Making lifestyle work : long-term effects in the prevention of type 2 diabetes

Making Lifestyle work

Long-term effects in the prevention of type 2 diabetes

The study presented in this thesis was performed within the Nutrition and Toxicology Research Institute Maastricht (NUTRIM) which participates in the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences), accredited by the Royal Netherlands Academy of Arts and Sciences.

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DISSERTATION

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De tijd

Soms droom ik dat zij stilstaat
Om van haar te genieten
Het moment dat nooit voorbij gaat

En andere keren hoop ik
Dat zij sneller vooruit loopt
Naar daar waar ik niet weet
Maar vooruit zodat ik vergeet

Heel langzaam of heel snel
Een ding weet ik wel
Hoe ik haar bekijk
Verandert niet haar voortbestaan
Zij zal niet bij mij blijven
Zij zal ook vergaan

Maar haar effect is niet onbemerkt
Zij raakt mij altijd even aan
In dat wat ik voel, wat ik denk
En hoe ik me beweeg
Met gevoel blijf ik achter
Soms vol, soms leeg

Content

1

GENERAL INTRODUCTION

Diabetes mellitus is a public health problem, which has reached pandemic proportions. The global prevalence of diabetes is expected to increase with 39% between 2000 and 2030, increasing absolute numbers to 366 million people (1).

Diabetes mellitus is a multi-factorial disease, characterized by hyperglycemia, resulting from a disturbed insulin secretion, insulin action or both. The long-term effects of diabetes mellitus include increased risk on complications such as foot ulcers, retinopathy, nephropathy and neuropathy. Even more problematic is the fact that 70-80% of people with diabetes die of cardiovascular disease (2). From a public health perspective, diabetes and its complications are relevant for treatment and prevention due to the related human suffering and disability and the huge socio-economic costs (3, 4) through premature morbidity (5) and mortality (6, 7). Clearly, there would be great benefits if research could provide evidence for effective prevention measures. The introduction of this thesis is composed of three main sections. First, the diagnosis and pathogenesis of type 2 diabetes are discussed, including the impaired glucose tolerance state (paragraph 1). The second section focuses on the prevention of type 2 diabetes by the use of different lifestyle intervention strategies and includes some aspects of implementation (paragraph 2). In the third section several mechanisms are discussed that may underlie the development of type 2 diabetes and that may be responsible for the lifestyle intervention-induced improvement in metabolic profile (paragraph 3). Finally, an outline is given of the chapters covering the present thesis (paragraph 4).

1. Type 2 Diabetes Mellitus

Several forms of diabetes exist of which type 1 and type 2 diabetes mellitus are the most common. Type 1 diabetes mellitus accounts for approximately 15% of all diabetes cases and results from pancreatic beta-cell dysfunction, causing insulin deficiency, and leading to the requirement of exogenously administrated insulin for survival. Type 2 diabetes mellitus is the most common form of diabetes accounting for approximately 85% of all cases. Type 2 diabetes mellitus results when pancreatic beta-cells fail to compensate for insulin resistance through an increased secretion of insulin by the beta-cells of the pancreas. Already several years ago, large scale analysis in 22 cohorts in Europe by the Diabetes Epidemiology Collaborative analysis Of Diagnosis criteria in Europe (DECODE) study group, showed a J shaped relationship, rather than showing a threshold effect, between high fasting glucose levels and all-cause mortality (8). Interestingly, all-cause mortality risks were similar in subjects with fasting glucose levels ≥ 7.0 mmol/l and in subjects with high 2-hr glucose levels of 10.01-11.09 mmol/l, when testing almost 30,000 people for their hazard ratio for death. Compared to the J shape of fasting glucose, the relationship between 2-hr glucose levels and cardiovascular mortality seemed to be graded and increasing (8). Overall, there seems to be a continuously graded risk for mortality with increasing glucose levels.

1.1 Diagnosis

In general, diagnosis is based upon the presence of clinical symptoms and gross hyperglycemia during a visit at the general medical practice. Indications for a visit at the general medical practice include diabetes symptoms like itching, tiredness, blurred vision and unintended weight loss. However, at present, health checks may be a reason for a visit. In the prediabetic state, in which symptoms are mostly absent, an oral glucose tolerance test (OGTT) can be performed (9). This is a blood glucose measurement during fasting and 2 hours after a 75-gram glucose bolus, dissolved in 250 ml water. According to the WHO, results can be interpreted as normal glucose tolerance (NGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and type 2 diabetes (table 1.1).

Table 1.1 WHO criteria for the diagnosis of impaired glucose metabolism (1999).

	WHO 1998			WHO 1985		
	Whole blood	Venous plasma	Capillary* (whole blood)	Whole blood	Venous plasma	Capillary (whole blood)
Diabetes mellitus						
Fasting or	≥ 6.1 (110)	≥ 7.0 (126)	≥ 6.1 (110)	≥ 6.7 (120)	≥ 7.8 (140)	≥ 6.7 (120)
2-hr glucose	≥ 10.0 (180)	≥ 11.1 (200)	≥ 11.1 (200)	≥ 10.0 (180)	≥ 11.1 (200)	≥ 11.1 (200)
Impaired glucose tolerance						
Fasting and	< 6.1 (110)	< 7.0 (126)	< 6.1 (110)	< 6.7 (120)	< 7.8 (140)	< 6.7 (120)
2-hr glucose	≥ 6.7 (120)	≥ 7.8 (140)	≥ 7.8 (140)	≥ 6.7 (120)	≥ 7.8 (140)	≥ 7.8 (140)
Impaired fasting glucose						
Fasting and	≥ 5.6 (100) < 6.1 (110)	≥ 6.1 (110) < 7.0 (126)	≥ 5.6 (100) < 6.1 (110)			
(if measured) 2-hr glucose	< 6.7 (120)	< 7.8 (140)	< 7.8 (140)			

1.2 Pathogenesis

In the pathogenesis of type 2 diabetes, two key features play a role, namely insulin resistance and beta-cell failure. Insulin resistance is characterized by the diminished ability of insulin to stimulate glucose uptake. Beta-cell failure is characterized by the inability to adequately produce sufficient amounts of the insulin. In the development of type 2 diabetes, which takes several decades, impairments in both features are believed to result in a transition from normal glycemia to hyperglycemia and hyperinsulinemia (10). Mild hyperglycemia can be classified as IFG when present in the fasted state and as IGT when present in the postprandial state (11). Subsequent deterioration of insulin resistance and beta-cell failure will lead to overt type 2 diabetes mellitus (figure 1.1) (11, 12). Insulin resistance and beta-cell failure are both under the influence of genetic variation and other factors, such as dietary intake, physical activity,

obesity. The relative contribution of these factors is strongly influenced by the patient population, e.g. ethnicity and age. With increasing age the risk for developing type 2 diabetes also increases.

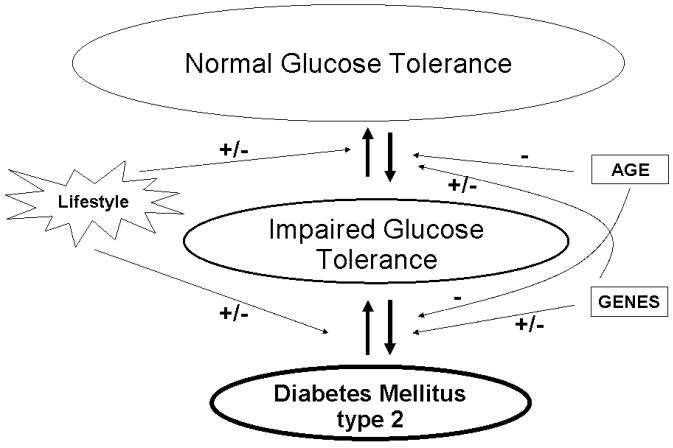

Figure 1.1 Progression from normal glucose tolerance to impaired glucose tolerance and type 2 diabetes mellitus (adapted from Saad et al. A two-step model for development of non-insulin-dependent diabetes. American Journal of Medicine. 1991;90(2):229-35). The progression can be effected by older age, genes and lifestyle, while changes towards a healthy lifestyle can delay or (partly) reverse the process.

1.3 Impaired glucose tolerance

The global prevalence of IGT is expected to increase from 314 million people in 2003 to 472 million in 2025, accounting for approximately 9% of the adult worldwide population (2). Depending on the characteristics of the population, e.g. age, BMI and ethnicity, the prevalence may range between 2% in rural areas and 20% in high-risk populations (13).

IGT is a strong predictor for the development of type 2 diabetes mellitus. In the Dutch Hoorn study, IGT, IFG and IGT/IFG combined predict the development of type 2 diabetes over 6 years with 9.1% progression for IFG, 32.5% progression for IGT and 64.5% for combined IGT/IFG (14). Besides the risk for development of type 2 diabetes, a graded positive relationship is also observed between postprandial glucose levels and cardiovascular mortality (8).

1.4 Risk factors for diabetes progression

Risk factors for the progression towards type 2 diabetes include lifestyle components such as body weight, dietary composition, and physical activity. Obesity is the most modifiable risk factor and BMI and body weight gain are strongly associated with diabetes risk (15). In addition to over-all obesity, body fat distribution and especially increased intra-abdominal fat mass is a predictor of type 2 diabetes (16, 17). Specifi-

cally an increased visceral fat depot, as shown by using magnetic resonance imaging and computed tomography, has been shown to be related to the presence of insulin resistance (18-21). Besides obesity, total fat intake and saturated fat intake are important risk factors for the development of type 2 diabetes. The energy-density of fat favors increased food intake and obesity. In the San Louis Valley Diabetes Study, a 40g higher fat intake corresponded with a 6-fold increase in diabetes risk in IGT subjects after adjustment for obesity and markers of glucose metabolism (i.e. fasting glucose and insulin) (22). Also, the kind of dietary fats seem to influence postprandial glucose metabolism and insulin resistance. The KANWU study reported an improved insulin sensitivity when replacing saturated fatty acids in the diet with mono-unsaturated fat in healthy volunteers during a period of 3 months, when a habitual diet low in fat was consumed. (23). In a recent study in subjects with normal glucose and triglyceride levels, beta cell function and insulin sensitivity progressively improved in the postprandial state as the proportion of mono unsaturated fatty acids as compared to saturated fatty acids in dietary fats increased (24). Also, a low glycemic-index diet and low glycemic load in type 2 diabetes patients during 6 months seems to moderately reduce body weight and HbA1c levels and increase high-density lipoprotein-cholesterol (HDL-cholesterol) (25). Besides the diet also other lifestyle factors influence diabetes risk. A recent systematic review and meta-analysis has shown that active smoking is associated with an increased risk for type 2 diabetes, with a greater risk for heavy smokers compared to light smokers (26). The causality and mechanism still need to be unraveled. Also lack of physical activity influences diabetes progression. Increasing physical activity has shown to decrease diabetes risk. For example, in IGT men from the Diabetes Prevention Study (DPS), individuals with the highest increase in their level of moderate-to-vigorous physical activity were 63-65% less likely to develop type 2 diabetes during the 4.1 year follow-up compared to individuals with the lowest increase in physical activity (27).

2. Prevention of Type 2 Diabetes

Over the past decades, several lifestyle intervention studies have been performed trying to reveal if, how and to what extent these programs could postpone or prevent deterioration of glucose tolerance and progression towards type 2 diabetes mellitus. These lifestyle intervention studies are composed of dietary intervention, exercise intervention, or the combination of diet and exercise.

2.1 The SLIM study: design, purpose and previous results

The SLIM study was designed to study the effect of a lifestyle intervention according to general public health recommendations regarding dietary intake and composition and physical activity on Dutch subjects at high risk for developing type 2 diabetes mellitus. Therefore, 147 subjects were randomized into an intervention and control group with stratification for sex and 2-hr glucose levels. Details of the design and screening has been described previously (28). A previous publication of this study show that the largest decrease in body weight and 2-hr glucose levels was observed after 1 year of

lifestyle intervention (29). Is was also shown that after 2 years, adherence to the diet and exercise recommendations gave the largest improvement in body weight, waist circumference, fasting insulin and 2-hr glucose levels, compared to adherence to the diet or exercise recommendations alone (29). This thesis describes the effects of the SLIM lifestyle intervention after 3-6 years as well as additional analyses on metabolic factors possibly associated with glucose tolerance, insulin resistance or the development of type 2 diabetes.

2.2 Dietary intervention

Several dietary components are related to body weight, insulin resistance and the development of type 2 diabetes (for more detail, see chapter 2). In normalweight and overweight subjects, ad libitum reduced-fat diet without energy restriction resulted in a moderate body weight reduction between 3-5 kilograms (30, 31). Besides reducing body weight and sustaining a body weight reduction, a high-fiber, low-fat diet also reduces the development of type 2 diabetes (32). In addition, the type of fatty acid may modify insulin sensitivity (23) and diabetes risk, e.g. by positive effects on skeletal muscle insulin sensitivity (33-35). In this thesis, we examined if dietary advice as part of a lifestyle intervention according to general public health recommendations has an effect on the dietary composition, glucose tolerance, insulin resistance and diabetes risk, and whether these changes are prolonged during a period of 3-6 years.

2.3 Exercise intervention within a lifestyle intervention

A recent meta-analysis has shown that lifestyle interventions aimed at increasing exercise combined with diet are able to decrease the incidence of type 2 diabetes mellitus in high risk groups (36) (for more detail, see chapter 2). Vigorous physical activities seem to give the largest reduction in diabetes risk, whereas also small but sustained increases in physical activity seem beneficial in the long-term (27, 37). There seems to be a dose-response relationship between physical activity and fitness (38), exemplifying that even a little exercise is good for health but that increasing exercise may even be better. Besides reducing diabetes risk and increasing fitness, physical activity may also prevent weight regain after weight loss. Currently, approximately 420 minutes of moderate intensity exercise (30 minutes a day) is recommended to prevent weight gain in overweight subjects (39). In this thesis, we examined if exercise recommendations and a physical activity program consisting of combined aerobic and resistance training, as part of a lifestyle intervention according to general public health recommendations, had an effect on maximal aerobic capacity, glucose tolerance, insulin resistance and diabetes risk and whether these changes are prolonged during a period of 3-6 years.

2.4 Diet and exercise intervention

Several combined diet and exercise lifestyle interventions have shown to reduce diabetes risk by ~40-60% during 3-6 years in European (40-42) and non-European IGT

people (43, 44) (for more detail, see chapter 2). Recently, the first studies have been published showing a significant diabetes reduction of 43% following 3-14 years cessation of the lifestyle intervention (45, 46). The effect of combined diet and exercise lifestyle interventions on 2-hr glucose levels has been less consistent (47, 48) (for more detail, see chapter 2).

Although combined diet and exercise lifestyle interventions have proven to be effective in reducing diabetes risk, it is not know if these lifestyle interventions can be effectively implemented in the general population. Currently, different approaches for lifestyle implementation are evaluated (49), which is a complex process that involves multiple factors that have to be aligned to achieve success. In chapter 2, more details regarding implementation are stated.

In this thesis, we assessed which factors are related to adherence to the diet and exercise recommendations and which factors are indicative for dropout to the lifestyle intervention program. Identification of these factors is the first step that can lead to increased effectiveness and efficacy of lifestyle interventions and can help to clarify difficulties that may interfere with successful implementation in the general population. In the next paragraph, metabolic mechanisms of importance in the etiology of diabetes, and thus possibly responsible for the lifestyle intervention effect, will be covered.

3. Metabolic disturbances associated with the development of type 2 diabetes

3.1 Ectopic fat accumulation

In the etiology of type 2 diabetes mellitus, several major organs and their interactions play a role like adipose tissue, liver, skeletal muscle and pancreas. Adipose tissue is the major organ for triglyceride storage. Normally, adipose tissue functions as a lipid buffering depot, taking up dietary fatty acids in the postprandial state, to release them later on when there is an increased need for fat as a fuel. In insulin resistance, this buffering capacity is disturbed leading to an excess of lipids (free fatty acids (FFA) and triglycerides) in the circulation. This increased lipid overflow to non-adipose tissue organs may result in ectopic fat deposition in tissues like liver, pancreas and skeletal muscle (33-35, 50-52) (figure 1.2). Elevated levels of FFA can cause peripheral insulin resistance (53, 54) and acute lowering of FFA by the antilipolytic drug acipimox enhances insulin action on glucose uptake in the periphery (55). Fat storage in the liver in the form of triacylglycerol (TAG), e.g. in non-alcoholic fatty liver disease (NAFLD), has been associated with all features of the metabolic syndrome including hepatic insulin resistance, type 2 diabetes and elevated triglyceride levels (56, 57). Hepatic insulin resistance results in a reduced inhibition of hepatic glucose production during fasting contributing to hyperglycemia. The increased TAG storage in liver seems to be largely due to increased delivery of free fatty acids from adipose tissue to the liver (58).

