assess the quality of the reported fish consumption data by comparing them with measured fish fatty acid concentrations (EPA and DHA) in breast milk in part of the study population. EPA and DHA concentrations in breast milk were positively associated with self-reported maternal fish consumption during pregnancy. This suggests that our measure of exposure was valid.

In large-scale cohort studies with a large number of repeated measurements during follow up, measurement of participants body weight by research staff is not always the most feasible option given the required number of person-hours and financial resources [30, 44]. In such cases, it is important to have quantitative indices of measurement errors to estimate the effect of differential misreporting on the observed association [41, 45]. Two studies that are part of this thesis (**chapter 2 and 4**) were conducted with data of the PIAMA birth cohort which includes information on children's weight and height reported by their parents. The validity of these data has been investigated at the children's ages of 4 and 8 years [46, 47]. Parents of children with a higher BMI tended to underreport, whereas parents of children with a lower

BMI tended to overreport their child's body weight with approximately 0.5 kilograms, which is a rather small amount. Based on this information, we estimated that the use of self-reported BMI data may have resulted in an underestimation of the energy gap in overweight young children by at most 13 kcal per day (**chapter 2**). The use of BMI reported by parents might have resulted in a small underestimation of the differences in BMI between fish consumption groups (**chapter 4**). Therefore, it is unlikely that underreporting of body weight has obscured a relevant beneficial effect of maternal fish consumption on children's BMI. Underreporting of BMI did not play a role in our research on genetic variation in glucose and fatty acid metabolism and overweight-phenotypes, because in the Doetinchem cohort study BMI and waist circumference were measured by research staff (**chapter 3**).

Public health relevance of the findings

Energy gap estimates are meant to represent the amount of excess daily energy intake over daily energy expenditure responsible for the observed weight gain among the population over a certain period of time. Such estimates provide valuable insight in the average changes in energy balance needed to prevent the upward shift in the distribution of BMI in the whole population [48, 49]. Energy gap estimates can be translated into required behavioral changes to prevent further excess weight gain or the development of overweight. It is important to recognize that population energy gap estimates are not directly applicable to individuals. For example, at the individual level a decrease in energy intake of, for example, 75 kcal per day can result in weight loss among individuals in the lower part of the population distribution of weight gain or still result in an excess weight gain among individuals in the upper part of the distribution.

This thesis shows that an energy gap of 75 kcal day was responsible for the development or maintenance of overweight in 90% of the young Dutch children who were overweight at the age of 5-7 years (**chapter 2**). Previous research showed that a daily energy gap of 50 kcal was responsible for the observed weight gain during 11 years in 90% of the Dutch adults [50]. Therefore, the required change in energy balance to prevent further excess weight gain in the population of children as well as adults is small relative to the daily energy intake. To illustrate, one glass of soft drink (approximately 200 ml) contains 75 kcal, one small bag (25 g) of potato crisps provides 135 kcal and one large candy bar (50 g) corresponds with 240 kcal [51]. This implies that a small excess energy intake is easily attained. To burn 50 kcal, adults have to walk for about 10 minutes. However, young children have a much lower body weight and have to walk twice as long to burn the same amount of kilocalories. Burning of the excess calories is thus more difficult for children than for adults. Being informed about the effect of even a

small energy gap may be useful in creating awareness among individuals, that is knowing, for example, that 1 extra glass of soft drink or going by bike instead of by car really makes a difference towards the development of overweight [52]. This concept was applied in the 'balansdag' intervention of the Dutch Nutrition Centre [53]. The so-called 'balance' approach accepts that overeating occasionally occurs, and focuses on making people aware of such occasions. In addition, this approach aims to motivate and enable people to compensate their overeating within a short span of time by moderation of food intake and/or physical activity to maintain a neutral energy balance on the longer term. The finding that the energy gap is often small in children shows that restoring the energy balance does not necessarily require large behavioral changes and suggests that prevention of overweight should be feasible. Creating an environment for children that supports the achievement and maintenance of a healthy dietary behavior is therefore essential [54].

As we observed in **chapter 3**, genetic variation in nutrient metabolism pathways can result in substantial differences in BMI and waist circumference between individuals. Nowadays, genetic studies have revealed more than 100 loci associated with BMI or waist circumference [11, 12]. In addition, those studies provided insight in important biological pathways involved in the etiology of overweight [55]. Part of this knowledge has already been used for the treatment of some rare cases of severe obesity, e.g. with the administration of leptin [56]. This thesis also supports a role in overweight etiology for the *SIRT1* gene, which has only rarely been linked with overweight phenotypes before (**chapter 3**). Despite the many associations that have been reported, our knowledge about relevant genetic risk factors is still limited. To date, only a small proportion (<5%) of BMI variability can be explained by common genetic variants [11, 56]. In addition, genetic factors cannot be modified [57]. Therefore, practical use of the knowledge about the genetics of obesity in developing a strategy to prevent overweight has been small so far. Currently, much of the unexplained interindividual variation in BMI is thought to be attributable to gene-environment interactions [58]. Epigenetic modification of genes by environmental factors might mediate gene-environment interactions and are now receiving much attention [59]. This possible epigenetic contribution to overweight would theoretically be preventable through minimization of the environmental triggers, which could include oversupply of energy dense foods and intrauterine determinants [57].

A potential dietary strategy to prevent overweight would therefore be improving the in utero nutrient environment through an optimal dietary intake by pregnant women. So far, scientific evidence for a role of maternal diet during pregnancy in the development of overweight among children is scarce [60]. Based on the limited number of studies conducted, there is no evidence that recommendation to pregnant women of fish consumption (**chapter 4**) or other

specific diets, foods or nutrients in general can prevent overweight in their children [61]. There is, however, some evidence that improved dietary intake by overweight pregnant women, can probably prevent the higher risk of overweight among their children. Results of the LIMIT trial show that antenatal lifestyle advices improved maternal dietary intake and reduced the child's risk of a high body weight at birth (>4 kg) with 20%, although no group differences were observed in maternal gestational weight [62, 63]. To date, there is clear evidence that children of mothers with a higher BMI during pregnancy are at higher risk of overweight [64]. So far, it remains unresolved whether observed associations really indicate in utero programming of maternal overweight or reflect similar food consumption habits among mother and child. The importance of a healthy body weight is also supported by analyses, presented in this thesis, in which the observed associations between maternal fish consumption during pregnancy and BMI in children at several ages could almost completely be explained by differences in maternal BMI before pregnancy (**chapter 4**). It is likely that the number of pregnant women with overweight will increase given the current prevalence of overweight among Dutch girls [65]. This may have severe consequences for the obesity epidemic in the future. Therefore, it is of great public health importance to prevent maternal overweight both before and during pregnancy.

In general, a healthy diet and lifestyle should be advocated to every pregnant woman and even before conception, since the first 1000 days of a child's life- the nine months in utero and the first two years after birth - are considered to be vital to its long-term health, including the risk of overweight [66]. In the Netherlands, there is growing attention for the importance of a healthy diet and body weight during pregnancy which is addressed in the factsheet on diet and pregnancy recently published by the Dutch Nutrition Centre [67]. In addition, the Health Council of the Netherlands is currently preparing an advisory report on diet for pregnant and lactating women. Midwives and other antenatal care givers play an important role in educating pregnant women about the importance of a healthy diet.

Besides diet during pregnancy, improving dietary intake during the entire life-course contributes to prevent a positive energy balance and overweight. Strategies focusing on reduction of energy density and portions sizes of food are likely to be more effective than those targeting specific macronutrients [68]. However, public health recommendations cannot be made simply on the basis of energy density values of individual foods and beverages [69]. For example, nuts or olive oil are energy dense foods, but studies have shown that individuals who consume these foods are not more likely to gain weights [70, 71]. Low energy dense diets are characterized by, amongst others, a high intake of fruit and vegetables (**chapter 5**) and consumption of those foods should be recommended. This is in line with current dietary guidelines to prevent overweight and chronic diseases [72]. Sugar-sweetened beverages also

have low energy density due to their liquid form, but there is accumulating evidence for a negative role of sugar-sweetened beverages in the development of overweight. Results of a recent Dutch 18-month blinded randomized controlled trial among normal-weight children showed that the group who received 250 ml (104 kcal) of a sugar-containing beverage per day had a 1 kg greater weight gain than the group of children who received a similar noncaloric beverage instead, while keeping their normal diet [73]. Substituting the consumption of one sugar-sweetened beverage by a noncaloric beverage could be a feasible strategy and sufficient to close the average daily energy gap of 75 kcal responsible for the development of maintenance of overweight among children (**chapter 2**). Considering this, the observed increase in the availability of water coolers at Dutch secondary schools is a positive development (**chapter 6**). To date, there is no direct evidence to advise low energy dense diets for the prevention of type 2 diabetes (**chapter 5**). However, a choice for low energy dense foods should also be recommended to people with type 2 diabetes, which is in line with the current recommendations for a healthy diet [72]. The importance of improving the energy density of foods is recognized nowadays. In 2014, the Dutch government, food industry and the hospitality and catering sector committed to gradually lowering the content of calories (from added sugar and fat) in foods and reducing portion size [74]. This is an important step forward.

Dietary behavior of individuals is modifiable. However, most people find it hard to change their dietary behavior and to maintain a healthier diet on the long term [75]. In addition, food choices of people are often not rational choices but are automatic, subconscious or driven by intuition [57, 76]. Therefore, effectiveness of, for example, nutrition education is often disappointing [76]. Moreover, the food environment largely drives dietary behavior of people [77]. Our current environment abundantly meets the demand for palatable, convenient and affordable food. Relatively cheap energy dense foods are more abundantly available and accessible than ever before [78, 79]. In our obesogenic food environment it is very hard to resist the many food temptations people are exposed to and to make responsible decisions on personal diet [76, 77]. This is supported by our biology, which has always been programmed to eat a lot, to avoid unnecessary activity and to store energy in order to survive periods of famine [80]. In addition, an obesogenic food environment facilitates the expression of individual genetic susceptibility to obesity [81]. This emphasizes the importance of making adherence to a healthy diet more easy by, for example, creating a supportive environment that contributes to closing the energy gap [79, 82].

A big step in overweight prevention can thus be made by improving the food environment [77, 83]. An important question is whether the food environment can be changed in such a way

that the energy balance is restored. This thesis provides evidence that the food environment at schools is modifiable and has become less obesogenic in recent years (**chapter 6**). As mentioned earlier, only small changes in energy intake or energy expenditure are sufficient (75 kcal) to prevent excessive weight gain (**chapter 2**). The presence of a water cooler at school or the availability of more healthy alternatives in vending machines or canteens can already make this difference. Although we did not have the possibility to investigate whether the observed changes in the school food environment resulted in improved dietary behavior or BMI among Dutch children, other research does support such a link [24, 84, 85]. With virtually all children exposed to the school environment for much of their childhood, improving the school food environment has the potential to be an important strategy for overweight prevention in children [24]. With unhealthy food choices marketed heavily to children through advertising in the retail food environment, a school environment that supports healthy dietary behaviors is essential for children to learn them to make healthy food choices [86].