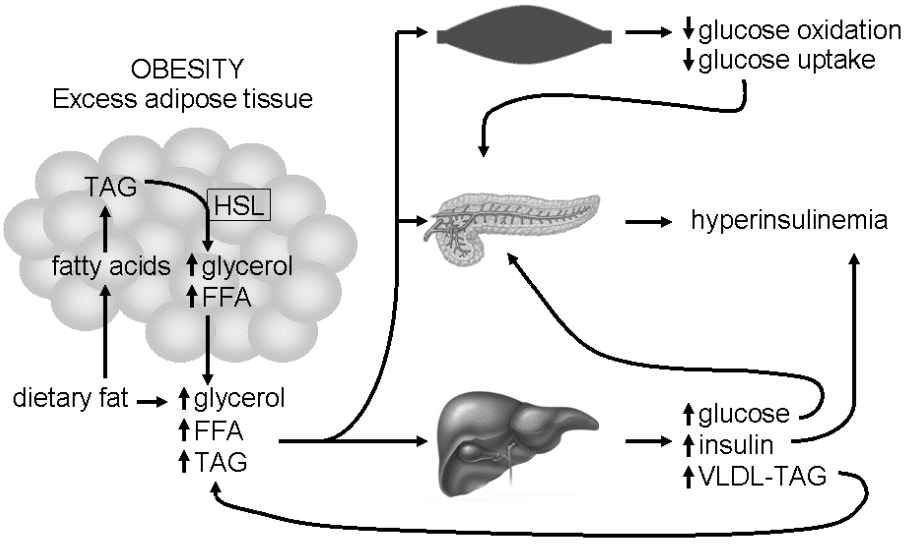

Figure 1.2 The normal function of adipose tissue is to buffer the daily influx of dietary fat. When the buffering capacity for lipid storage in adipose tissue is decreased, as in obesity (when the fat cells are overloaded), other tissues like skeletal muscle, pancreas and liver are exposed to an excessive influx of fatty acids and TAG. TAG storage in these tissues may result in conditions related to insulin resistance, such as glucose intolerance, hyperinsulinemia and hyperlipidemia (adapted from Frayn, KN. Adipose tissue and the insulin resistance syndrome. The Proceedings of the Nutrition Society. 2001; 60(3): 375-80 and Goossens, G. PhD thesis: The renin-angiotensin system in obesity, metabolic and hemodynamic effects. ISBN-13: 978-905278-542-4)

In skeletal muscle, accumulation of lipids as intramyocellular lipid (IMCL) has been strongly linked with skeletal muscle insulin resistance in both lean and obese subjects (59-62). In particular lipid intermediates like diacylglycerol (DAG) and ceramides, may interfere with insulin signaling or induce inflammatory pathways thereby reducing insulin action (63-66).

Also, the insulin resistant muscle may be characterized by a reduced fat oxidation (67-69) or an inability to adjust fat oxidation to different metabolic conditions (metabolic inflexibility, (70)). Mitochondrial dysfunction has been proposed to contribute to a reduced or insufficient capacity to oxidize fatty acids and to skeletal muscle insulin resistance, (71, 72), while evidence is increasing that this relationship is not as straightforward as originally thought (73). Previous studies have suggested that one of the underlying mechanisms by which physical activity may contribute to type 2 diabetes prevention is via improved capacity to oxidize fatty acids (74-76).

3.2 Adipose tissue as endocrine organ

During recent years it has become increasingly clear that adipose tissue is not only a lipid buffering depot, but also produces many peptides and hormones, which may act locally (autocrine and/or paracrine) or may be secreted in the circulation to act as

endocrine factors. These adipo(cyto)kines may provide a link between obesity and insulin resistance and related metabolic complications (figure 1.3). As adiposity increases adipocytes become hypertrophied and the ability of adipose tissue to function as an endocrine organ and secrete multiple biologically active proteins is affected (77).

Among others, adipose tissue secretes adiponectin, leptin and resistin. Several prospective studies have shown that low adiponectin levels are predictive of the development of insulin resistance and type 2 diabetes mellitus (78-82). Adiponectin has been shown to inhibit TNF-α (83) and activate AMP-activated protein kinase (AMPK) and, in turn, modulate inflammatory signals (84). High-molecular weight (HMW) adiponectin has been proposed to be the biologically active form of the hormone (85), although although HMW adiponectin and total adiponectin had similar ability to predict the presence of insulin resistance (86).

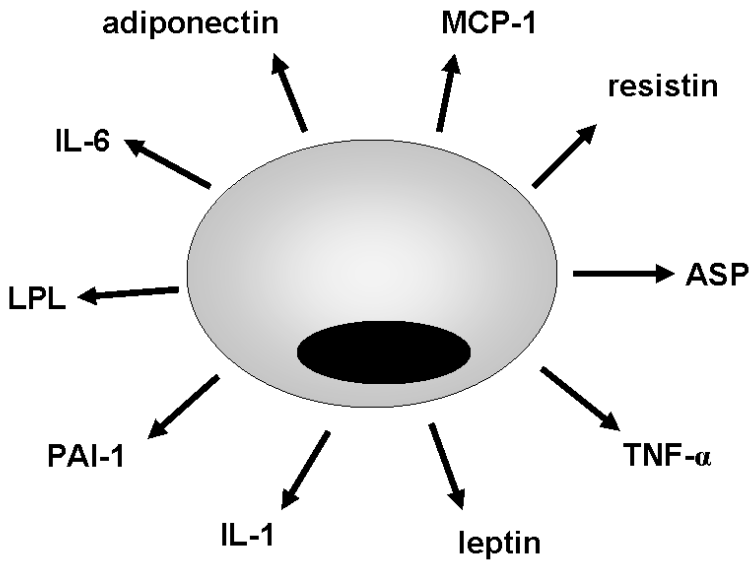

Figure 1.3 Adipocytes secrete a variety of substances which can have autocrine/paracrine and endocrine functions. Some of these factors may be associated with disturbances in obesity, glucose intolerance and insulin resistance (adapted from Goossens, Physiology and Behavior, 2008).

Leptin was originally thought to be a hormone whose primary role was to inhibit obesity by reducing food intake (87, 88). This idea was driven by the observation that rodents and humans lacking leptin or functional leptin receptors develop hyperphagia and obesity (87). The hypothesis was questioned when it was observed that obesity is typically associated with high leptin levels (89, 90) and fasting (or weight loss) induces a rapid fall in leptin levels, e.g. in obese subjects (91). A fall in leptin levels induces changes in energy balance and hormone levels (88, 89). Recently is was shown that in 6 obese subjects, restoration of leptin levels to the original levels before body weight loss, causes maintenance of weight loss and reversal of changes in brain control of

food intake (91), indicating a potential of leptin to induce and sustain body weight loss (92).

With regard to resistin, conflicting data have been provided that do (93) and do not (94, 95) find an association with insulin resistance. For the complement system, data are more conclusive about a linear correlation with insulin resistance, in particular complement factor 3 (C3) and complement factor 4 (C4) (96, 97). Besides the liver, adipose tissue may be a source of C3 production, through activated macrophages (98) and adipocytes (99), thereby contributing to the development of type 2 diabetes. C-reactive protein (CRP), which is also produced by the liver, has also shown to be a predictor of future risk of type 2 diabetes (100-102). Lifestyle interventions have shown to be able to reduce CRP levels (103, 104).

In recent years, the metabolic role of IL-6 regained interest with the new concept of skeletal muscle producing cytokines (myokines), e.g. IL-6, during physical activity (105). IL-6 seems to be paradoxically related to both an enhanced and reduced insulin action. On one hand, IL-6 is markedly produced and released in the post-exercise period (106) when insulin action is enhanced but (107-109), on the other hand, IL-6 has been associated with obesity (110), diabetes and atherosclerotic vascular disease (111). It seems that chronic elevation of IL-6 is not desirable (110), while short elevations of IL-6 do not seem to be harmful.

A discussion on the mechanisms for the increased inflammatory state in obesity, insulin resistance (112, 113), and type 2 diabetes (101, 114-116), would go beyond the scope of this introduction. In this respect, it has been demonstrated that hypertrophied adipocytes secrete macrophage chemoattractants, including monocyte chemoattractant protein-1 (MCP-1). MCP-1 recruits macrophage infiltration which, in turn, leads to a pro-inflammatory state in which macrophages secrete, among others, large amounts of tumour necrosis factor-α (TNF-α) (77, 117, 118). The pro-inflammatory state may in turn increase lipolysis, e.g. by interfering with the insulin signaling, and decrease triglyceride synthesis within the adipocyte by downregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ), and thereby increase lipid overflow to other organs (77).

Furthermore, although recent progress has been made in the understanding by which mechanisms biologically active proteins act on adipose tissue and skeletal muscle, a lot of questions remain to be answered to reveal the complex process of the pathogenesis of type 2 diabetes, thereby creating exciting opportunities in science.

3.3 Iron metabolism

The iron metabolism has been associated with insulin resistance and the development of type 2 diabetes (119). Until recently, however, it was not clear whether elevated iron stores predicted the risk of development of insulin resistance and type 2 diabetes. Proof of concept was provided by a large prospective study in healthy women that showed that higher iron stores (reflected by ferritin concentrations and the ratio of transferrin receptors to ferritin) were associated with an increased risk of type 2 diabetes, independently of known diabetes risk factors (120). Epidemiological studies have further demonstrated that both ferritin and transferrin predict the development of type 2 diabetes (121-123). The mechanism by which ferritin and transferrin may

contribute to insulin resistance is still unclear. Transferrin has been shown to be a determinant of lipolytic activity in human adipocytes (124), and adipose tissue lipolysis has been recognized as a major determinant of insulin resistance (125). Also, ferritin has been suggested to affect insulin action (126) or catalyze hydroxyl radicals (124, 127). Ferritin may stimulate lipolysis and subsequent lipid overflow towards liver and skeletal muscle, and the development of insulin resistance, thereby affecting the pathogenesis of type 2 diabetes.

4. Outline of the thesis

The leading thread through this thesis is the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (the SLIM-study) and each chapter will address a different question with respect to this study. In chapter 2 an overview is given about lifestyle intervention studies in the prevention of type 2 diabetes with respect to the main outcome and determinants of outcome and adherence, recent cost-effectiveness analyses and future implementation strategies. After 1 and 2 years, results of the SLIM study have shown that most improvement in glucose tolerance is achieved after 1 year of intervention and that adherence to the diet and exercise recommendations gives the largest improvement in body weight, waist circumference, fasting insulin and 2-hr glucose levels, compared to adherence to the diet or exercise recommendations alone (29). Chapter 3 addresses the 3-yr effects of on glucose tolerance, insulin resistance and the effect on metabolic cardiovascular risk factors, i.e. factors associated with the Metabolic Syndrome. Long-term effects of a lifestyle intervention (mean 4.2 years) as well as determinants of the main outcome and characteristics of dropout are evaluated in chapter 4. Non-adherence to lifestyle interventions is a widespread problem (128) in need of clarification so that the success of lifestyle interventions involves the majority of the target group. Therefore, the study in chapter 5 aims to assess adherence to the physical activity and nutrition advices and to determine personal and environmental factors that may have affected adherence. In the chapters thereafter, factors are examined, which may be associated with the success of the lifestyle intervention. This may reveal predictors of intervention outcome and/or clues underlying the mechanism of the pathogenesis of type 2 diabetes mellitus. The 1-year and 3-year lifestyle-associated changes in inflammatory and immune markers with insulin resistance and glucose tolerance are discussed in chapter 6 and 7. Increasing adipose tissue (adiposity), as observed in obesity, is the main risk factor for type 2 diabetes. As adiposity increases, the ability of adipose tissue to function as endocrine organ and secrete multiple biologically active proteins, also called adipokines, is affected. In chapter 8, the relationship between the iron metabolism and insulin resistance after 1 year of lifestyle intervention is discussed. In chapter 9, the results and conclusions of the previous chapters are integrated and discussed in a broader perspective and implications for future research are given.

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2

LIFESTYLE INTERVENTION IN THE PREVENTION OF TYPE DIABETES

Determinants of success for future implementation

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Abstract

Lifestyle interventions are reported to reduce the risk of type 2 diabetes in high-risk individuals after mid- and long-term follow-up. Information on determinants of intervention outcome and adherence and the mechanisms underlying diabetes progression are valuable for a more targeted implementation. Weight loss seems a major determinant of diabetes risk reduction, whereas physical activity, dietary composition may also independently contribute. Also, body composition and genetic variation may affect the response to intervention. Lifestyle interventions are cost-effective and should be optimized to increase adherence and compliance, especially for the high-risk group with a low socio-economic status, so that public health policy can introduce targeted implementation programs nationwide. The aim of this review is to summarize the mid- and long-term effects of lifestyle interventions on impaired glucose tolerance and type 2 diabetes mellitus and to provide determinants of intervention outcome and adherence which can be used for future implementation of lifestyle interventions.

Introduction

The World Health Organization predicts a global increase in diabetes prevalence of 39% between 2000 and 2030, increasing absolute numbers to 366 million people (1). Impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and IGT/IFG combined predict the development of type 2 diabetes over 6 years with 9.1% progression for IFG, 32.5% progression for IGT and 64.5% for combined IGT/IFG (2). IGT is characterized by insulin resistance and reduced β-cell glucose sensitivity (3-5), which may develop over many years. Lifestyle intervention may improve the metabolic profile and reverse the progression towards diabetes (figure 2.1) (5).

In this manuscript, we summarize the mid- and long-term effects of lifestyle interventions in subjects with IGT to lifestyle intervention outcome, i.e. changes in glucose tolerance or diabetes incidence. To develop implementation strategies it is important to know which factors determine the effectiveness and adherence to the intervention. Therefore, we provide information on lifestyle factors, i.e. (central) obesity, physical activity, dietary patterns (2, 6-9), on metabolic factors and genetic variation. Implementation of lifestyle interventions in general health care services will only start after lifestyle interventions have shown to be cost-effective. Therefore, this subject is discussed in a separate section. To conclude, this manuscript provides conclusive paragraphs regarding the long-term effects of lifestyle interventions and the major determinants of intervention outcome and adherence. In addition this manuscript discusses the cost-effectiveness of lifestyle interventions and concludes with a summary on these paragraphs and additional implementation strategies for the future, which also include social-economic, social and psychological factors.

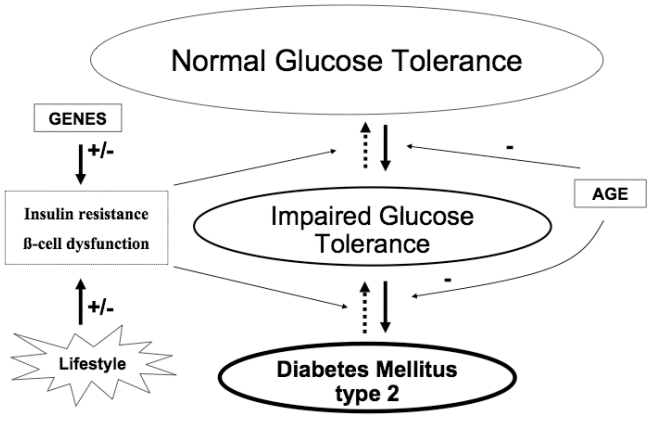

Figure 2.1 Transition from normal glucose tolerance to diabetes mellitus type 2: three step model (adapted from Saad et al. (1999) (5)). The development of normal glucose tolerance to impaired glucose tolerance and type 2 diabetes mellitus can be affected by age, genes and lifestyle. It is (partly) reversible by healthy changes in lifestyle.

Lifestyle interventions: effect on diabetes incidence and 2-hour glucose concentrations

In table 2.1 and 2.2 a summary is given of lifestyle intervention studies to prevent type 2 diabetes in IGT subjects. Only studies were included, that investigated the effect of exercise and/or dietary intake on 2-hour glucose concentration as an intermediate endpoint (table 2.1) or diabetes incidence as primary endpoint and that lasted more than 1 year (table 2.2).