However, the influence of individuals in modifying the food environment is limited. This underlines the necessity of involving all stakeholders in the food system (e.g. government, food production, food marketing, retailers, schools and individuals) to successfully change the food environment [54].

The importance of improving the food environment at schools is widely supported. This is illustrated by the goal of the Dutch Government to have 100% healthy school canteens in 2015 [87, 88]. Recent research showed that schools that participate in the healthy school canteens programme offer more healthy products in their canteens and took more actions to improve dietary behavior than non-participating schools [89]. However, at the same time also the less healthy foods were still available in all schools. The same effects were observed in this thesis (**chapter 6**). Therefore, further reducing the availability of unhealthy foods at schools and also in other environmental settings (e.g. hospitals, homes, workplaces, community) should be supported.

The school setting is also part of the French EPODE (Ensemble Prévenons l'Obésité Des Enfants) community intervention to prevent childhood obesity [90]. EPODE is a model that enables community stakeholders to implement effective and sustainable strategies to prevent childhood obesity. It aims to change environments, behaviors and social norms step by step using social marketing, scientific evaluation, ensuring political support and the involvement of all sectors of the community (schools, families, public and private parties). In the Netherlands, the EPODE approach was adopted and converted into "Jongeren op Gezond Gewicht" (JOGG). Today, 91 Dutch municipalities have implemented JOGG. For several JOGG municipalities a decrease in obesity prevalence among children has been observed [91]. So far, community strategies seem to be an effective approach to combat childhood obesity [92]. Therefore, extending the use of such strategies should be recommended and supported.

Future research

A lot of knowledge has become available about the composition of a healthy diet (e.g. low energy dense) to prevent overweight [68, 73, 93]. Currently, the major challenge for people is to adhere to a healthy diet within our obesogenic environment [77]. The scope of future overweight research should therefore be primarily aimed at improving dietary intake by tackling the food environment.

Although the relevance of the food environment in the development of overweight is more and more acknowledged, strong evidence for this influence is still limited [82, 94]. Further research that provides insight in important modifiable overweight determinants related to the food environment is therefore of great importance. In particular, there is a need for high quality intervention studies to provide more conclusive evidence [24]. First of all, such studies should include measurements of obesity phenotypes as observed changes in diet do not necessarily lead to changes in body weight. Secondly, future research on the role of the food environment should include actual measures of food availability instead of self-reported data only. Finally, it is important to include measurements of dietary behavior, because observed changes in the food environment do not necessarily lead to changes in food consumption. Considering this, it is also relevant to take into account food consumption in other environmental settings than the one that is observed in the study to get insight into possible compensatory behavior [24]. A lot of research has already been performed at schools[24, 95]. Future studies should also be extended to other settings (e.g. hospitals, sport canteens, (check out queues at) shops).

As the power of individuals to change the food environment is limited, it is important to empower people to cope with the obesogenic environment and to control the amount of food they consume[96]. Behavioral strategies, such as 'don't' buy jumbo-sized packages' or 'stop eating when you are satisfied, even if you have not cleared your plate'', could help increase people's ability to self-regulate their amount of food selected and consumed [97]. A recent study shows that such strategies are perceived as feasible in daily life and useful in the weight management of individuals [98]. More research is needed to investigate to what extent behavioral strategies are effective in the long term to control energy intake and the prevention of overweight.

In addition, it is important to start the prevention of overweight as early in life as possible [99]. This requires more insight in relevant modifiable in utero determinants [100]. Whether or not maternal diet during pregnancy can play a relevant role in the early prevention of overweight among children requires more research [101]. Relatively few epidemiological studies have been conducted on this topic so far. More knowledge about the optimal dietary composition during pregnancy is needed [66]. Furthermore, future research should include research on the effects of maternal diet during pregnancy on the child's body weight in the long term,

preferably into adulthood [100]. Moreover, the underlying mechanisms need to be unraveled to answer the question whether the observed associations really reflect in utero programming of body weight [60].

Knowledge about the genetics of overweight provides valuable insight in the etiology of overweight which may be used in the treatment of overweight [56]. Future studies are needed to confirm or falsify the observed associations between genetic variants related to glucose and fatty acid metabolism and BMI and waist circumference **(chapter 2)**. In addition, future genetic studies should focus on the potential epigenetic mechanisms that could be involved in the development of overweight [59]. As the epigenome can be influenced by environmental factors, it is therefore a potential target for prevention [57].

Conclusion

The research described in this thesis shows that small daily changes in energy balance are in the long term responsible for the development of overweight in children and that genetic variation in glucose and fat metabolism affects overweight phenotypes in adults. In general, improving dietary intake can contribute to restoring the energy balance and prevent overweight and its consequences. This thesis studied the relation between maternal fish consumption during pregnancy and BMI in children and the relation between consumption of high energy dense diets and risk of type 2 diabetes among adults, but found no evidence for such relations. This thesis further shows that the food environment at Dutch secondary schools is modifiable and has become less obesogenic in recent years, which is of great public health importance. Creating a supportive food environment can facilitate individuals to adhere to a healthy diet and may suppress the effect of an individual's genetic susceptibility to overweight. Future overweight prevention research should primarily focus on effective strategies that support the healthy choice by tackling the obesogenic environment.

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Summary

Overweight (body mass index (BMI) ≥ 25 kg/m^2) is still one of the most important global public health problems. Overweight is an established risk factor for various serious health disorders including cardiovascular diseases, many types of cancer, musculoskeletal disorders and type 2 diabetes. The primary cause of overweight is a long term disturbance of the energy balance, where energy intake exceeds energy expenditure and the excess energy is stored in the body primarily as fat. Many factors directly or indirectly affect the underlying components of the energy balance. Among those are factors related to nutrient metabolism, maternal diet, individual dietary intake and the food environment, so diet can influence the energy balance in many ways. Although overweight is a preventable condition, to date no country, including the Netherlands, has reversed its obesity epidemic. More insight in relevant determinants of overweight that are related to diet, their effect size and modifiability, may provide clues for prevention. Aim of this thesis was to study the role of a wide range of dietary factors on the development of overweight from a population perspective. The aim of this thesis has been further specified into three objectives.

The first objective was to quantify the energy gap responsible for excess weight gain in Dutch children. The energy gap reflects the excess daily energy intake over the daily energy expenditure that produced weight gain at the population level over a certain period of time. Chapter 2 addresses the estimation of the energy gap responsible for the development or maintenance of overweight during 4-years of follow-up in 2-year old Dutch children from the PIAMA birth cohort (n=2190). Hereby, it is taken into account that part of the positive energy balance is essential for normal growth. We found that 10% of the children were overweight at the age of 5-7 years. For these children, median weight gain during 4-years follow-up was 13.3 kg, as compared to 8.5 kg in the group of children who had a normal weight at the end of the study. A daily energy gap of 75 kcal (e.g. 1 glass of soft drink) was responsible for the excess weight gain in 90% of the children who were overweight at the age of 5-7 years. Based on our results and other research performed on this topic, we concluded that the change in daily energy balance required to stop the trend of increasing prevalence of overweight in Dutch children is small relative to the daily energy intake.

The second objective of this thesis was to investigate possible associations between on the one hand determinants of nutrient metabolism (genetic variants), maternal diet (fish consumption during pregnancy) and individual dietary intake (energy density of diets), and on the other hand overweight and its important consequence type 2 diabetes.

Chapter 3 presents associations between 327 genetic variants in 239 candidate genes from regulatory pathways that control fatty acid and glucose metabolism, and repeated measurements of BMI and waist circumference. In this explorative study we used data of 3575 Dutch adults from the Doetinchem cohort study who were examined 3 times in 11 years. We detected strong associations between adult BMI and/or waist circumference and five single nucleotide polymorphisms (SNPs) located across the *NR1H4, SIRT1, SMARCA2, SCAP* and *IL6* genes. Observed differences in mean BMI between genotypes ranged from 0.4 to 5.9 kg/m^2 and for mean waist circumference from 1.1 to 18.5 cm. Based on our results and findings from whole genome and candidate gene studies, we concluded that the potential roles for *NR1H4, SMARCA2, SCAP* and *IL6* in the development of (abdominal) obesity remain unclear. There is, however, growing evidence for a role of sirtuin 1 (SIRT1) in the regulation of body weight.

Chapter 4 describes the longitudinal association between maternal fish consumption during pregnancy and BMI in children. Fish fatty acids are thought to have an anti-obesogenic effect by blocking the maturation of adipocytes. A previous study showed that children of mothers with higher fish consumption during pregnancy were at lower risk of obesity at 3 years of age. We studied the long term effect of maternal fish consumption during pregnancy on the child's BMI between birth and 14 years of age in 3684 children of the PIAMA birth cohort. BMI was reported 11 times and important maternal and child characteristics were taken into account. We observed that higher maternal fish consumption during pregnancy was associated with a lower BMI in the children at several ages. However, it was likely that other healthy maternal characteristics were responsible for this association, especially a lower maternal BMI before pregnancy. We cannot rule out completely an effect of maternal fish consumption on BMI development in children, because our study was conducted in a population with relatively low fish consumption. Because other studies on long term effects are lacking, we concluded that there is currently no clear evidence to support an independent association between maternal fish consumption during pregnancy and BMI in children up to 14 years of age.

Chapter 5 reports on the effect of energy density of diets and risk of type 2 diabetes. The energy density of daily diets reflects the amount of available energy (kcal or kJ) per unit weight of foods or meals and is mainly determined by fat, water and fibre content. Consumption of diets with a higher energy density is a known risk factor for overweight and may also be associated with risk of type 2 diabetes as observed in a previously conducted small study. In our prospective study with more than 12,000 incident type 2 diabetes cases embedded in a large European population (EPIC-InterAct), we found no association between energy density of the diet and the risk of type 2 diabetes. We concluded that there is currently no conclusive evidence for such an association. However, we found some indication that methodological issues such as overweight related underreporting of dietary intake may have obscured a

positive association between dietary energy density and risk of type 2 diabetes, and that such an association, if any, would most likely be small.

The third objective of this thesis was to assess changes in the Dutch school food environment. Previous research showed that unhealthy drinks and foods were widely available at Dutch secondary schools and only a small proportion of schools had a policy on the prevention of overweight in 2006/2007. Recently, in the Netherlands several national policies were launched or intensified to promote changes in the school food environment. However, little scientific evidence was available as to whether such policies are effective.
Chapter 6 describes four year changes in the food environment at Dutch secondary schools (n=187) based on data from two nation-wide surveys. We observed favorable changes in the food supply, attitudes and beliefs regarding overweight and policies on health and nutrition at schools between 2006/2007 and 2010/2011. For example, a higher proportion of Dutch secondary schools reported that vending machines and canteens contain a more favorable balance between healthy and unhealthy foods and drinks after four years of follow-up. The presence of water coolers increased (12% vs 33%). More schools indicate that it is forbidden to sell certain unhealthy foods in the canteen (56% vs 38%) and that parents are informed about unhealthy dietary behavior of their child (18% vs 9%). However, less favorable developments were also observed after four years of follow-up. For example, more schools had vending places of unhealthy foods in their vicinity (73% vs 85). Only a small, statistically non-significant, increase in the proportion of schools with a policy on (healthy) nutrition was found (57% vs 49%). There were no differences in the opinion of schools about the responsibility for the prevention of overweight among students. About 40% of schools considered themselves partly responsible. We conclude that our results suggest that the food environment at Dutch secondary schools has become less obesogenic. However, schools should be encouraged to contribute to the prevention of overweight, or to continue to do so.