Glucose tolerance

There is evidence that lifestyle intervention leads to an improvement in glucose tolerance, measured as the 2-hr plasma glucose concentration after a 75-g glucose load. Lifestyle interventions generally aim to achieve a body weight loss of at least 5 percent, through a healthy diet and energy restriction and to increase physical activity of moderate intensity to at least 30 minutes a day (table 2.3). The healthy diet most often refers to a total fat intake of less than 30 percent of energy consumed (E%), a saturated fat intake of less than 10 E% and an increase in fiber intake to at least 15 g per 1000 kcal. In the Diabetes Prevention Study (DPS), 2-hour glucose levels tended to decrease more in the intervention as compared to the control group after 3 years of lifestyle intervention (10). In New Zealand, a 1-year lifestyle intervention directed at reducing fat intake, by education of IGT participants on dietary fat intake, showed a lower increase in 2-hour glucose levels in the intervention group after a follow-up of 5 years (11) ($p < 0.01$, table 2.1). Three smaller diet and exercise intervention studies, one in Swedish participants, one in Japanese Americans and one in British subjects with IGT, demonstrated a reduced body weight (12-14) and an improved insulin sensitivity, but no significant differences in 2-hour glucose changes after 2 years (13, 14). In Japanese Americans, central adiposity measured with computed tomography was reduced and the incremental area under the curve for glucose during an oral glucose tolerance test (OGTT) was improved after 6 months in the diet plus endurance training group versus the diet plus stretching (control) group (13). The Dutch Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM) evaluated a combined diet-and-exercise lifestyle intervention in IGT subjects. After 3 years, the postprandial glucose concentration was 0.8 mmol/l lower in the lifestyle group compared to the control group, representing a substantial diabetes risk reduction (15).

Most, but not all studies show a beneficial effect of lifestyle intervention on glucose tolerance. Sample size and a short follow-up of 1-2 years may explain part of the variation in effect size. Differences in baseline characteristics between intervention and control group, like waist circumference (13), fasting glucose and insulin levels (12) and percentage of subjects engaging physical activity at least once a week (14) may have influenced the effect of lifestyle interventions on 2-hr glucose levels. Interesting is that a lack of significant effect on 2-hr glucose is not necessarily related to a lack of effect on diabetes risk reduction. In the DPS, the reduction in 2-hr glucose levels was not significant (10) (0.4 mM difference between intervention and control), but risk reduction was 58% (table 2.2). Part of the risk reduction may be mediated via other pathways than 2-hr-glucose, e.g. via sub-clinical inflammatory factors (16) or preservation of β-cell function (4).

Table 2.1 Lifestyle interventions to prevent type 2 diabetes with a minimal follow-up of 1 year and with endpoint change in 2-hr glucose.

Author (year) (reference #)	Country	Study design	Follow-up	Inclusion criteria	Type intervention	EX intervention	DIET intervention	Change in 2-hr plasma glucose
Lindström et al. (2003) (10)	Finland, DPS, Kuopio	RCT	3 yrs	IGT Age 40-64 yrs BMI ≥ 25 kg/m^2	EX & DIET	Encouragement to increase overall physical activity + circuit exercise sessions offered	Weight reduction through a healthy diet	EX & DIET -0.5 ± 2.4 mmol/l vs. -0.1 ± 2.2 mmol/l CON, n.s.
Swinburn et al. (2001) (11)	New Zealand	RCT	1 yr intervention and 4 yr follow-up	IGT	DIET	N/A	Monthly group education sessions of reduced fat-eating	DIET After 5 years 1.02 ± 0.40 mmol/l vs. 2.30 ± 0.54 mmol/l CON, P < 0.05
Lindahl et al. (1999) (12)	Sweden	RCT	1 yrs	IGT Age 30-60 yrs BMI ≥ 27 kg/m^2	EX & DIET	Encouragement to increase physical activity + free exercise sessions first month	Weight reduction through healthy low-energy, low-fat diet	EX & DIET -0.7 mmol/l, n.s.
Carr et al. (2005) (13)	USA	RCT	2 yrs	IGT	EX & DIET	Walking/ jogging at 70% of HR for 1 hr on 3 days/week	Encouragement to follow energy-balanced American Heart Foundation Step 2 diet	EX -0.6 mmol/l, n.s.
Oldroyd et al. (2006) (14)	England	RCT	2 yrs	IGT	EX & DIET	Encouragement to 20-30 min aerobic activity 2-3 days/week + discount local gyms.	Weight reduction through healthy low-energy, low-fat diet	EX & DIET 0.2 mmol/l, n.s.
Roumen et al. (2008) (15)	The Netherlands, SLIM study	RCT	3 yrs	IGT Age >40 yrs BMI ≥ 25 kg/m^2	EX & DIET	Encouragement to 30 min/day moderate intensity exercise + free exercise sessions	Weight reduction through healthy low-energy, low-fat diet	EX & DIET -0.04 mmol/l, P < 0.05

n.s., no significant difference between groups. BMI, Body mass index; DIET, Dietary; DM2, type 2 Diabetes Mellitus ; DPS, Diabetes Prevention Study; EX, Exercise; HR, Heart Rate reserve; IGT, Impaired glucose tolerance; LCD, Low Calory Diet; LTPA, Leisure time physical activity; N/A, not applicable; RCT, Randomized Controlled Trial; SLIM, Study on Lifestyle intervention and Impaired glucose tolerance Maastricht; yrs,years

Table 2.2 Lifestyle interventions to prevent type 2 diabetes with a minimal follow-up of 1 year and with endpoint diabetes incidence.

Author (year) (reference #)	Country	Study design	Follow-up	Inclusion criteria	Type intervention	EX intervention	DIET intervention	Relative risk reduction of type 2 diabetes vs. Control (%)
Eriksson and Lindgärde (1991) (17)	Sweden, Malmö Feasibility Study	Non-RCT	5 yrs	IGT	EX & DIET	Encouragement to increase physical activity	Healthy dietary advice	63% #
Pan et al. (1997) (18)	China, Da Qing IGT and Diabetes Study	RCT	6 yrs	IGT	EX EX & DIET	Encouragement to 1 unit exercise/day*	BMI ≥ 25 kg/m^2 weight loss through reduced energy intake	42% #
Tuomilehto et al. (2001) (19)	Finland, DPS	RCT	3.2 yrs	IGT	EX & DIET	Encouragement to 30 min/day moderate intensity exercise	Weight reduction ≥ 5% through healthy low-energy	58% #
Knowler et al. (2002) (20)	USA, DPP	RCT	2.8 yrs	IGT	EX & DIET Metformin	Encouragement to 150 min/week moderate intensity exercise	Weight reduction > 7% through healthy low-energy, low-fat diet	58% EX & DIET # 31% Metformin #
Lindstrom et al. (2006) (21)	Finland, DPS, Kuopio	RCT	7 yrs (4 yrs intervention + 3 yrs follow-up)	IGT Age 40-64 yrs BMI ≥ 25 kg/m^2	Post-hoc analysis	Encouragement to increase overall physical activity + circuit exercise sessions offered	Weight reduction through a healthy diet	43% #
Li et al. (2008) (22)	China, Da Qing IGT and Diabetes Study	RCT	20 yrs (6 yrs intervention + 14 yrs follow-up)	IGT	EX EX & DIET	Encouragement to 1 unit exercise/day*	BMI ≥ 25 kg/m^2 weight loss through reduced energy intake	43% #
Kosaka et al. (2005) (23)	Japan	RCT, intensive vs non-intensive lifestyle interven-tion	4 yrs	IGT men	EX & DIET	Encouragement to 30-40 min/day moderate intensity exercise	BMI < 22 kg/m^2 through a healthy diet	67% #

Author (year) (reference #)	Country	Study design	Follow-up	Inclusion criteria	Type intervention	EX intervention	DIET intervention	Relative risk reduction of type 2 diabetes vs. Control (%)
Ramachandran et al. (2006) (24)	India, IDPP-1	Prospective community-based	30 months	IGT	EX & DIET Metformin EX & DIET + Metformin	Encouragement to 30 min/day moderate intensity exercise	Healthy dietary advice	29% EX & DIET # 28% EX & DIET + Metformin #
Hamman et al. (2006) (30)	USA, DPP	Cox hazard regression in intervention arm	3.2 yrs	IGT, only intervention group	EX & DIET	Encouragement to 150 min/week moderate intensity exercise	Weight reduction > 7% through healthy low-energy, low-fat diet	16% per kg weight loss #
Laaksonen et al. (2005) (52)	Finland, DPS	RCT	4.1 yrs	IGT Age 40-64 yrs BMI ≥ 25 kg/m^2	EX & DIET	Encouragement to 30 min/day moderate intensity exercise	Weight reduction ≥ 5% through healthy low-energy	EX LTPA to moderate-vigorous or strenuous-structured 63-65% #

*1 unit = 30 min. mild exercise, 20 min. moderate exercise, 10 min strenuous exercise or 5 min very strenuous exercise. DIET, Dietary; DM2, type 2 Diabetes Mellitus ; DPP, Diabetes Prevention Program; DPS, Diabetes Prevention Study; EX, Exercise; HR, Heart Rate reserve; IGT, Impaired glucose tolerance; LCD, Low Calory Diet; LTPA, Leisure time physical activity; N/A, not applicable; RCT, Randomized Controlled Trial; yrs,years
= $p < 0.05$, significant difference between groups.

Diabetes incidence

The Malmö-study (17) and the Chinese Da Qing IGT and Diabetes study (18) were two of the first studies to show a reduced type 2 diabetes risk of more than 50% and 42%, respectively, by a diet and exercise program during a follow-up of 6 years. More recently, both the Finnish Diabetes Prevention Study (DPS) (19) and the Diabetes Prevention Program (DPP) in the USA (20) confirmed the beneficial effect of lifestyle and showed a diabetes risk reduction of 58% in IGT subjects that received a lifestyle intervention consisting of dietary and exercise advice during a follow-up of 3.2 and 2.8 years, respectively. Interestingly, in an additional follow-up, the DPS showed that even after 3 years cessation of a 4-year lifestyle program (total follow-up 7 years), diabetes risk was still reduced with 43% (21). The Chinese Da Qing IGT and Diabetes study found the same diabetes risk reduction after a 14-year follow-up of a 6-year lifestyle intervention (22). A 4-year randomized controlled trial in Japan reduced diabetes risk with 67.4% by a combined dietary and exercise advice to IGT men (23) and the Indian Diabetes Prevention Program (IDPP-1) reduced diabetes risk by 28.5% in the diet and exercise intervention after 3 years (24). Thus, lifestyle changes in IGT subjects reduce or delay the development of type 2 diabetes. The risk reduction of studies that follow the lifestyle criteria listed in table 2.3 (the DPP (20), DPS (19) and SLIM (15)) is approximately 58% after 3 years follow-up. Although all studies show a beneficial lifestyle effect, the risk reduction ranges from 28% to 67%. Differences in risk reductions may be due to different population characteristics, ethnicity, duration of the lifestyle intervention and follow-up, and the lifestyle goals accomplished.

A recent systematic review and meta-analysis shows that lifestyle interventions seem at least as effective as pharmacological interventions, e.g. with orlistat, a drug which reduces intestinal fat absorption (25). In the DPP, metformin, a drug which suppresses hepatic glucose production, was less effective to reduce type 2 diabetes risk (–31%) than lifestyle intervention (20).

In general, lifestyle changes in subjects with IGT considerably reduce or delay the development of type 2 diabetes and is at least equally effective as pharmacological interventions.

Table 2.3 Common features of lifestyle interventions.

Body weight loss	≥ 5 %
Dietary guidelines	
Carbohydrates	± 55 E%
Total Fat	< 30 E%
- Saturated Fat	≤ 10 E%
- Cholesterol	< 33mg/MJ
Proteins	10-15 E%
Fiber	3 g/MJ a day
Exercise guidelines	30 minutes of moderate physical activity a day for at least 5 days a week

Determinants of intervention outcome and adherence

The progression towards type 2 diabetes can be influenced by lifestyle components such as body weight loss, dietary composition, and physical activity. These factors may all contribute to the improved glucose metabolism and the response and adherence to the lifestyle intervention.

Body weight and diabetes risk

Body mass index (BMI) and body weight gain are strongly associated with diabetes risk (26). Weight gain of 11-20 kg in 14 years of follow-up increased age-adjusted relative diabetes risk 5.4-fold in female nurses who had normal weight at age 18 (BMI 22-25 kg/m^2) relative to nurses with normal weight at age 18 y who had no weight gain or loss of more than 5 kg. In addition to over-all obesity, body fat distribution and especially increased intra-abdominal fat is a predictor of type 2 diabetes (27, 28). The BMI of middle-aged women at midlife strongly predicts 3-year incidence rates of type 2 diabetes (29), whereas short-term weight changes during this period did not significantly affect the onset of type 2 diabetes (29).

The effect of changes in body weight on diabetes risk

In the lifestyle intervention group of the DPP, weight loss was the dominant predictor of reduced diabetes risk with a 16% reduction for every kilogram weight loss during the 3.2 year follow-up (30). Thereafter, in the 4-year follow-up, insulin sensitivity in the entire group (intervention plus control) improved by 64% in the highest tertile of weight loss, but deteriorated with 24% in those who gained weight, while insulin secretion remained unchanged in IGT subjects who managed to loose weight (31). Overall, weight loss is a major contributor in the prevention of type 2 diabetes mellitus.

Besides weight loss, changes in body fat distribution may influence diabetes risk. It has been shown that a relatively minor loss of body weight (-3%) which was accompanied by a major reduction in visceral fat mass (-12%) and liver fat content (-33%) was associated with improved insulin sensitivity (32, 33). However, to bring the visceral depot to normal levels, subjects with upper body obesity, those with a larger visceral depot compared to subjects with lower body obesity, may need to loose larger amounts of body weight compared to subjects with lower body obesity (34). The loss of visceral fat seems to be predominantly determined by the initial amount of fat in the visceral fat depot (35, 36). Subjects with high visceral adipose tissue and liver fat or high BMI may more easily loose body weight and visceral fat, while this does not imply metabolic benefits (32). Even after weight loss, fat deposition in these subjects is above average, which may not make a metabolic difference, because an improvement in insulin sensitivity may require a reduction in ectopic fat deposition below a specific threshold (32). Overall, with regard to body fat distribution, lifestyle interventions have lead to a reduced diabetes risk, in parallel with reductions in preferentially visceral fat but also subcutaneous fat and total body fat (13, 37). It is still unclear to what extent body fat distribution adds to the metabolic benefits of lifestyle interventions. There-

fore, no clear suggestions can be made at this point regarding body fat distribution as determinant of intervention outcome.

The relationship between increased adiposity and insulin resistance and diabetes may, on one hand be explained by an increased lipid overflow in the circulation. Normally, adipose tissue functions as a 'metabolic buffer' trapping dietary fatty acids, whilst in insulin resistant conditions this buffering action is impaired resulting in exposure of non-adipose tissues like muscle and liver to excessive fluxes of lipids. Accumulation of lipids in these tissues plays a critical role in the etiology of insulin resistance and type 2 diabetes mellitus (38-43). On the other hand, the relationship between adiposity and insulin resistance may be explained by an alteration in the production of inflammatory factors. Inflammatory factors, which can be produced by adipose tissue but also numerous other cell types, may have autocrine effects (i.e. effects on gene expression, lipid and glucose metabolism) (44) or may be secreted as endocrine factors influencing energy and substrate metabolism and insulin sensitivity in other tissues like skeletal muscle and liver (45). For example, baseline adiponectin levels, an adipose-derived adipokine, seems a strong predictor of incident diabetes in the DPP (46). Considerable weight loss seems to restore high adiponectin levels (47, 48), but lifestyle interventions according to general guidelines do not seem to have a relevant effect on adiponectin in either diabetic patients (49), IGT subjects (50) or obese subjects (51). Leptin, another adipokine, seems also related to improvements in insulin sensitivity, independent of changes in body composition (50).