The public health relevance of the findings presented in this thesis and directions for future research are discussed in chapter 7. Our finding of a small energy gap in children (chapter 2) shows that on the population level restoring the energy balance does not necessarily require large behavioral changes, like consuming 1 glass of soft drink per day less or increase walking for about half an hour per day. This suggests that prevention of excess weight gain and subsequently overweight should be feasible. Today many associations between genetic variants and overweight measures have been reported (including chapter 3). However, knowledge about relevant genetic risk factors is still limited. In addition, genetic factors cannot be modified. Therefore, practical use of the knowledge about the genetics of overweight in developing a strategy to prevent overweight has been limited so far. Im-

proving the in utero nutrient environment through an optimal dietary intake by pregnant women is thought to be a potential dietary strategy to prevent overweight. So far, scientific evidence for a role of maternal diet during pregnancy in the development of overweight among children is scarce. Based on the limited number of studies conducted, there is no evidence that the recommendation to pregnant women to eat fish (chapter 4) or other specific diets, foods or nutrients in general can prevent overweight in their children. Another dietary strategy to prevent overweight is improving individual dietary intake. Strategies focusing on the reduction of energy density and portions sizes of food are likely to be more effective than those targeting specific macronutrients. To date, there is no conclusive evidence for an association between dietary energy density and risk of type 2 diabetes (chapter 5). However, choosing low energy dense foods should also be promoted to people with type 2 diabetes as they support current recommendations of the World Health Organization to prevent chronic diseases. Although dietary behavior of individuals is modifiable, most people find it hard to change their dietary behavior and to maintain a healthier diet on the long term. This emphasizes the importance of making adherence to a healthy diet easier by, for example, creating a supportive environment that contributes to closing the energy gap. This thesis provides evidence that the food environment at schools is modifiable and has become less obesogenic at several aspects in recent years (chapter 6). There is supportive evidence that changes in the school food environment can result in a more favorable dietary intake and BMI among children. However, more high quality interventions are needed to provide conclusive evidence. The influence of individuals in modifying the food environment is limited. This underlines the necessity of involving all stakeholders in the food system (e.g. government, food production, food marketing, retailers, schools and individuals) to successfully change the food environment. Nowadays, a lot of knowledge has become available about the composition of a healthy diet. Currently, the major challenge for people is to adhere to a healthy diet within our obesogenic environment. The scope of future overweight research should therefore be primarily aimed at improving dietary intake by tackling the food environment.

Overall conclusion of the thesis entitled "Diet and Overweight. Epidemiological studies on intake, environment and genetics" by Saskia van den Berg, 2016.

The research described in this thesis shows that small daily changes in energy balance are in the long term responsible for the development of overweight in children and that genetic variation in glucose and fat metabolism affects body mass index and waist circumference in adults. In general, improving dietary intake can contribute to restoring the energy balance and prevent overweight and its consequences. This thesis studied the relation between maternal fish consumption during pregnancy and body mass index in children and the relation between consumption of high energy dense diets and risk of type 2 diabetes among adults, but found no evidence for such relations. This thesis further shows that the food environment at Dutch secondary schools is modifiable and has become less obesogenic in recent years, which is of great public health importance. Creating a supportive food environment can facilitate individuals to adhere to a healthy diet and may suppress the effect of an individual's genetic susceptibility to overweight. Future overweight prevention research should primarily focus on effective strategies that support the healthy choice by tackling the obesogenic environment.

Samenvatting

Overgewicht (body mass index (BMI) ≥ 25 kg/m^2) is nog steeds één van de grootste volksgezondheidsproblemen. Wereldwijd komt overgewicht veel voor. Mensen met overgewicht hebben een hoger risico op verschillende ernstige gezondheidsproblemen waaronder hart- en vaatziekten, verschillende soorten kanker, spier- en gewrichtsproblemen en type 2 diabetes. De primaire oorzaak van overgewicht is een lange termijn verstoring van de energiebalans, waarbij de energie-inname groter is dan het energieverbruik. Het teveel aan energie wordt, voornamelijk als vet, opgeslagen in het lichaam. Veel factoren beïnvloeden, direct of indirect, de onderliggende componenten van de energiebalans (inname, verbruik en opslag). Voeding speelt een belangrijke rol. Hierbij valt te denken aan factoren die te maken hebben met het verteren en opslaan van voedingsstoffen (stofwisseling of metabolisme), de voedselconsumptie van aanstaande moeders tijdens de zwangerschap, het voedingspatroon van een individu zelf, en de beschikbaarheid en aanbod van voedsel in de omgeving. Voeding kan dus op veel verschillende manieren de energiebalans beïnvloeden. Overgewicht kan voorkómen worden. Tot op heden is het echter nog niet gelukt om de overgewichtsepidemie te keren. Meer inzicht in belangrijke risicofactoren voor overgewicht die samenhangen met voeding, hun effectgrootte en veranderbaarheid levert mogelijk aanwijzingen op voor preventie. Het doel van dit proefschrift was om de rol van een breed scala aan voedingsfactoren bij het ontstaan van overgewicht te bestuderen vanuit een populatieperspectief. Dit doel is verder gespecificeerd in 3 onderliggende doelstellingen.

De eerste doelstelling was om de energiekloof, verantwoordelijk voor het teveel aan gewichtstoename bij Nederlandse kinderen, te berekenen. De energiekloof geeft het dagelijks overschot aan energie-inname ten opzichte van energieverbruik weer dat verantwoordelijk is geweest voor de gewichtstoename in een populatie over een bepaalde tijdsperiode.
Hoofdstuk 2 beschrijft de schatting van de energiekloof verantwoordelijk voor het ontstaan of behoud van overgewicht gedurende vier jaar voor tweejarige Nederlandse kinderen afkomstig uit het PIAMA geboortecohort (n=2190). Bij deze schatting is rekening gehouden met het feit dat een deel van de positieve energiebalans noodzakelijk was voor normale groei. We vonden dat op 5-7 jarige leeftijd 10% van de kinderen overgewicht had. Deze groep kinderen had een mediane[1] gewichtstoename van 13,3 kg in vier jaar tijd. Voor de groep kinderen met een normaal gewicht aan het eind van de studie was de gewichtstoename 8,5 kg. Een dagelijkse energiekloof van 75 kcal (≈ 1 glas frisdrank) was verantwoordelijk voor het teveel aan gewichtstoename bij 90% van de kinderen met overgewicht op 5-7 jarige leeftijd.

[1] Mediaan is de waarde waar 50% van de groep boven en 50% van de groep onder zit.

Gebaseerd op onze resultaten en ander onderzoek uitgevoerd op dit terrein concludeerden we dat de verandering in de dagelijkse energiebalans die nodig is om de toename van het aantal kinderen met overgewicht in Nederland te stoppen relatief klein is ten opzichte van de dagelijkse energie-inname.

De tweede doelstelling van dit proefschrift was om mogelijke verbanden te onderzoeken tussen factoren die te maken hebben met het nutriëntenmetabolisme (genetische factoren), voedselconsumptie van aanstaande moeders (visconsumptie tijdens de zwangerschap) en het voedingspatroon van individuen zelf (energiedichtheid van de voeding) enerzijds, en overgewicht en de belangrijkste complicatie namelijk type 2 diabetes, anderzijds.
Hoofdstuk 3 beschrijft de samenhang tussen 327 genetische variaties in 239 kandidaatgenen die een rol spelen bij het regelen van de vetzuur- en glucosestofwisseling en BMI en middelomtrek. Voor deze verkennende studie hebben we gegevens gebruikt van 3575 Nederlandse volwassenen afkomstig uit de Doetinchem cohortstudie die drie keer onderzocht zijn in 11 jaar tijd. Wij vonden sterke verbanden tussen BMI en middelomtrek en vijf genetische variaties in het *NR1H4, SIRT1, SMARCA2, SCAP* en *IL6* gen. De verschillen in de gemiddelde BMI tussen de genotypen varieerden van 0,4 tot 5,9 kg/m^2 en voor de gemiddelde middelomtrek van 1,1 tot 18,5 cm. Gebaseerd op onze resultaten en de bevindingen uit andere studies, waaronder genoombrede associatiestudies, concludeerden wij dat de rol van *NR1H4, SMARCA2, SCAP* en *IL6* bij de ontwikkeling van (abdominaal) obesitas onduidelijk blijft. Er is echter wel toenemend bewijs voor een rol van sirtuin 1 (SIRT1) bij de regulatie van het lichaamsgewicht.
Hoofdstuk 4 beschrijft het verband tussen visconsumptie door aanstaande moeders tijdens de zwangerschap en de BMI ontwikkeling van hun kinderen. Visvetzuren beschermen mogelijk tegen overgewicht door het blokkeren van de rijping van vetcellen van de foetus. Een eerdere studie liet zien dat op drie jarige leeftijd kinderen van moeders met een hogere visconsumptie tijdens de zwangerschap een lager risico op ernstig overgewicht (obesitas: BMI $\geq$ 30 kg/m^2) hadden. Wij onderzochten het lange termijn effect van visconsumptie door zwangere vrouwen op de BMI van hun kinderen tussen geboorte en 14 jarige leeftijd bij 3684 kinderen afkomstig uit het PIAMA geboortecohort. De BMI werd 11 keer nagevraagd en er werd gecorrigeerd voor belangrijke andere karakteristieken van moeder en kind. We vonden dat een hogere visconsumptie door moeders tijdens de zwangerschap samenhing met een lagere BMI van hun kinderen op verschillende leeftijden. Het was echter aannemelijk dat andere gezondheidskarakteristieken van de moeder verantwoordelijk waren voor dit verband, voornamelijk een lagere BMI van de moeder zelf voor de zwangerschap. Wij kunnen een effect van visconsumptie door zwangere vrouwen op de BMI ontwikkeling van hun kind echter niet volledig uitsluiten omdat onze studie is uitgevoerd binnen een populatie waarin relatief weinig vis gegeten wordt. Omdat er geen andere lange termijn studies uitgevoerd zijn, hebben wij geconcludeerd dat er op dit moment

geen duidelijk bewijs is voor een onafhankelijk verband tussen visconsumptie van zwangere vrouwen en de BMI van hun kinderen.
Hoofdstuk 5 beschrijft de relatie tussen de energiedichtheid van de voeding en het krijgen van type 2 diabetes. De energiedichtheid van de dagelijkse voeding geeft de hoeveelheid beschikbare energie (kcal of kJ) per eenheid gewicht van de gegeten voedingsmiddelen en maaltijden weer en wordt voornamelijk bepaald door de hoeveelheid vet, water en vezel. Het eten van voedsel met een hoge energiedichtheid verhoogt het risico op het krijgen van overgewicht en hangt mogelijk ook samen met het risico op type 2 diabetes zoals in een eerder uitgevoerde kleine studie werd gevonden. In onze studie met meer dan 12,000 personen met type 2 diabetes afkomstig uit een grote Europese studie (EPIC-InterAct), vonden we geen verband tussen de energiedichtheid van de voeding en het risico op het krijgen van type 2 diabetes. We hebben geconcludeerd dat er op dit moment geen overtuigend bewijs is voor een dergelijk verband. Beperkingen in de gebruikte onderzoeksmethode, zoals onderrapportage van de voedselconsumptie door personen met overgewicht, zouden een mogelijke positief verband tussen de energiedichtheid van de voeding en het risico op type 2 diabetes echter vertroebeld kunnen hebben. Als een dergelijke relatie wel bestaat is die waarschijnlijk zeer klein.