The effect of changes in physical activity on diabetes risk

To design optimal guidelines for physical activity as part of lifestyle programs, it is important to define the independent effect of physical activity on the risk of type 2 diabetes. Post-hoc analysis of 487 IGT men and women in the DPS on whom data of leisure-time physical activity were available revealed that individuals with the highest increase in their level of moderate-to-vigorous physical activity were 63-65% less likely to develop type 2 diabetes during the 4.1 year follow-up compared to individuals with the lowest increase in physical activity (52). Similarly, in the DPP, indexes that reflect autonomic function and fitness improved (i.e., heart rate decreased and heart rate variability increased) during a 3.2 year intervention and were inversely related to diabetes risk, independent of weight change (53). In the Chinese Da Qing IGT and Diabetes Study (18), diet and exercise were equally effective as exercise alone to prevent the progression of IGT to type 2 diabetes. Results from the Dutch SLIM study (54) and the Oslo Diet and Exercise study (55) showed most effect in improving glucose tolerance and preventing type 2 diabetes by the combination of diet and exercise. These post-hoc analyses show that exercise contributes to improve glucose metabolism, independent of weight loss (52) and therefore seems to have an additive effect on diabetes prevention. Besides reducing diabetes risk, increasing exercise training results in an improved fitness in a dose dependent manner (56), which may positively influence daily-life activities. Physical activity is also an important predictor of weight maintenance after weight loss (57). To conclude, physical activity is besides body weight loss an independent contributor to improve glucose metabolism. There are

indications that especially moderate-to-vigorous physical activity help prevent type 2 diabetes.

One of the underlying mechanisms by which physical activity may contribute to type 2 diabetes prevention is via improved capacity to oxidize fatty acids (58-60). Free fatty acid (FFA) oxidation is reduced in subjects with type 2 diabetes mellitus and impaired glucose tolerance (IGT) and it has been shown that a combined diet and physical activity intervention program can prevent further deterioration of impaired fatty acid oxidation during exercise in subjects with IGT (61). An improved utilization of fatty acids for energy may reduce the accumulation of lipids and lipotoxic intermediates in skeletal muscle and thereby improve insulin sensitivity (62-64). Additionally, it has also been suggested that training can bring about improvements in the regulation of hepatic glucose output (65, 66).

With respect to duration and intensity, the guidelines of the International Diabetes Federation (IDF) and the American College of Sports Medicine prescribe at least 30 minutes of moderate-intensity physical activity on most, preferably all days of the week (6, 67). However, in formerly obese or overweight subjects 60-90 minutes/day or 45-60 minutes/day of moderate intensity physical activity are recommended, respectively, to prevent further weight gain (68). To date, a recent review indicated that 30 min/day of moderate- or high-level physical activity is an effective and safe way to prevent type 2 diabetes in all populations (69). There is enough evidence to justify that physical activity has an additive effect when combined with a dietary intervention and should be included in lifestyle programs, despite the uncertainty about the exact duration and intensity to effectively prevent diabetes (70).

With respect to the kind of physical activity, a recent trial showed most improvement in hemoglobin A1c levels of patients with type 2 diabetes when the physical activity consisted of combined aerobic and resistance training (71). Recent guidelines on physical activity and public health in older adults from the American College of Sports Medicine and American Heart Association also recommend muscle strengthening and balance exercise as an integrated part of physical activity intervention in older adults (72, 73).

Lifestyle interventions face the challenge of increasing adherence and compliance to the program and it seems that men and women with a low initial BMI may be more likely to meet activity goals (74). However, reaching the recommended physical activity level for the majority of people at risk for diabetes may be quite a challenge, since the majority (61%) of US patients with diabetes or at highest risk for diabetes do not engage in regular physical activity (75). On the other hand, in the Nurses' Health Study it was shown that even among women who did not perform vigorous physical activity, diabetes risk was reduced with 26% after 8 years of follow-up for those who had walked most relative to those who walked least (76). This seems to indicate that even small but sustained increases in physical activity are beneficial in the long-term; exemplifying that physical activity should be included in lifestyle programs. Since there is a high heterogeneity in high-risk subjects, i.e. with regard to co-morbidity and muscle strength, as is the case for diabetic patients, the exercise intervention should become more individualized to optimize its therapeutic value (73). A personalized activity plan may be one of the ways to achieve the physical activity recommendations (72).

The effect of changes in dietary composition on diabetes risk

Dietary factors related to the development of type 2 diabetes include, besides energy intake restriction, total fat intake, fat quality, fiber, glycemic index/glycemic load, alcohol consumption and coffee consumption (7). A western diet, emphasizing red meat, French fries and high fat dairy products has been associated with increased diabetes risk (77, 78), while a 'prudent' diet, emphasizing fruits, vegetables, fish and whole grains has been associated with a reduced diabetes risk (79-82). The Women's Health study, a large prospective study, showed a relative risk of 1.28 for women in the highest quintile of red meat use, versus the lowest quintile (83). Short-term dietary changes towards high-carbohydrate and low glycemic index seem to improve β-cell function in IGT subjects (84). Dietary fibers can reduce the rate of glucose absorption in the intestine, thereby lowering postprandial glycemic and insulinemic responses (85). Also the total fat intake is an important risk factor for the development of type 2 diabetes as its energy-density favors increased food intake and obesity. In the San Louis Valley Diabetes Study, a 40g higher fat intake corresponded with a 6-fold increase in diabetes risk in IGT subjects after adjustment for obesity and markers of glucose metabolism (i.e. fasting glucose and insulin) (9). An ad libitum reduced-fat diet without energy restriction has been shown to produce a moderate weight reduction between 3-5 kilograms (86, 87). In agreement with these findings, results from the DPS in 500 IGT subjects (intervention plus control group) during a 4.1 year follow-up suggest that a high-fiber, low-fat diet predict sustained weight loss and reduces the development of type 2 diabetes, even after adjustment for other risk factors such as physical activity (88). In addition, the quality of dietary fat modifies diabetes risk. In particular, an excess of saturated fat may have detrimental effects on skeletal muscle insulin sensitivity (38-40). Insulin sensitivity improved when saturated fatty acids in the diet were replaced either by mono-unsaturated fat (89) or poly-unsaturated fat (90). Unsaturated fatty acids may influence diabetes risk by increasing the fluidity of membranes, thereby facilitating membrane signaling, including insulin signaling (91) and/or by influencing the regulation of genes involved in the breakdown and oxidation of fatty acids (92). The relation between insulin sensitivity and n-3 poly-unsaturated fatty acids (PUFAs) is less clear, but most studies using a euglycemic hyperinsulinemic clamp have found no effect of n-3 PUFAs on insulin sensitivity in healthy or diabetic subjects (89, 91, 93). Recent interest has developed for specific fatty acids, and for enzymes that alter the saturation of ingested fatty acids, the so-called desaturase enzymes (94). In the SLIM study, lifestyle-induced changes in insulin sensitivity were partly related to changes in fatty acid profile of serum cholesteryl esters, and in particular to changes in desaturase activities (95).

To conclude, multiple components of a healthy diet, e.g. high fiber and a low saturated fat intake, reduce diabetes risk, and contribute to sustained weight loss and should therefore be included in long-term lifestyle interventions. Composition of the diet plays a key role in diabetes prevention in the first place to sustain weight loss in the long-term and in the second place to initiate weight loss and diabetes risk reduction.

Genetic susceptibility

Genetic variability may partly explain why there are non-responders to lifestyle treatment despite compliance and adherence to the lifestyle intervention program. In the DPS a large number of genes have been examined for their effect on diabetes incidence and intervention success (for review of genes investigated in the DPS, see (96)). An interaction with intervention outcome has been found for the TT genotype of SNP rs12255372 in the TCF7L2 gene which was associated with 2.85-fold increased risk of incident type 2 diabetes in the control subjects of the DPS, but not in the intervention subjects (97). The TCF7L2 gene is presumed to play a role in the first-phase insulin release (97). A similar pattern was found for a single nucleotide polymorphism (SNP), the X/Ala genotype of the PPARγ-2 Pro12Ala SNP, which was associated with increased diabetes risk in the control group, whereas there was no newly diagnosed diabetes in the Ala/Ala genotype subjects of the intervention group (98). One explanation is that subjects with the Ala12 allele are more responsive to weight reduction and physical activity than subjects with the Pro/Pro genotype, and thus suffer more from bad lifestyle habits but profit more from the lifestyle intervention as well.

A large study that investigates the interaction between exercise training and genes, is the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study (99). Multiple genes related to the response of an exercise program were identified, including several Quantitative Trait Loci (QTL) on chromosome 1p, 3q, 6p, 7q, 10p, 12q and 19q in white participants (100, 101), the -514 C>T SNP in the Hepatic Lipase gene (LIPC) in both white and black individuals (101) and the rs2180062 and rs9018 of the Four and a Half LIM domains 1 (FHL1) gene (102).

The Tübinger Lifestyle Intervention Program (TULIP) (103) followed the lifestyle protocol from the Finnish DPS study (weight loss, increase physical activity and healthy diet) (19). They showed that the minor G allele of SNP rs2267668 in PPARD and the minor serine-encoding allele of the common Gly482Ser SNP in PPARGC1A were independently associated with less increase in individual anaerobic threshold (104), indicating that these alleles impair the effectiveness of aerobic training. In addition, low skeletal muscle mitochondrial function in vitro was detected in young carriers of the G allele of the rs2267668 SNP of PPARD. They also showed that variation in the adiponectin receptor 1 (ADIPOR1) gene predicted the improvement of insulin sensitivity and the reduction of liver fat after lifestyle intervention (105). The adiponectin receptor 1 may have a putative role in the development of body size, as has been suggested by the Finnish DPS (106).

To conclude, genetic variation can play a role in the response to a lifestyle intervention. Some SNP's may increase the vulnerability for lifestyle factors. This means that these individuals run an increased risk for developing type 2 diabetes with adverse lifestyle behavior, and at the same time may benefit more from lifestyle intervention. Other SNP's increase diabetes risk independent of lifestyle factors. Studies involving interactions between genes and lifestyle intervention response are still limited and need confirmation in large cohorts. When more conclusive evidence is provided, the effectiveness of tailored lifestyle programs should be tested in subgroups with genotypes that are associated with adequate and impaired metabolic response to a lifestyle intervention (96).

Costs and cost-effectiveness

The potential benefits of lifestyle intervention are substantial, but so are the costs for implementing such programs. In return, the potential for cost savings from the prevention or delay of type 2 diabetes and its complications is also considerable. Although several studies have shown effectiveness over longer periods, up to 6 years, it is important for policy makers and health care providers to predict its effectiveness in 10, 20 or 30 years time. To assist policy makers, researchers have developed computer models that simulate the progression of diabetes, expenditures on diabetes care, and effects of interventions. Two principal types of diabetes models exist. The Markov model simulates transitions from one disease state to another (e.g. from IGT to type 2 diabetes) as chance events. A second novel type of model, named Archimedes, integrates detailed biological and administrative information in complex differential equations to simulate pathophysiological processes (e.g. postprandial glucose disposal) that change over time and can lead to disease (107).

The results of the DPP and DPS have been extensively used for cost-effectiveness analyses using both models, resulting in different outcomes. The outcome depends on many factors, such as the assumptions for the natural progression of glycemia and the effectiveness of the lifestyle intervention, the time horizon that is used (30 years or until death after diagnosis), characteristics of included patients (age, degree of obesity, ethnicity, gender), and the costs of the lifestyle program, which depends on the approach (group or individual counseling) and country where the implementation will take place (108).

The DPP developed an analysis based on the Markov model, lifetime horizon and a societal perspective. The cost was $8800 per quality-adjusted life-years (QALY) for the lifestyle intervention and $29.900 for metformin that would be saved (109), which fall both within a range generally accepted as being cost-effective (110). When using the Archimedes model with time horizons of 10, 20 and 30 years and a societal perspective, the cost per QALY of beginning the intensive lifestyle intervention in subjects with IGT was $62.600, and $35.400 for metformin over 30 years, when compared with no intervention (111). The cost per QALY for an intensive lifestyle intervention started after the onset of diabetes was $24.500 (111). The Archimedes model adopted more conservative assumptions for the development of diabetes and its complications, which may have reduced cost-effectiveness. Both studies underline that lifestyle intervention will substantially reduce the proportion of patients at risk to develop diabetes, it will postpone the onset of diabetes from 7 or 8 to 14 or 18 years, and it will lead to fewer complications, longer life, and improved quality of life.

When the DPS results were applied to a Swedish setting, the cost-effectiveness analysis based on the Markov model predicted that the program would be cost-saving from the healthcare payers' perspective (112). In the Netherlands, two different approaches for health care interventions were evaluated: a community based approach targeted at the general population with a high number needed to treat (300-1500 individuals) but low costs per individual (113), and a health care intervention approach aimed at high risk individuals with a low number needed-to-treat (7-30 individuals) but relative high costs per individual (54). Both approaches were evaluated using a Markov-type, multistage transition model, which describes the development

over time of demography, risk factor prevalence, disease incidence, and mortality in the Dutch population (114). Both approaches were cost-effective in the range of €5600- €9900 (114).

To design cost-effective lifestyle interventions, the effectiveness of lifestyle intervention for different subgroups may need to be taken into account. The results from the DPP show no differences in progression towards type 2 diabetes between ethnic groups, once they are identified as impaired glucose tolerant (115). Age may be important: metformin was only cost-effective in IGT subjects under the age of 65, but lifestyle intervention was effective in all age groups (109). Metformin had a better impact on costs in younger and more obese subjects, whereas lifestyle intervention was more effective in subjects with a BMI below 30 kg/m^2 (108). Further identification of factors that modify intervention outcome may increase the efficacy of tailored advice, improving cost-effectiveness.

In general, the implementation of lifestyle intervention as a therapy to prevent and postpone type 2 diabetes and its complications looks promising and cost-effectiveness seems acceptable. In particular differences in the effectiveness of life-style intervention between groups and countries, and approach for implementation (group or individual counseling), will be of influence on the cost-effectiveness. In Finland, a large implementation trial to evaluate different approaches for lifestyle intervention in the health care system started recently (116). It will provide valuable information about the barriers, strategies and costs of lifestyle implementation in a real-life setting.

Implementation strategies

Lifestyle interventions are cost-effective in reducing the risk for type 2 diabetes on the long-term. The next question is how to implement a lifestyle intervention in the general public health setting in a way that is most successful. The high-risk approach has the advantage over a community-based strategy that tailored advice can increase personal risk awareness of diabetes as well as the magnitude and durability of behavioral changes, whereas a community-based strategy will prompt the individual to a lesser extent to undertake action.

Recently, the IDF has proposed a simple three-step plan for the prevention of type 2 diabetes in high-risk individuals: first, identification of those who may be at increased risk, second, measurement of risk and third, intervention to prevent the development of type 2 diabetes (6). For identification the IDF recommends brief questionnaires with criteria of obesity, family history, age, cardiovascular history, gestational history and drug history to assess the level of risk. These questionnaires are simple, practical, non-invasive and inexpensive. In Finland, a type 2 diabetes risk assessment form has already been developed which assesses diabetes risk based on age, BMI, waist, physical activity, fruit and vegetable intake, blood pressure medication, previous high blood glucose (i.e. during pregnancy or illness) and family history of type 2 diabetes, the so-called FINDRISC score (117). A recent post-hoc analysis of the DPS results shows that the FINDRISC may be useful in identifying high-risk groups most likely to benefit from intensive lifestyle intervention to prevent type 2 diabetes (118).

For measurement of risk the IDF recommends the measurement of venous fasting plasma glucose levels. If plasma glucose levels are above 6.1 mmol/l, a 2-hour OGTT is recommended to detect cases of IGT and undiagnosed diabetes. For intervention the IDF recommends gradual weight loss, change in dietary composition and increasing physical activity to 30 minutes moderate intensity physical activity per day, on most days of the week (6). Similar recommendations are made in recent reviews evaluating diet, glucose tolerance and type 2 diabetes (119, 120). Furthermore, although smoking is not usually included as part of a lifestyle intervention program, it is an important lifestyle-related risk factor for diabetes. Since quitting smoking may lead to weight gain (121), special attention is necessary with regard to weight control (122). Lifestyle intervention programs can be suitable for this.

However, merely providing the method of implementation will not be sufficient for successful implementation of lifestyle interventions. For achieving and sustaining lifestyle changes throughout the lifespan, intervention mapping can be used as a tool for the development of health promotion interventions (123). However, efforts from the individual, the practitioner, the community and policy makers fulfill a key role in giving high-risk individuals tailored advice and encouragement to decrease their diabetes risk.

To increase sustainable lifestyle changes, first, the individual should be aware of his/her increased risk for type 2 diabetes and his/her lifestyle. This can be achieved by e.g. the general practitioner who asks individuals at risk if they have family or friends who suffer from the disease. In a recent cross-sectional study, African Americans with a family history of diabetes were more aware of diabetes risk factors and were more likely to engage in certain health behaviors than were subjects without a family history of the disease (124).

Second, motivational interviewing by nurse practitioners can be applied to assist individuals to achieve a positive attitude towards changes in diet, physical activity or the social aspects associated with it. The intentional behavior stage of an individual, as identified by the transtheoretical model of behavior change, is of influence on the preparedness for lifestyle changes (for review with regard to exercise see (125)). In subjects who have developed type 2 diabetes, being in the action stage was associated with healthier eating, compared to those in a pre-action stage (126). In the action stage people are active as in undertaking action for less than 6 months and in the pre-action stages (precontemplation, contemplation and preparation), individuals are not active and have no intention to become active or, in the latter two cases, have the intention to become active within 6 months or 1 month, respectively (127). Knowing the behavioral stage of the individual gives the advantage of formulating specific advice. Altogether, tailored information and advice may increase the intention to adopt a certain lifestyle aspect (125, 128).

Third, general practitioners should be aware and convinced of the clinical significance of IGT and reducing type 2 diabetes incidence by targeting lifestyle interventions at patients at risk (129). Practitioners or dieticians should inform subjects about reading food labels and understanding portion size (130). Therefore, it is desired that practitioners have skills that include assessment of dietary history and physical activity counseling, or refer patients to professionals who have those skills, such as dieticians and physiotherapists. A Finnish study evaluating lifestyle counseling in primary care

has recently shown a lack of communication skills of nurses and physicians counseling subjects with diabetes and IGT with regard to diet and physical activity (131). Improving communication skills, e.g. by teaching the motivational interviewing approach to health care professionals, may therefore improve intervention outcome.

To conclude, high-risk screening and tailored advice seem to have potential for success. In addition, it is acknowledged that community-based strategies involving the food industry and government policies may be necessary as an integral component of diabetes prevention to create an environment supportive of an active and healthy lifestyle (6).

Conclusion

The studies mentioned in this review clearly illustrate that improvements in lifestyle can have a large beneficial impact on diabetes risk. Very promising are the new data on long-term effects of lifestyle programs, showing a sustained diabetes risk reduction, even after counseling was stopped (21, 23). Lifestyle intervention can additionally have a positive impact on cardiovascular risk profile (132) and features of the metabolic syndrome (133). Therefore, we conclude that lifestyle interventions can reduce the diabetes risk substantially in subjects with impaired glucose tolerance, especially when existing of a combined diet and exercise program. We underscore the importance of cost-effective changes in lifestyle that are sustainable over a long period.

With regard to determinants of intervention outcome, weight loss seems the most important factor for reducing diabetes risk. At the same time, physical activity has shown to be an important contributor, independent of obesity, as well. Relatively small changes in physical activity that are prolonged over several years already seem to contribute to a diabetes risk reduction, although moderate-to-vigorous physical activity seems to have a larger effect. Changes in dietary composition are in the first place important to sustain achieved weight loss in the long-term and in the second place to initiate weight loss and reduce diabetes risk. Body composition and genetic variation seem to modulate the effect of lifestyle intervention on metabolic benefits. However, on both subjects no conclusive data are yet available for evidence-based recommendations. More large studies are necessary to elucidate the major modulators and the way they interact with lifestyle effect.

With respect to determinants of adherence, this paper shows that lifestyle intervention outcome is not just the result of the individuals' motivation, but the result of a variety of metabolic, genetic and socio-economic factors, i.e. low social economic class (134-136). Increasing self-efficacy, motivational readiness, social activities (137) and decreasing perceived stress (126), exemplifying the importance of prevention and giving tailored personal advice may be a first step in increasing adherence. As an alternative for non-effective lifestyle intervention, subscription of medication or change of policy to adjust the environment to a more favorable one may be necessary. Furthermore, concise and conclusive reporting on risk genotypes and reasons for non-adherence in future publications is essential, so that 1) lifestyle interventions can be adjusted for those less likely to adhere and benefit from it or so that 2) the environ-

ment can be adjusted to increase adherence and compliance, especially for the low social-economic classes or 3) preferentially both.

To conclude, lifestyle intervention programs are feasible and cost-effective for the long-term and will probably be the most important tool to alleviate the burden of diabetes and related complications in the future, and to sustain healthy ageing. Thus, the question is no longer whether lifestyle interventions might be effective, but under which circumstances they will be most effective.

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3

IMPACT OF 3-YEAR LIFESTYLE INTERVENTION ON POSTPRANDIAL GLUCOSE METABOLISM

The SLIM study

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Abstract

Objective
To evaluate the effect of a 3-year diet- and exercise lifestyle intervention, based on general public health recommendations, on glucose tolerance, insulin resistance and metabolic cardiovascular risk factors in Dutch subjects with impaired glucose tolerance (IGT).

Method
Subjects 147 IGT subjects (75 male, 72 female) were randomized to the intervention group (INT) or control group (CON). 106 subjects (52 INT, 54 CON) completed 3 years of intervention. Annually, glucose, insulin and free fatty acid (FFA) concentrations were determined during fasting and after an oral glucose tolerance test and measurements of body weight, serum lipids, blood pressure and maximal aerobic capacity.

Results
According to our completers analysis, the lifestyle intervention improved body weight (INT: -1.08 ± 4.30 kg; CON: +0.16 ± 4.91 kg, p=0.01), HOMA index for insulin resistance and 2-hour FFA. 2-Hour glucose concentrations improved in INT, most pronounced after 1 year with a regain to baseline values after 3 years, from 8.59 ± 1.55 mM to 8.55 ± 0.34 mM, but deteriorated in CON: from 8.46 ± 1.84 mM to 9.35 ± 2.50 mM (p=0.02). For INT, diabetes incidence was reduced by 58% (p=0.025).

Conclusion
Our lifestyle intervention showed a sustained beneficial effect on 2-hr glucose concentrations, insulin resistance and 2-hr FFA, even after 3 years. Our lifestyle intervention is efficacious, but for implementation more information is warranted on determinants of adherence.

Introduction

Impaired glucose tolerance (IGT) is an obligatory transition state between normal glucose tolerance and type 2 diabetes, and from a health perspective IGT subjects are an important high-risk target group for the prevention of type 2 diabetes and related complications.

We demonstrated previously in the SLIM Study (Study on Lifestyle intervention and IGT Maastricht) (1), that a combined diet- and exercise lifestyle intervention can improve the postprandial glucose metabolism and insulin resistance in IGT subjects with a relatively high level of physical activity and low prevalence of obesity. The Diabetes Prevention Study (DPS) showed a tendency towards an improved glucose tolerance during a 3-yr lifestyle intervention program (0.4 mM difference in 2-hr glucose) (2). Also, the DPS as well as the Diabetes Prevention Program (DPP) showed that changes in lifestyle can prevent the onset of type 2 diabetes in IGT subjects by 58% over a 3-yr period (3-4). Although the SLIM study is relatively small compared to the DPS and DPP, its advantage is that we performed a detailed phenotyping of HOMA-IR, 2-hr glucose, 2-hr insulin, 2-hr FFA concentrations and performed a test for maximal aerobic capacity (VO_2 max) at baseline and follow-up. We have performed mechanistic studies to underscore our intervention results (5-6). Additionally, we have a well-controlled exercise program in which subjects participated at an intensity of at least 70% of their maximal peak oxygen consumption (VO_2max).

Elevated 2-hr glucose concentrations are an important risk factor for the development of type 2 diabetes, but also for cardiovascular disease (CVD) (7). Prolonged postprandial glucose excursions can be considered a risk marker of insulin resistance and dyslipidemia that collectively impact on CVD risk (8). In IGT subjects, disturbances in fatty acid utilization are already present (5) and increased plasma free fatty acids (FFA) have been associated with type 2 diabetes, insulin resistance and CVD risk (9). Moreover, IGT has been related to the Metabolic Syndrome (MetS), which is a clustering of pathologies associated with insulin resistance, type 2 diabetes and CVD risk (10-12).

The SLIM project offers the opportunity to address the impact of lifestyle changes on glucose tolerance and maximal aerobic capacity, as an objective measure for physical fitness. Furthermore, we provide specific data regarding lifestyle efficacy and effectiveness. Our aim was to investigate the impact of a 3-year lifestyle intervention on glucose homeostasis after an oral glucose tolerance test (OGTT) in IGT subjects. In addition, we assessed the changes in metabolic CVD risk factors and evaluated the impact of the lifestyle intervention on the incidence of type 2 diabetes.

Patients and methods

The SLIM project (Study on Lifestyle Intervention and Impaired Glucose Tolerance Maastricht) is a randomized controlled trial, designed to study whether a 6-yr combined dietary and physical activity intervention program can improve glucose tolerance in IGT subjects (13). In addition, changes in body composition, body fat distribution, fasting and 2-hr insulin and plasma glucose concentrations, FFA levels, serum lipids,

blood pressure and maximal aerobic capacity are determined. This report concerns the results after 3 years of intervention.

Study design and subjects

The study design has been described in detail previously (13). Briefly, subjects with an increased risk for glucose intolerance were selected from a cohort in the area of Maastricht, and invited to undergo a capillary standard OGTT. Those subjects with a 2-hr blood glucose concentration > 7.8 mM were invited for a second venous OGTT. For inclusion, mean 2-hr glucose concentration of both OGTTs had to be between 7.8 and 12.5 mM and fasting glucose concentration < 7.8 mM. Data obtained during the second (venous) OGTT were used as baseline values. Exclusion criteria were known diabetes, glucose concentrations outside the inclusion criteria, chronic illness, medication known to interfere with glucose tolerance, participation in a vigorous exercise and/or diet program.

Screening and inclusion of subjects for the SLIM study occurred between March 1999 and May 2000 and in 2002. In total, 147 subjects were included in our study. All analyses in this paper were adjusted for the screening period. Subjects were randomized with stratification for sex and mean 2-hr plasma glucose concentration to the intervention group (INT: 74 subjects; 38 male, 36 female) or the control group (CON: 73 subjects; 37 male, 36 female). It was calculated, according to the preliminary results after 1 year of the Finnish DPS (14), that 50-60 subjects per group would be sufficient to detect a 1.0 mmol/l difference in 2-hr glucose concentration between groups. Data analyses of the 3-year results include those subjects still participating in the study (completers, n=106: 52 INT subjects and 54 CON subjects). 41 Subjects (22 INT, 19 CON) were unable to adhere for 3 years of study of whom 28 subjects, (14 INT and 14 CON) completed the first year. From all dropouts, 32 subjects discontinued the study (16 INT, 16 CON) due to medical reasons in 10 cases, lack of time in 7 cases, lack of motivation in 7 cases, dissatisfaction in 3 cases, no response in 1 case, no transportation in 1 case, unknown in 2 cases and death in 1 case. 9 Subjects did not attend all annual measurements.

Lifestyle Intervention

The intervention program consisted of a dietary and physical activity part. Dietary recommendations were based on the Dutch guidelines for a healthy diet (Dutch Nutrition Council). A skilled dietician gave personal dietary advice during a one-hour counseling session every 3 months, after consideration of a 3-day food record. In addition, subjects received individual advice on how to increase their level of physical activity to at least 30 minutes a day for at least 5 days a week. A body weight loss of 5-7% was the objective. Dietary intake and physical activity were documented in 3-day records and new goals were set and documented for future reference. Furthermore, subjects were encouraged to participate in a combined aerobic- and resistance exercise program in which subjects participated at an intensity of at least 70% of their maximal peak oxygen consumption (VO_2max). Three times a year subjects were asked to participate

in the exercise program using a heartbeat watch to validate the exercise intensity. Control subjects were only briefly informed about the beneficial effects of a healthy diet and physical activity, whereas no individual advice was provided.

Measurements

Body weight was measured with an electronical scale to the nearest 0.1 kg. Waist was measured to the nearest 0.5 cm, with the subject in standing position at the level midway between the lowest rib and the iliacal crest. Body fat percentage was determined by using a bio impedance (Hydra S ecf/icf, rev1.0d, Xitron technologies).

A 3-day weighed food record (two weekdays and 1 weekend day) was kept before the annual visit. Nutrient intake was calculated using the Dutch food table (NEVO version 1996). An incremental exhaustive exercise test was performed on an electronically braked bicycle ergometer to determine maximal peak oxygen consumption (VO_2max).

Changes in glucose tolerance were studied using an OGTT. Plasma glucose, FFA and serum lipids were measured in the fasting state and after 2 hours, with a standard enzymatic technique, automated on a Cobas Fara centrifugal analyzer. Plasma insulin concentration was measured with a Radio Immuno Assay (Cat. #HI-14K, Linco Research) that shows no cross-reactivity with pro-insulin. HBA1c was determined in a fasting serum sample with high-performance liquid chromatography. The HOMA-IR index for insulin resistance was calculated as described by Matthews et al., (1985) (15). Low-density lipoprotein (LDL) cholesterol was calculated according to the formula of Friedewald (16). Blood pressure was measured with a Maxi Stabil 3 pressostabil (CE0047, Speidel en Keller) in duplo with the subject in supine position, after 10 minutes of rest.

The MetS was defined according to the NCEP criteria (17) as having 3 or more of the following conditions: waist circumference greater than 102 cm in men and greater than 88 cm in women; fasting serum triglyceride levels of at least 1.7 mM (≥150mg/dl); high-density lipoprotein (HDL) cholesterol level less than 1.03 mM (<40mg/dl) in men and less than 1.30 mM (<50mg/dl) in women; blood pressure of 130/85 mm Hg or greater; and fasting glucose levels of at least 6.2 mM (110mg/l). Subjects using antihypertensive drugs or lipid lowering medication were classified positive for the respective criterion. The incidence of type 2 diabetes was determined by one OGTT according to WHO criteria of 1999 (18).

Statistical analysis

Data analysis was conducted using SPSS for Macintosh (version 10.0) on subjects who completed 3 years of lifestyle intervention (n=106, completers analysis). In addition, we analyzed all available data of all 147 subjects, including those who dropped out before the 3-yr examination, to indicate intention-to-treat. Insulin, serum lipids and FFA concentrations were not normally distributed and were ln-transformed. Data are presented as mean ± SD. Differences between groups were tested with a Student's t

test for independent samples or by a χ^2 test when applicable. Changes over time between groups were assessed using ANOVA for repeated measures for the completers analysis and with MIXED linear models for the intention-to-treat analysis. A p-value of less than 0.05 was considered statistically significant. All tests were two-sided.

Statement of ethics

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The Medical Ethical Review Committee of Maastricht University approved the study protocol and all subjects gave their written informed consent before the start of the study.

Results

Table 3.1 shows the baseline characteristics of the subjects who completed the 3-yr period. Age was higher in CON compared to INT (p=0.001), whereas no differences were seen in other baseline characteristics. Within each study group, men had a significantly higher weight, waist-to-hip ratio and maximal aerobic capacity and a lower fat percentage and HDL cholesterol levels, compared to women (p_{all} <0.05).

After 1 year, INT subjects had a mean weight change of –2.77 ± 3.69 kg versus -0.62 ± 3.92 kg in CON subjects (p=0.003, time x group interaction) (table 3.2). After 3 years, the weight change between groups became smaller but remained statistically significant different (p=0.01, time x group interaction). Moreover, after 3 years, the increase over time in body fat percentage and body fat mass was smaller in INT compared to CON, (p=0.006 and p=0.016, respectively, time x group interaction). Furthermore, INT subjects improved their maximal aerobic capacity (VO_2 max), while that of CON aggravated (p=0.02) (table 3.2). Intention-to-treat analysis did not change these results (table 3.2). Exercise intensity was regularly monitored during the exercise program by means of heart rate recording. After 3 years, the mean heart frequency during the exercise program was 76% of the maximal heart frequency, indicating the moderate to high intensity. Intervention subjects who participated in the exercise program during at least the second and third year (n=17) increased their maximal aerobic capacity and tended to increase more compared to those who did not participate in the exercise program (n=25), +0.17 l/min versus -0.03 l/min, respectively (p=0.08). The intervention group increased in the number of days that they were at least 30 minutes physically active in doing bicycling, gardening or doing sports with 0.89±2.75 days while those of the control group decreased with -0.55±3.31 days, p=0.046. In the total study population, an increase in the number of days at least 30 minutes physically active in doing bicycling, gardening or doing sports was significantly associated with an increase in aerobic capacity (correlation coefficient R=0.34, p=0.01). In line with these results, fat intake decreased significantly more in INT vs. CON (p interaction<0.001) and carbohydrate intake increased more in INT compared to CON (p interaction=0.001). Fiber intake increased more in INT compared to CON (p interaction=0.05).