De derde doelstelling van dit proefschrift was het onderzoeken van veranderingen in de voedingsomgeving op Nederlandse scholen. Eerder onderzoek uitgevoerd in de periode 2006/2007 heeft laten zien dat ongezonde voedingsmiddelen en dranken ruim beschikbaar waren op middelbare scholen in Nederland en dat maar een klein deel van de scholen beleid voert op de preventie van overgewicht. Recentelijk is in Nederland beleid geïmplementeerd of geïntensiveerd op het gebied van de preventie van overgewicht dat, onder andere, veranderingen in de voedingsomgeving op scholen stimuleert. Er is echter weinig wetenschappelijk bewijs voor de effectiviteit van dergelijke beleidsaanpassingen.
Hoofdstuk 6 beschrijft veranderingen in de voedingsomgeving op Nederlandse middelbare scholen (n=187) in vier jaar tijd, gebaseerd op gegevens afkomstig uit twee nationale enquêtes. Wij vonden gunstige veranderingen in het voedselaanbod, houdingen en opvattingen ten aanzien van overgewicht en beleid op het gebied van gezondheid en voeding op middelbare scholen tussen 2006/2007 en 2010/2011. Meer scholen gaven na vier jaar bijvoorbeeld aan dat automaten en kantines een aanbod hebben met een gunstigere balans tussen gezonde en ongezonde voedingsmiddelen en dranken. Er was een toename in de aanwezigheid van waterkoelers op scholen (12% vs 33%). Meer scholen gaven daarnaast aan dat het verboden is om bepaalde ongezonde voedingsmiddelen te verkopen in de schoolkantine (56% vs 38%) en dat ouders aangesproken worden op ongezond eetgedrag van hun kind (18% vs 9%). Echter, minder gunstige ontwikkelingen werden ook gezien in deze vier jaar. Meer scholen

hadden bijvoorbeeld een verkooppunt van ongezond voedsel in hun nabije omgeving (73% vs 85%). Er werd maar een klein (niet statistisch significant) verschil gevonden in het aantal scholen dat beleid voert op (gezonde) voeding (57% vs 49%). Er was na vier jaar geen verschil in de opvatting van scholen over de verantwoordelijkheid voor de preventie van overgewicht onder scholieren. Ongeveer 40% van de scholen beschouwde zichzelf als gedeeltelijk verantwoordelijk. We hebben geconcludeerd dat onze resultaten suggereren dat de voedingsomgeving op middelbare scholen minder obesogeen is geworden. Het blijft belangrijk dat scholen worden aangemoedigd om een bijdrage te leveren aan de preventie van overgewicht of om hiermee door te gaan.

Het belang voor de volksgezondheid van de onderzoeksresultaten beschreven in dit proefschrift en richtingen voor toekomstig onderzoek zijn bediscussieerd in hoofdstuk 7. Onze bevinding van een kleine energiekloof bij kinderen (hoofdstuk 2) laat zien dat de veranderingen in gedrag die noodzakelijk zijn om de energiebalans op populatieniveau te herstellen niet groot zijn. Hierbij valt te denken aan het drinken van één glas frisdrank minder per dag of 30 minuten meer lopen per dag. Dit suggereert dat preventie van een ongewenste gewichtstoename haalbaar zou moeten zijn. Vandaag de dag is veelvuldig aangetoond dat genetische verschillen tussen mensen kunnen leiden tot verschillen in BMI en/of middelomtrek (ook in hoofdstuk 3). De kennis over relevante genetische variaties die een rol spelen bij de gevoeligheid voor overgewicht is echter nog steeds beperkt. Daarnaast zijn genetische factoren niet veranderbaar. De praktische toepassing van de kennis over erfelijke factoren voor overgewicht bij het voorkómen van overgewicht is daarom op dit moment beperkt. Het verbeteren van de nutriëntenvoorziening in de baarmoeder door een optimale voedselconsumptie door zwangere vrouwen wordt gezien als een mogelijke strategie om overgewicht te voorkómen. Wetenschappelijk bewijs voor een verband tussen voedselconsumptie van zwangere vrouwen en het ontstaan van overgewicht bij hun kinderen is tot nu toe schaars. Gebaseerd op het bewijs uit het beperkte aantal studies dat over dit onderwerp is uitgevoerd, is er op dit moment geen reden om zwangere vrouwen aan te bevelen vis te eten (hoofdstuk 4), of andere specifieke diëten, voedingsmiddelen of nutriënten aan te raden ter voorkóming van overgewicht bij hun kinderen. Een andere strategie om overgewicht te voorkómen is het verbeteren van het individuele voedingspatroon. Strategieën die zich richten op het verlagen van de energiedichtheid en portiegroottes van voedingsmiddelen zijn waarschijnlijk effectiever dan strategieën die zich richten op specifieke macronutriënten. Tot op heden is er geen overtuigend bewijs voor een verband tussen de energiedichtheid van de voeding en het risico op type 2 diabetes (hoofdstuk 5). Echter, het eten van voedingsmiddelen met een lage energiedichtheid zou toch moeten worden geadviseerd aan mensen met type 2 diabetes omdat dit in lijn is met de huidige aanbevelingen ter preventie van chronische ziekten