Table 3.1 Baseline characteristics of the subjects participating in the 3-yr SLIM lifestyle intervention study.

		Intervention group	Control group
n (male/female)		52 (28/24)	54 (30/24)
Age	(years)	54.2 ± 5.8	58.4 ± 6.8*
Weight	(kg)	87.5 ± 13.7	83.0 ± 11.7
BMI	(kg/m^2)	29.6 ± 3.8	29.2 ± 3.3
Waist	(cm)	103.2 ± 10.6	102.4 ± 9.2
Body fat percentage	(%)	38.4 ± 6.3	37.5 ± 6.4
Body fat mass	(kg)	33.4 ± 7.2	31.0 ± 6.6
Body fat free mass	(kg)	54.1 ± 10.4	51.9 ± 9.4
VO_2 max	(l/min)	2.22 ± 0.61	2.13 ± 0.55
Fasting glucose	(mmol/l)	5.97 ± 0.87	5.90 ± 0.70
2-hr Glucose	(mmol/l)	8.59 ± 1.55	8.46 ± 1.84
HBA1c	(%)	5.6 ± 0.5	5.8 ± 0.5
Fasting insulin	(mU/l)	18.0 ± 6.3	17.1 ± 6.9
2-hr Insulin	(mU/l)	103.3 ± 73.0	96.4 ± 89.9
HOMA-IR		4.82 ± 2.04	4.55 ± 2.05
Triglycerides	(mmol/l)	1.56 ± 1.33	1.49 ± 0.88
Total Cholesterol	(mmol/l)	5.17 ± 0.78	5.25 ± 0.84
HDL Cholesterol	(mmol/l)	1.14 ± 0.30	1.11 ± 0.27
LDL Cholesterol	(mmol/l)	3.40 ±0.78	3.47 ± 0.71
FFA	(µmol/l)	589 ± 231	551 ± 172
2-hr FFA	(µmol/l)	115 ± 79	102 ± 42
Diastolic blood pressure	(mm Hg)	90 ± 9	88 ± 7
Systolic blood pressure	(mm Hg)	142 ± 16	145 ± 14

Data are Mean ± SD, n= 106. * p= 0.001 (Student's t-test).

2-Hour plasma glucose levels decreased in INT from 8.59 ± 0.24 mM at baseline to 7.96 ± 0.29 mM after 1 year and returned to 8.55 ± 0.34 mM after 3 years (figure 3.1, open circles). In CON an increase was seen from 8.46 ± 0.23 mM at baseline to 8.83 ± 0.29 mM after 1 year and to 9.35 ± 0.33 mM after 3 years (p=0.023 time x group interaction) (figure 3.1, black squares). In the intention-to-treat analysis, there remained a tendency towards a difference between groups (p=0.086). The difference between groups was 0.87 mM after 1 year and remained relatively constant throughout the study (p interaction=0.014).

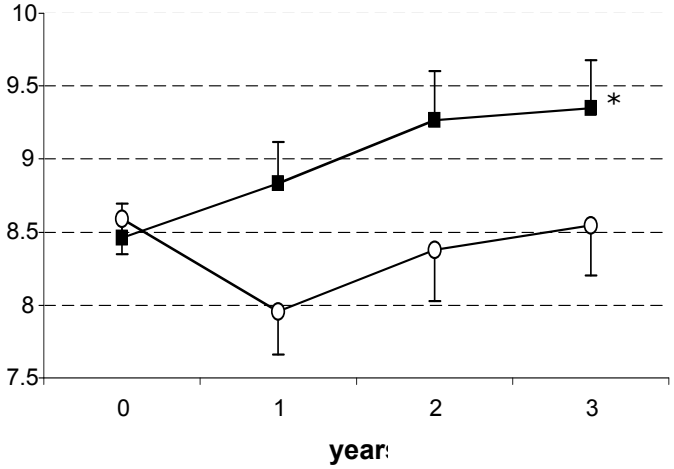


Figure 3.1 2-hr Plasma glucose levels for the intervention group (open circles) and control group (black squares) at baseline and after 1, 2 and 3 years of follow-up. Data are mean ± SEM, n= 106: 52 INT, 54 CON. P= 0.023* for the difference between the groups over time (GLM ANOVA for repeated measures).

Multiple linear regression analysis revealed that a decrease in body weight was correlated with a decrease in 2-hr glucose levels (β= 0.257 kg, p=0.020), and a decrease in 2-hr glucose levels was correlated with an improvement in VO_2max (β= -0.220 l/min, p=0.048), although not independent of bodyweight loss (β= -0.174 kg, p=0.117). No differences were observed in fasting plasma glucose and HBA1c in the completers analysis, whereas the change in fasting glucose did become significantly different between groups in the intention-to-treat analysis (p=0.04, table 3.2).

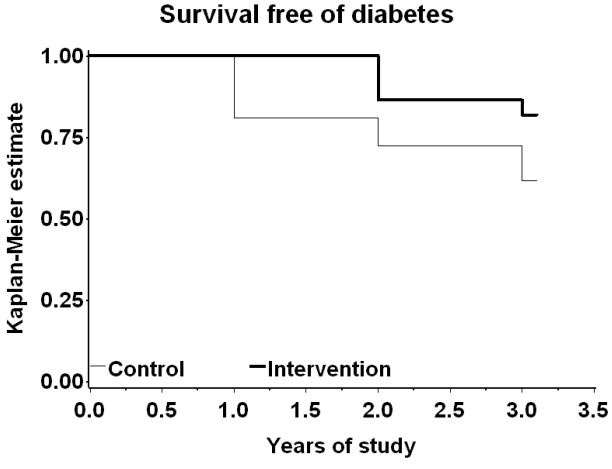
Insulin resistance, as indicated by the HOMA index for insulin resistance (HOMA-IR), decreased in the INT after 3 years (-0.19: -1.11; 1.12), while there was an increase in CON (+0.37: -0.97; 1.52); p interaction=0.04). Fasting insulin decreased in both groups and 2-hr insulin levels tended to increase more in CON compared to INT (p interaction=0.07). Using intention-to-treat analysis, differences between groups in insulin levels and HOMA-IR were reduced. Multiple linear regression analysis revealed that the change in body weight was significantly correlated with the change in HOMA-IR (β= 0.470 kg, p<0.001), whereas the change in VO_2 max was not predictive (β= -0.073 l/min, p=0.513). 2-Hr FFA levels decreased more in INT, compared to CON (p interaction=0.04), whereas the 3-yr decrease in fasting FFA level was not different between groups (table 3.2).

Triglyceride levels were decreased after 2 years in INT versus an increase in CON, but these differences disappeared after 3 years. Total cholesterol, HDL and LDL concentrations did not change over time, and did not differ between groups (p_{all} >0.05). Both in INT and CON a decline in time was observed in diastolic blood pressure (p<0.001) and systolic blood pressure (p=0.001), which was not different between groups (p=0.14 and p=0.92, respectively). Results on serum lipids and blood pressure were similar for the intention-to-treat analysis and after taking lipid lowering and antihypertensive medication into account respectively.

Within each study group, men and women did not significantly differ in change over 3 years, except for waist-to-hip ratio and percentage body fat. The waist-to-hip ratio increased in women, but remained relatively stable in men ($p<0.01$ for differences between sex in each study group). In both study groups, the percentage body fat increased slightly in women, whereas a slight decrease was observed for men ($p<0.01$ for differences between sex in each study group).

Prevalence of the MetS rose from 61.4% at baseline to 66.0% after 3 years in INT (n = 44, 24 male/20 female) and from 58.3% to 68.8% in CON (n = 48, 27 male/21 female) ($p=0.630$ for difference between groups). More women (33/41) than men (29/51) had the MetS ($p=0.02$), since more women than men met the criteria for a high waist circumference ($p=0.004$) and a low HDL cholesterol ($p<0.001$).

Although the primary outcome of this study was change in 2-hr plasma glucose concentration, we also examined the cumulative diabetes incidence during the study, although the results have to be interpreted with caution, since our study was under-powered for these analyses. In the analysis of the subjects who completed the full 3 years of the study, the cumulative incidence was 18% (8/44) in the intervention group and 38% (18/47) in the control group. The p-value of the log-rank test amounted to 0.025, and the relative risk was 0.42 (95% CI 0.18-0.96) (figure 3.2). In the intention-to treat-analysis the cumulative incidence of diabetes in the intervention group was 18% (11/61) compared to 32% (19/60) in the control group. The p-value from the log-rank test comparing the survival curves was 0.07, the relative risk amounted to 0.52 (95% CI 0.25-1.10). Adjustment for age and mean weight did not change the outcome of this study.



INT	**44**	**44**	**38**	**35**
CON	**47**	**38**	**34**	**29**

Total subjects at risk for each study group during 3 years of study

Figure 3.2 Proportion of subjects without diabetes during the study. The relative risk of diabetes for subjects in the intervention group, as compared with those in the control group, was 0.42 ($p=0.025$ for the comparison between groups).

Table 3.2 Changes in body composition and glucose metabolism after 1, 2 and 3 years of SLIM lifestyle intervention compared to baseline.

			Δ Year 1	Δ Year 2	Δ Year 3	Δ Year 3 95% CI interval	P*	P §
Weight	(kg)	INT	-2.77 ± 3.69	-1.76 ± 4.34	-1.08 ± 4.30	(-2.28; 0.11)	0.011	0.045
		CON	-0.62 ± 3.92	-0.11 ± 3.26	0.16 ± 4.91	(-1.18; 1.50)		
BMI	(kg/m2)	INT	-0.94 ± 1.25	-0.61 ± 1.49	-0.36 ± 1.47	(-0.77; 0.05)	0.014	0.047
		CON	-0.20 ± 1.39	-0.02 ± 1.17	0.08 ± 1.80	(-0.41; 0.57)		
Waist	(cm)	INT	-3.31 ± 4.33	-1.49 ± 4.59	-0.33 ± 6.77	(-2.21; 1.56)	0.505	0.670
		CON	-1.72 ± 4.99	-0.64 ± 4.78	1.30 ± 7.38	(-0.72; 3.31)		
Body fat percentage (n=76)	(%)	INT	-1.66 ± 2.68	-0.74 ± 2.54	-1.03 ± 3.38	(-1.97; -0.09)	0.006	0.002
		CON	-0.84 ± 1.90	-0.63 ± 2.04	-0.41 ± 2.72	(-1.16; 0.34)		
Body fat mass (n=75)	(kg)	INT	-2.40 ± 3.21	-1.26 ± 3.28	-1.16 ± 3.80	(-2.21; -0.10)	0.016	0.010
		CON	-1.03 ± 2.81	-0.64 ± 2.68	-0.24 ± 3.99	(-1.34; 0.86)		
Body fat free mass (n=75)	(kg)	INT	-0.37 ± 2.20	-0.34 ± 2.20	0.07 ± 3.44	(-0.88; 1.03)	0.087	0.138
		CON	0.33 ± 1.87	0.44 ± 1.68	0.33 ± 2.33	(-0.31; 0.98)		
VO_2 max (n=76)	(l/min)	INT	0.13 ± 0.25	0.10 ± 0.25	0.05 ± 0.35	(-0.06; 0.16)	0.019	0.009
		CON	0.02 ± 0.21	-0.05 ± 0.23	-0.06 ± 0.21	(-0.12; 0.12)		
Fasting glucose	(mmol/l)	INT	-0.11 ± 0.54	0.05 ± 0.66	0.32 ± 0.83	(0.08; 0.55)	0.169	0.040
		CON	0.02 ± 0.63	0.40 ± 0.84	0.55 ± 0.82	(0.33; 0.78)		
2-hr Glucose	(mmol/l)	INT	-0.63 ± 1.55	-0.21 ± 2.13	-0.05 ± 2.02	(-0.61; 0.52)	0.023	0.086
		CON	0.37 ± 2.19	0.80 ± 2.66	0.89 ± 1.90	(0.37; 1.40)		
HBA1c (n=99)	(%)	INT	-0.24 ± 0.39	-0.09 ± 0.62	-0.09 ± 0.43	(-0.21; 0.03)	0.952	0.838
		CON	-0.19 ± 0.32	-0.11 ± 0.38	-0.10 ± 0.38	(-0.20; 0.00)		
Fasting insulin	(mU/l)	INT	-0.69 (-5.98; 1.58)	-3.66 (-10.85; 0.77)	-1.17 (-5.23; 2.46)	-	0.077	0.613
		CON	0.24 (-2.79; 3.45)	-2.42 (-9.07; 0.92)	-0.35 (-3.74; 2.96)	-		
2-hr Insulin (n=95)	(mU/l)	INT	-9.28 (-30.66; 14.60)	-10.44 (-45.97; 11.31)	7.77 (-26.35; 61.60)	-	0.071	0.388
		CON	12.06 (-6.27; 32.77)	1.25 (-13.23; 23.70)	15.57 (-5.83; 49.01)	-		

			Δ Year 1	Δ Year 2	Δ Year 3	Δ Year 3 95% CI interval	P*	P §
HOMA-IR		INT	-0.26 (-1.97; 0.57)	-1.05 (-2.62; 0.41)	-0.19 (-1.11; 1.12)	-	0.039	0.346
		CON	0.13 (-0.74; 1.11)	-0.41 (-2.27; 0.56)	0.37 (-0.97; 1.52)	-		
Triglycerides (n=97)	(mmol/l)	INT	0.00 (-0.29; 0.17)	-0.01 (-0.43; 0.18)	0.06 (-0.31; 0.24)	-	0.034	0.023
		CON	0.02 (-0.23; 0.38)	0.01 (-0.36; 0.50)	0.01 (-0.33; 0.47)	-		
Total cholesterol (n=98)	(mmol/l)	INT	-0.00 ± 0.69	0.22 ± 0.81	0.41 ± 0.86	(0.16; 0.67)	0.157	0.213
		CON	0.10 ± 0.57	0.32 ± 0.75	0.26 ± 0.94	(-0.00; 0.52)		
HDL cholesterol	(mmol/l)	INT	0.00 ± 0.16	0.08 ± 0.16	0.10 ± 0.17	(0.05; 0.15)	0.476	0.720
		CON	-0.01 ± 0.18	0.05 ± 0.16	0.06 ± 0.14	(0.02; 0.10)		
LDL cholesterol (n=96)	(mmol/l)	INT	-0.06 ± 0.55	0.17 ± 0.77	0.22 ± 0.79	(-0.02; 0.46)	0.430	0.554
		CON	0.06 ± 0.56	0.22 ± 0.66	0.13 ± 0.75	(-0.08; 0.33)		
FFA	(µmol/l)	INT	-117 (-195; -15)	-85 (-154; -6)	-90 (-185; 5)	-	0.369	0.419
		CON	-83 (-163; 29)	-49 (-116; 36)	-92 (-165; -1)	-		
2-hr FFA	(µmol/l)	INT	-24 (-49; -10)	-10 (-41; 6)	-18 (-46; 23)	-	0.041	0.199
		CON	-12 (-29; 9)	-4 (-21; 19)	-6 (-22; 16)	-		
Diastolic blood pressure	(mm Hg)	INT	-2.8 ± 6.4	-4.9 ± 7.5	-6.2 ± 7.4	(-8.3; -4.1)	0.135	0.775
		CON	0.2 ± 6.7	-2.7 ± 7.4	-3.1 ± 8.2	(-5.4; -0.8)		
Systolic blood pressure	(mm Hg)	INT	-4.7 ± 15.4	-5.7 ± 14.1	-3.6 ± 15.8	(-8.0; 0.9)	0.922	0.997
		CON	-4.2 ± 13.6	-5.9 ± 16.9	-3.5 ± 15.6	(-7.8; 0.9)		

Data are mean ± SD. Fasting insulin, 2-hr insulin, HOMA-IR, triglycerides, free fatty acids are expressed as median (25th percentile; 75th percentile) n= 106: 52 INT, 54 CON, unless stated otherwise. *P-value for the completers analysis regarding the difference between the groups over 3 years time (group x time interaction). § P-value for the intention-to-treat analysis regarding the difference between the groups over 3 years time (group x time interaction).

At baseline, metabolic characteristics of dropouts (n=28) were similar between INT and CON except for 2-hr insulin levels, which were 81.68 mU/l in INT and 131.96 mU/l in CON (p=0.02). The reasons for dropout were similar between INT and CON and no differences in dropout were observed between men and women. Dropouts had a higher baseline BMI, 2-hr plasma glucose levels and a lower maximal aerobic capacity than subjects who completed all 3 years of study (p_{all} <0.05 for difference between dropouts and completers). As specific dropout can affect the result of our analysis, we performed an intention-to-treat analysis (n=147; table 3.2). This analysis affected 2-hr glucose, insulin and HOMA-IR (p>0.05 for differences between INT and CON), while no significant changes were observed in BMI, waist, body fat mass and distribution, HBA1c VO_2 max, serum lipids, blood pressure and dietary intake.

Table 3.3 Changes in energy intake, based on 3-day food records, after 1, 2 and 3 years compared to baseline.

		Baseline	Δ Year 1	Δ Year 2	Δ Year 3	Δ Year 3 95% CI interval	P
Energy							
(MJ/day)	INT	8.8 ± 2.2	-0.7 ± 1.7	-0.9 ± 2.1	-1.1 ± 1.9	(-1.6; -0.5)	0.231
	CON	8.7 ± 2.2	-0.2 ± 2.0	-0.3 ± 1.8	-0.3 ± 1.8	(-0.8; 0.2)	
Total fat							
(E%)	INT	36.2 ± 6.7	-4.8 ± 6.3	-4.3 ± 7.6	-4.7 ± 5.9	(-6.4; -3.0)	<0.001
	CON	35.0 ± 6.8	-0.2 ± 7.1	0.2 ± 7.0	-0.5 ± 5.8	(-2.1; 1.1)	
Saturated fat							
(E%)	INT	13.6 ± 2.9	-2.4 ± 2.9	-2.3 ± 3.7	-2.9 ± 3.2	(-3.8; -2.0)	<0.001
	CON	13.6 ± 3.5	-0.2 ± 3.2	0.1 ± 3.1	-0.7 ± 3.1	(-1.6; 0.1)	
Carbohydrates							
(E%)	INT	41.0 ± 7.4	4.5 ± 5.5	5.3 ± 6.9	4.8 ± 5.6	(3.3; 6.4)	0.001
	CON	43.4 ± 7.3	0.3 ± 6.7	0.3 ± 7.0	1.2 ± 5.4	(-0.3; 2.8)	
Fiber							
(g/MJ)	INT	2.7 ± 0.8	0.5 ± 0.7	0.4 ± 0.8	0.5 ± 0.6	(0.3; 0.7)	0.050
	CON	2.7 ± 0.9	0.1 ± 0.8	0.2 ± 0.8	0.2 ± 0.8	(0.0; 0.5)	
Alcohol							
(E%)	INT	6.2 ± 7.1	-0.6 ± 5.4	-1.6 ± 4.6	-1.5 ± 3.7	(-2.5; -0.4)	0.304
	CON	5.6 ± 5.5	-0.0 ± 5.6	-0.4 ± 3.7	-0.1 ± 4.5	(-1.4; 1.2)	

Data are mean ± SD, n= 96: 47 INT, 49 CON.

Discussion

The SLIM study shows that a lifestyle intervention program according to general guidelines was effective in improving dietary composition (reduction fat intake), and increasing VO_2 max, and resulted in a beneficial effect on glucose tolerance and insulin sensitivity in a Dutch population at high risk for type 2 diabetes. After 3 years, the difference between groups in 2-hr plasma glucose levels remained as high as 0.8 mmol/l (similar as after 1 year), which was associated with a 58% reduction in the incidence of diabetes. In addition, HOMA-IR and 2-hr FFA concentrations improved more in the INT group. However, no significant changes were found in components of the metabolic syndrome.

Our lifestyle intervention showed a modest weight reduction after 1 year of -2.8 kg, with a gradual regain in the following years, which is consistent with data from the Finnish DPS: after a weight reduction of -4.5 kg during the first year, a regain of approximately 1 kg after 3 years was observed (2). Furthermore, INT subjects improved their dietary habits by increasing carbohydrate and fiber intake, decreasing fat intake and they improved their VO_2 max, indicating that diet and exercise recommendations were (at least partly) followed.

Despite the relatively small weight changes in our study, substantial improvement in 2-hr glucose concentration was observed in the INT, and the difference between groups persisted throughout the 3 years of lifestyle intervention. Although our results seem less impressive than for example the 1.6 mmol/l reduction in 2-hr glucose by rosiglitazone from the DREAM trial (19), a similar lifestyle intervention from the DPS has recently shown that beneficial lifestyle changes were sustained during a follow-up period for a median of 3 years without individual lifestyle counseling (20), whereas this has not been proven yet for a pharmacological intervention. Furthermore, our lifestyle intervention reduced average weight with -1.08 kg, whereas the rosiglitazone group in the DREAM trial increased in weight by 2.2 kg, which may be considered disadvantageous for diabetes and CVD risk over the long term.

In contrast to 2-hr glucose, we did not observe a significant difference in fasting glucose concentrations in the completers analysis, which is in agreement with the hypothesis that fasting and postchallenge hyperglycemia may represent phenotypes with distinct natural histories in the evolution of type 2 diabetes (21). Whereas postprandial glucose disposal may be the main defect in IGT, impaired fasting glucose (IFG) may only reflect an abnormal glucose setpoint, but not an abnormal postprandial glucose response (22). Cumulative diabetes incidence was 38% in CON, but only 18% in INT, reflecting 58% less progression towards type 2 diabetes. Intention-to-treat analysis did not appreciably change these results, although the relative risk decreased to 48% for INT compared to CON (p=0.07). Although our study was not designed to investigate diabetes incidence and thus lacked the statistical power, the results are comparable to results of the Finnish DPS and American DPP, both showing a 58% diabetes risk reduction after approximately 3 years time (3-4).

The natural progression towards type 2 diabetes, as is observed in the CON, is very high in our IGT group. Our results are in agreement with the Hoorn study (23), which showed a progression of 34% in isolated IGT subjects during a follow-up of 6.4 years. In the study of Meigs and colleagues (21), 21% of the subjects with baseline

IFG/IGT progressed towards type 2 diabetes during a 5-year follow-up. Our study underscores the importance of lifestyle intervention in subjects with IGT, since they are clearly at high risk for developing type 2 diabetes.

In addition, HOMA-IR declined in our completers analysis, indicating improvement in insulin sensitivity. Furthermore, our intervention not only improved glucose homeostasis and insulin sensitivity but also reduced postprandial circulating FFA. Elevation of (postprandial) FFA concentration may induce peripheral (skeletal) muscle and hepatic insulin resistance (24). Thus, reduced FFA concentrations may be one of the mechanisms for an improved insulin sensitivity in these subjects. Furthermore, previous studies showed that in subgroups of these IGT subjects, skeletal muscle fat oxidation capacity and fat oxidation improved after one year of lifestyle intervention, which may also be related to an improved insulin sensitivity (25).

IGT has been associated with an increased CVD risk (26-27) and although our study showed a marked improvement in 2-hr glucose, this was not accompanied by changes between groups in NCEP MetS prevalence. An explanation can be the relatively small number of subjects in each study group, combined with only minor changes in waist circumference and fasting plasma glucose concentrations and no changes in the other MetS criteria. When we applied the MetS criteria of the International Diabetes Federation (IDF) (28) the results were essentially similar.

2-Hr glucose concentrations and HOMA-IR seem more sensitive to minor changes in weight and lifestyle and these criteria may be required for prediction of CVD risk, since type 2 diabetes alone may convey a much greater risk on CVD than the presence of MetS (29). Furthermore, the NCEP MetS does not include CVD risk factors such as age, physical activity or history of CVD events and does not weigh the severity of each criterion, which may be critically important for adequate prediction of CVD risk [29]. Also, endothelial dysfunction, possibly induced by oxidative stress and inflammation, may be associated with IGT and recent discovered CVD risk markers involved in inflammation and coagulation/fibrinolysis like CRP (30), IL-6 and PAI-1 (31) await to be determined.

After 3 years, 28% of all study subjects (22 out of 74 in INT and 19 out of 73 in CON) discontinued their participation, compared to a dropout rate of 8% and 7.5% in the DPS and DPP, respectively. Several explanations for the difference in dropout rate between the DPS, DPP and SLIM can be given. First, our study population was originally recruited from the general population and they may have had less internal motivation to participate in the study, as compared to subjects recruited via advertisements or high-risk screening, as was done in the DPS (14). The DPP recruited subjects from a variety of resources, including informational mailings, advertisements, open screenings and referrals from health care professionals (32). Second, no weight loss program was offered when subjects did not loose weight which may have led to dissatisfaction for participants. Interestingly, our dropout percentage is similar to that observed in the DREAM trial (19), with 29.3% (772/2635) dropout in the rosiglitazone group and 25.0% (658/2634) in the control group. Our dropouts were in a worse metabolic condition at baseline than completers, but the dropout rate was not different between study groups. Intention-to-treat analysis revealed that the difference in 2-hr glucose tolerance, HOMA-IR and 2-hr FFA was less pronounced, which suggests selective drop-out according to subjects in the intervention group with smaller improvements. To in-

crease effectiveness of our lifestyle intervention in a clinical setting, we underscore the importance of more insight in the unknown determinants to participate in a lifestyle-intervention and to remain adherent.

In conclusion, our lifestyle intervention program, aimed at increasing physical activity and using a healthy diet, resulted in a sustained beneficial effect on 2-hr glucose concentrations, insulin resistance and 2-hr FFA in a population at high risk for type 2 diabetes, even after 3 years, which was associated with a reduced risk for developing type 2 diabetes. Although there was some selective dropout, intention-to-treat analysis still showed a similar trend in results with a significant difference in fasting glucose and a tendency towards a difference in 2-hr glucose and diabetes incidence. No effect was observed on components of the metabolic syndrome or on fasting glucose concentrations, suggesting that fasting and postchallenge hyperglycemia may represent phenotypes with distinct natural histories in the evolution of type 2 diabetes. Our lifestyle intervention is efficacious, but due to the relatively high dropout rate of subjects in worse metabolic condition, additional information on determinants of adherence and dropout for increased effectiveness and successful implementation is needed.

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4

PREDICTORS OF INTERVENTION OUCOME AND DROPOUT

The SLIM study

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submitted

Abstract

Objective
To evaluate the effect of a 4.1 year (range 3-6 years) lifestyle intervention according to general public health recommendations on glucose tolerance and dropout in a Dutch population with impaired glucose tolerance (IGT).

Method
In the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM), 147 Caucasian IGT subjects were randomized to an intervention group (n=74, 38 male, 36 female (INT)) and control group (n=73, 37 male, 36 female (CON)). Annually, subjects underwent measurements of body weight, anthropometry, glucose tolerance (OGTT), insulin resistance (HOMA-IR), maximal aerobic capacity (VO_2 max), blood lipids and blood pressure. INT received individual advice regarding a healthy diet and physical activity.

Results
INT decreased their saturated fat intake ($p<0.05$) and VO_2 max ($p=0.04$), versus CON. Body weight did not change between groups. After a decrease, 2-hr glucose levels overall increased in INT (+0.11 mmol/l), but significantly less than CON (+1.18 mmol/l; $p=0.04$). Diabetes incidence was lower in INT versus CON (30% versus 56%, $p=0.04$). Change in body weight was associated with change in 2-hr glucose levels ($\beta=0.399$ mmol/l, $p=0.02$). Dropouts (n=32) had a lower maximal aerobic capacity and social economic status and a higher BMI and 2-hr glucose, compared to non-dropouts (n=115).

Conclusion
Prolonged feasible changes in diet and physical activity prevent deterioration of glucose tolerance and reduce diabetes risk. Body weight changes is the strongest predictor of changes in 2-hr glucose levels. Low social economic status, low maximal aerobic capacity and high BMI and 2-hr glucose are indicative of dropout to the program.

Introduction

Type 2 diabetes needs effective prevention measures for curbing the growing burden worldwide. Diabetes incidence is 10–20 times greater in those with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) than those with normal glycemia (1). Lifestyle interventions in IGT are an efficacious (2, 3) and cost-effective (4) way to prevent type 2 diabetes (5, 6), even after active counseling is stopped (7).

Lifestyle changes towards general guidelines for diet and physical activity seems inversely associated with diabetes risk (8), indicating that the more strict compliance to the regime, the better the outcome. The Diabetes Prevention Program (DPP) has shown that a lifestyle-induced reduction in body weight was most associated with a reduced diabetes risk (9). This study, the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM), showed that subjects who adhered to both the dietary as well as the physical activity recommendations had the greatest one-year improvement in bodyweight, waist and fasting insulin (10) and prevented deterioration of 2-hr glucose levels after 3 years (3).

The dropout rate in lifestyle interventions is an important factor associated with a decreased efficacy of the program. Overall, dropout rate varies highly between lifestyle interventions with or without medication (5, 6, 11-13), depending on the patient population, the medical condition, the form of treatment, but also the complexity of the regime and the period of time. The SLIM regime has a complex regime and a long-term follow-up. Therefore, this study offers the opportunity to increase our knowledge on determinants of dropout and intervention outcome (2-hr glucose tolerance) may contribute to a more efficient and targeted intervention to prevent and/or treat type 2 diabetes in the future.

The aim of the present SLIM study was to 1) assess the effectiveness of a lifestyle intervention on glucose tolerance and related cardiovascular risk factors during a mean follow-up of 4.1 years, 2) to examine which lifestyle behaviors changed during the course of the intervention, 3) to examine whether change in these lifestyle behaviors was associated with change in glucose tolerance and 4) to determine factors associated with dropout to the program. The present paper extends previously published 3-year results by providing novel information on determinants of intervention outcome and dropout, which can help optimize identification, lifestyle effect and adherence in high-risk subjects.

Methods

The SLIM study (Study on Lifestyle intervention and Impaired glucose tolerance Maastricht) is a randomized controlled trial, evaluating the effect of a combined dietary and physical activity intervention program on glucose tolerance in IGT subjects (10). Changes in body composition, fasting and 2-hr insulin and plasma glucose concentrations, serum lipids, blood pressure and maximal aerobic capacity are determined annually. The Medical Ethical Review Committee of Maastricht University approved the study protocol and all subjects gave their written informed consent before the start of the study.

Study design and subjects

The study design has been described in detail previously (10). Briefly, subjects with an increased risk for glucose intolerance, e.g. family history of diabetes, age >40 years, BMI > 25 kg/m^2, were selected from a cohort in the area of Maastricht, and were invited to undergo a capillary standard Oral Glucose Tolerance Test (OGTT) (response rate 46.2%, see reference (14)) at the University of Maastricht. Those subjects with a 2-hr blood glucose concentration > 7.8 mmol/l were invited for a second venous OGTT. For inclusion, mean 2-hr glucose concentration of both OGTTs had to be between 7.8 and 12.5 mmol/l and fasting glucose concentration < 7.8 mmol/l. Data obtained during the second (venous) OGTT were used as baseline values. Exclusion criteria were known diabetes, chronic illness, medication known to interfere with glucose tolerance, participation in a vigorous exercise and/or diet program. The incidence of type 2 diabetes was determined according to WHO criteria of 1999 (15).

Screening and inclusion started in 1999. Originally, the study follow-up was 3 years, but this was extended to 6 years in 2002. In 2002, a second screening period was performed, and an additional 33 subjects were included in the study. In total, the study population consists of 147 subjects. It was calculated, according to the preliminary results after 1 year of the Finnish DPS (16), that 50-60 subjects per group would be sufficient to detect a 1.0 mmol/l difference in 2-hr glucose concentration between groups. The study was completed in June 2006 (see figure 4.1). Subjects were randomized, with stratification for sex and mean 2-hr plasma glucose concentration, to either the intervention group (INT: 74 subjects; 38 male, 36 female) or the control group (CON: 73 subjects; 37 male, 36 female). At the end of the intervention, 58 INT (78%) and 57 CON (78%) completed at least 3 years of lifestyle intervention, of whom 6 INT and 3 CON did not attend all measurements. This means that the participants in the INT group attended the quarterly meetings with the dietician in addition to the annual meetings and that the participants in the CON group attended the annual meetings. In total 32 subjects (16 INT, 16 CON) discontinued study participation and were classified as dropout. Reason for discontinuation did not differ between study groups (p=0.85). The SLIM flow-chart is presented in figure 4.1.