van de Wereldgezondheidsorganisatie. Ondanks het feit dat voedingsgedrag veranderbaar is, vinden veel mensen het lastig om hun ongezonde eetgewoontes aan te passen en dit op de lange termijn vol te houden. Daarom is het belangrijk om het bewerkstelligen en het volhouden van een gezond voedingspatroon makkelijker te maken door bijvoorbeeld een omgeving te creëren die gezonde keuzes stimuleert en zo kan bijdragen aan het dichten van de energiekloof. Dit proefschrift levert bewijs dat de voedingsomgeving op scholen veranderbaar is en dat de schoolomgeving op een aantal aspecten minder obesogeen geworden is in de afgelopen jaren (hoofdstuk 6). Daarnaast hebben andere studies laten zien dat verbeteringen in de voedingsomgeving op scholen kunnen resulteren in een gunstigere voedselconsumptie en BMI van kinderen. Er zijn echter meer kwalitatief goede interventiestudies nodig om hiervoor overtuigend bewijs te leveren. De invloed van individuen om hun leefomgeving te veranderen is beperkt. Dit benadrukt het belang van het betrekken van alle partijen betrokken bij het voedselsysteem (e.g. overheid, voedselproducenten, markerteers, verkopers, scholen en individuen) om de voedingsomgeving succesvol te verbeteren. Vandaag de dag is er veel kennis beschikbaar over de samenstelling van een gezond voedingspatroon. De grootste uitdaging voor mensen op dit moment is echter om een gezond voedingspatroon vol te houden in onze obesogene samenleving. Toekomstig onderzoek zou zich daarom vooral moeten richten op het verbeteren van de voedselconsumptie door het aanpakken van de obesogene omgeving.

Conclusie van het proefschrift getiteld "Voeding en Overgewicht. Epidemiologische studies naar inname, omgeving en genen" geschreven door Saskia van den Berg, 2016.

Het onderzoek beschreven in dit proefschrift laat zien dat kleine dagelijkse veranderingen in de energiebalans op de lange termijn verantwoordelijk zijn voor het ontwikkelen van overgewicht bij kinderen en dat genetische verschillen in glucose en vetmetabolisme tussen mensen samenhangen met BMI en middelomtrek op volwassen leeftijd. Het is bekend dat het verbeteren van het voedingspatroon kan bijdragen aan het herstellen van de energiebalans en het voorkómen van overgewicht. In dit proefschrift is het verband onderzocht tussen visconsumptie door moeders tijdens de zwangerschap en de BMI van hun kinderen en het verband tussen het eten van een voeding met een hoge energiedichtheid en het risico op type 2 diabetes bij volwassenen. Voor beide verbanden werd geen bewijs gevonden. Dit proefschrift laat verder zien dat de voedingsomgeving op Nederlandse middelbare scholen veranderbaar is en dat de schoolomgeving in de afgelopen jaren minder obesogeen geworden is. Dit is van groot belang voor de volksgezondheid. Het creëren van een ondersteunende voedingsomgeving kan mensen faciliteren om gezond te eten en onderdrukt mogelijk iemands genetische gevoeligheid voor overgewicht. Toekomstig onderzoek zou zich hoofdzakelijk moeten richten op effectieve strategieën om gezonde voedselkeuzes te stimuleren door het aanpakken van de obesogene omgeving.

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Saskia

About the author

Saskia van den Berg was born in Amersfoort, the Netherlands on April 17, 1979. After completing secondary school she studied Nutrition and Dietetics at Amsterdam University of Applied Sciences (Hogeschool van Amsterdam) from 1996-2000, and obtained her Bachelor of Science degree. Subsequently she studied Nutrition and Health at Wageningen University from 2000-2002, and obtained her Master of Science degree with a major in epidemiology.
After her graduation she started working as a researcher at the National Institute for Public Health and the Environment (RIVM), Center for Nutrition and Health in Bilthoven, The Netherlands. At the RIVM she performed epidemiological research on a wide range of topics within the area of diet and overweight. Key words regarding this work are (in random order): energy gap, low-fat and low-carbohydrate diets, dietary energy density, snacks, diet products, genetics, birth weight, maternal food consumption, weight cycling 'jojo-en', food pricing policy, and school food environment. The work described in this thesis is a result of several of these activities.

Saskia completed her thesis under the supervision of prof. dr. H.A. Smit (Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht) and dr. ir. J.MA. Boer (RIVM). As part of the PhD Epidemiology programme she attended the courses 'meta-analysis', 'public health epidemiology' and 'prognostic research' at the Utrecht University Graduate School of Life Sciences, the course 'longitudinal data-analysis' at the VU Medical Center, and the course 'pregnancy and programming and later risk of obesity' at the University of Copenhagen in Denmark. In addition, she presented part of the work described in this thesis at national and international conferences, such as the European (2009) and International (2010) Congress on Obesity, the European Congress of Epidemiology (2013) and the Power of Programming conference (2014).

After completion of this PhD thesis, Saskia will continue her work as a researcher at the Centre for Nutrition, Prevention and Health Services at the RIVM. Her current research activities focus on the effects of diet on Attention Deficit Hyperactivity Disorder (ADHD) symptoms in children and on monitoring selected key indicators of sports and physical activity.

Saskia is married to Frister Tammenga. They live in Amersfoort together with their daughters Meike (2009) and Nyne (2012).

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