Lifestyle Intervention

The intervention program consisted of a dietary and physical activity part. Dietary recommendations were based on the Dutch guidelines for a healthy diet (Dutch Nutrition Council). A skilled dietician gave personal dietary advice during a one-hour counseling session every 3 months, based on a 3-day food record. A 3-day weighed food record (two weekdays and 1 weekend day) was kept before the annual visit. Nutrient intake was calculated using the Dutch food table (NEVO version 1996). After consideration of a 3-day physical activity record, subjects received personalized advice by the researcher and/or dietician on how to increase their level of physical activity to at least 30 minutes a day for at least 5 days a week. Subjects were encouraged to participate in a free, supervised combined aerobic- and resistance exercise program, especially designed for this study, which was offered at 3 different days and time points every week until the end of the study. Intensity of the program was evaluated three times a

year, by wearing a heart rate monitor during the training sessions. The intensity of the program was at least 70% of the maximal peak oxygen consumption (VO_2max). Control subjects received no individual advice, only annual general information about the beneficial effects of a healthy diet and physical activity.

Measurements

In both groups, several measurements were performed annually including an OGTT, insulin levels, VO_2max, body weight, waist circumference, body fat percentage, blood pressure, HBA1c, cholesterol and HDL. Body weight was measured with an electronical scale to the nearest 0.1 kg. Waist was measured to the nearest 0.5 cm, with the subject in standing position at the level midway between the lowest rib and the iliacal crest. Body fat percentage was determined by skinfold measurements (17). Blood pressure was measured in duplo with a Maxi Stabil 3 pressostabil (CE0047; Speidel en Keller, Jungingen, Germany) with the subject in supine position, after 10 minutes of rest. The mean of both measurements was used in the analyses. On a separate occasion, an incremental exhaustive exercise test was performed on an electronically braked bicycle ergo meter to determine maximal peak oxygen consumption (VO_2max). Changes in glucose tolerance were studied using an OGTT. Blood samples were drawn after an overnight fast and again 2 hours after an oral glucose load (75 gram glucose). Plasma glucose was measured, with a standard enzymatic technique, automated on a Cobas Fara centrifugal analyzer. Plasma insulin concentration was measured with a Radio Immuno Assay (catalogue no. HI-14K; Linco Research, St Charles, MO, USA) that shows no cross-reactivity with pro-insulin. The HOMA-IR index for insulin resistance was calculated as described by Matthews et al., (1985)(18). Glycated hemoglobin (HBA1c) was determined in a fasting serum sample with high-performance liquid chromatography (reference values for our laboratory 4.4-6.2%). Total cholesterol, HDL cholesterol, and triglycerides levels were measured, with a standard enzymatic technique, automated on a Cobas Fara centrifugal analyzer. Low-density lipoprotein (LDL) cholesterol was calculated according to the formula of Friedewald (19). In a questionnaire, subjects were asked to fill in their highest educational background. At baseline and annually until year 3, physical activity was measured with the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH) in all participants, which proved to be fairly reliable and reasonably valid (20). This Dutch questionnaire contains 10 questions about the number of days spent per week, the average time spent per day and intensity regarding four activity categories: commuting activities, leisure time activities, household activities and activities at work and school. The questionnaire categorizes all activities into light, moderate and vigorous intensity activities, based on MET (metabolic equivalent) values defined by Ainsworth's compendium of physical activities (21) and the respondents' age.

Statistical analysis

Data analysis was conducted using SPSS for Windows (version 14.1). Insulin and serum lipid concentrations were not normally distributed and were ln-transformed. Data are

presented as mean ± SD in the tables and text, and as mean ± SEM in the figures to improve graphical presentation. Differences between groups at baseline were tested with a Student's t test for independent samples or a chi-square test. Changes over time between groups were assessed using mixed model analysis on intention to treat, which included all available observations, including those from later dropouts. P-values of interaction between group and time were used to indicate differences between the groups as a result of the lifestyle intervention. Survival analysis was used to determine the hazard ratio for diabetes development and to produce a Kaplan-Meier graph. Stepwise backward linear regression analysis was used to determine which parameters were associated with changes in glucose tolerance. A p-value of less than 0.05 was considered statistically significant. All tests were two-sided.

Results

Effectiveness of the lifestyle intervention

At baseline, no differences between the randomized groups were seen apart from age, which was higher in the control subjects (58.8 ± 8.4 years) compared to the intervention subjects (55.0 ± 6.5 years, p=0.001 for differences between groups; table 4.1). The age difference remained similar between groups during the lifestyle intervention period.

The SLIM intervention had a beneficial effect on 2-hr glucose levels and diabetes risk. 2-Hr glucose levels decreased in INT in the first 4 years of the study and increased slightly at the end of the study. In CON, 2-hr glucose increased from 8.80±2.09 mmol/l to 9.38 ± 2.45 mmol/l, p=0.041 for average difference between groups (figure 4.2D). Diabetes incidence was higher in the control group compared to the intervention group with a p-value of the log-rank test that amounted to 0.04 and a relative risk of 0.53 (95% CI 0.29-0.97) (figure 4.3).

Lifestyle-induced behavior change

During the lifestyle intervention, intervention subjects decreased their total fat intake (p=0.01 time x group interaction, figure 4.2A) and increased their carbohydrate intake from 40.9±7.5 E% at baseline to 46.7±5.9 E% at the end of the study (p=0.002), whereas the increase was smaller in CON (+1.2 E%). INT decreased their saturated fat intake with 2.4%, while a minor decrease of 0.9% was observed for CON (p<0.001). Fiber intake increased in INT from 2.7±0.7 mg/MJ to 3.4±1.1 mg/MJ and increased less in CON from 2.7±0.9 mg/MJ to 3.3±1.2 mg/MJ (p=0.05 between groups). Changes in energy, cholesterol, protein and alcohol intake were similar between study groups (p>0.05, data not shown). Data on energy intake from baseline to year 3 have been published previously (3).

During the lifestyle intervention, body weight decreased in INT after 1 year (-2.47 kg) and during the first 4 years (-0.32 kg) (table 4.1), but increased to baseline value at the end of the study, whereas body weight did not change in CON (p=0.20, figure 4.2B).

Maximal aerobic capacity (VO_2 max) improved more in INT, compared to CON, p=0.042 (figure 4.2C). To verify the association between physical activity and aerobic capacity we looked at the number of days that subjects were at least 30 minutes physically active doing walking, bicycling, gardening or doing sports after 3 years. Statistical analyses revealed that the number of active days per week increased significantly in the intervention group from 2.9±2.4 days at baseline to 3.8±2.5 days after 3 years (change: 0.9±2.8 days), while those of the control group decreased from 3.0±2.6 days at baseline to 2.5±2.7 days (change: -0.55±3.31 days (mean±SD)), p=0.05 between groups. In the total study population, the increase in number of days was significantly associated with an increase in aerobic capacity (Pearson's correlation coefficient R=0.343, p=0.01). No differences between groups or over time were observed in fasting glucose, fasting insulin and 2-hr insulin, HOMA-IR, triglycerides, total cholesterol, HDL and LDL cholesterol, diastolic and systolic blood pressure or medication use (p>0.05, table 4.1).

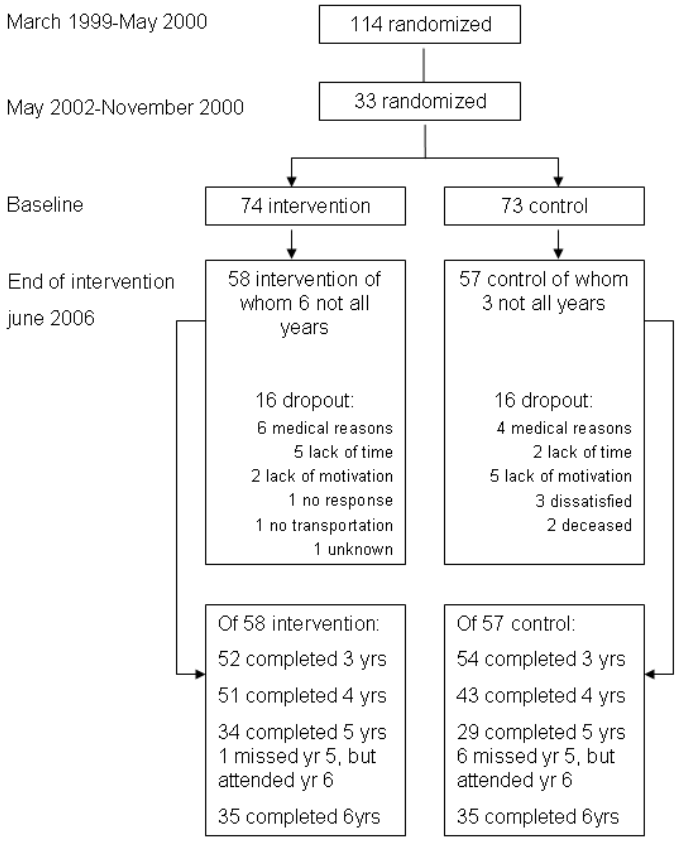

Figure 4.1 Trial profile of the SLIM intervention study in the Netherlands between the year 2000 and 2006. Participants who dropped out of the program were treated as censored observations in the intention-to-treat analyses.

Table 4.1 Changes in body composition, glucose metabolism and blood lipid profile during the SLIM intervention period in the Netherlands between the year 2000 and 2006.

Parameter			Baseline	Year 1	Year 2	End	P Group	P Time	P Group x Time
N		INT	74	64	56	58	-	-	-
		CON	73	65	58	57	-	-	-
Age	(years)	INT	55.0 ± 6.5	-	-	-	<0.01	-	-
		CON	58.8 ± 8.4	-	-	-		-	-
Low Social economic status	N (% of total)	INT	37 (50%)	-	-	-	0.50	-	-
		CON	40 (55%)	-	-	-		-	-
Smoking status	N (% of total)	INT	8 (11%)	-	-	-	0.60	-	-
		CON	10 (14%)	-	-	-		-	-
Weight	(kg)	INT	86.83 ± 13.24	84.36 ± 13.18	85.98 ± 13.52	85.74 ± 13.56	0.30	<0.01	0.20
		CON	84.08 ± 12.06	83.47 ± 11.38	83.32 ± 11.15	84.04 ± 11.99			
BMI	(kg/m2)	INT	29.89 ± 4.16	28.78 ± 3.86	29.16 ± 3.84	29.19 ± 3.90	0.90	0.06	0.46
		CON	29.65 ± 3.42	29.35 ± 3.22	29.19 ± 3.14	29.37 ± 3.32			
Waist	(cm)	INT	103.6 ± 11.3	100.1 ± 11.2	102.1 ± 10.7	102.9 ± 11.1	0.70	<0.01	0.71
		CON	103.6 ± 9.7	101.6 ± 9.8	102.3 ± 9.5	104.2 ± 8.5			
VO_2 max	(l/min)	INT	2.18 ± 0.59	2.38 ± 0.63	2.39 ± 0.62	2.35 ± 0.63	0.03	<0.01	0.04
		CON	2.06 ± 0.57	2.14 ± 0.60	2.04 ± 0.59	2.08 ± 0.61			
Fasting glucose	(mmol/l)	INT	6.01 ± 0.84	5.96 ± 0.88	6.05 ± 1.09	6.30 ± 1.07	0.31	<0.01	0.19
		CON	5.92 ± 0.70	5.94 ± 0.64	6.31 ± 0.84	6.48 ± 0.86			
2-hr Glucose	(mmol/l)	INT	8.85 ± 2.01	8.24 ± 2.04	8.50 ± 2.51	8.66 ± 2.38	0.10	<0.01	0.04
		CON	8.80 ± 2.09	8.79 ± 2.25	9.35 ± 2.64	9.38 ± 2.45			
Fasting insulin	(mU/l)	INT	17.97 ± 8.72	16.03 ± 7.41	12.04 ± 6.61	16.51 ± 8.15	0.84	<0.01	0.68
		CON	17.39 ± 6.89	17.56 ± 8.04	13.31 ± 7.92	16.39 ± 7.26			
HBA1c		INT	5.92 ± 0.48	5.69 ±0.42	5.79 ± 0.59	6.27 ± 0.79	0.85	<0.01	0.91
		CON	5.96 ± 0.51	5.75 ±0.44	5.85 ± 0.44	6.19 ± 0.72			
2-hr Insulin	(mU/l)	INT	96.94 ± 65.96	91.79 ± 80.61	85.18 ± 47.23	118.28 ± 67.46	0.27	<0.01	0.52
		CON	105.91 ± 87.56	103.88 ± 64.25	91.35 ± 65.45	113.36 ± 75.94			
HOMA-IR		INT	4.89 ± 2.83	4.32 ± 2.24	3.40 ± 1.94	4.75 ± 2.64	0.44	<0.01	0.54

Parameter			Baseline	Year 1	Year 2	End	P Group	P Time	P Group x Time
		CON	4.65 ± 2.09	4.74 ± 2.47	3.74 ± 2.24	4.79 ± 2.39			
Glucose lowering	N (%)	INT	0 (0%)	0 (0%)	1 (2%)	5 (9%)	0.98		Chi-square End†
medication		CON	0 (0%)	0 (0%)	0 (0%)	5 (9%)			
Triglycerides	(mmol/l)	INT	1.52 ± 1.18	1.5 ± 1.39	1.24 ± 0.56	1.74 ± 1.87	0.29	<0.01	0.12
		CON	1.44 ± 0.79	1.61 ± 1.19	1.45 ± 1.60	1.53 ± 1.06			
Total cholesterol	(mmol/l)	INT	5.17 ± 0.83	5.14 ± 0.81	5.40 ± 0.85	5.51 ± 0.82	0.58	<0.01	0.43
		CON	5.27 ± 0.85	5.39 ± 0.85	5.55 ± 0.89	5.48 ± 0.97			
HDL cholesterol	(mmol/l)	INT	1.14 ± 0.30	1.14 ± 0.30	1.21 ± 0.33	1.25 ± 0.37	0.25	<0.01	0.41
		CON	1.11 ± 0.28	1.10 ± 0.30	1.16 ± 0.30	1.18 ± 0.31			
LDL cholesterol	(mmol/l)	INT	3.39 ± 0.81	3.35 ± 0.80	3.59 ± 0.81	3.57 ± 0.86	0.30	0.03	0.39
		CON	3.51 ± 0.75	3.60 ± 0.81	3.66 ± 0.81	3.57 ± 0.72			
Blood lipid									
lowering	N (%)	INT	6 (8%)	5 (8%)	6 (11%)	12 (21%)	0.97		Chi-square End†
medication		CON	5 (7%)	8 (12%)	8 (14%)	12 (21%)			
Diastolic blood									
pressure	(mm Hg)	INT	89.0 ± 9.4	87.6 ± 7.3	87.6 ± 7.3	83.8 ± 7.9	0.22	<0.01	0.52
		CON	89.1 ± 7.8	88.6 ± 8.0	85.4 ± 8.0	84.9 ± 7.6			
Systolic blood									
pressure	(mm Hg)	INT	141.6 ± 16.7	138.0 ± 14.7	137.2 ± 14.7	138.6 ± 14.3	0.07	<0.01	0.91
		CON	145.0 ± 14.6	141.0 ± 16.0	139.9 ± 13.5	141.2 ± 13.9			
Blood pressure									
lowering	N (%)	INT	21 (28%)	21 (33%)	18 (32%)	29 (51%)	0.07		Chi-square End†
medication		CON	18 (25%)	16 (25%)	18 (31%)	19 (33%)			

Data are mean ± SD. *P-value using intention-to-treat analysis using all available data regarding the difference between the groups over a mean follow-up of 4.1 (3-6) years. P-value for fasting glucose, 2-hr glucose, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, diastolic and systolic blood pressure were adjusted for medication use. †Data on medication use are analysed using a chi-square test, presenting the p-value at the end of intervention for differences between study groups.

Associations between changes in lifestyle behavior and changes in glucose tolerance

Regression analysis in INT (n=49) revealed that in a model including Δbody weight, Δmaximal aerobic capacity, Δtotal fat intake and Δfiber intake as covariates, only Δbody weight was significantly associated with changes in 2-hr glucose levels (β=0.303 mmol/l, p=0.04). When replacing Δbodyweight with Δwaist circumference, this parameter was slightly more strongly associated with Δ2-hr glucose (β=0.316 mmol/l, p=0.03). In the control group, suprailiacal skinfold thickness and age at baseline predicted worsening in 2-hr glucose levels (β=0.19 cm, p=0.05; β=0.23 mmol/l, p=0.01).

Factors associated with dropout to the program.

At the end of the study, 115 (58 INT/57 CON) subjects were still participating and 32 (16 INT/16 CON) had dropped out. At baseline, adherent subjects had a higher VO_2 max ($p<0.05$), were higher educated and had a lower BMI and 2-hr glucose levels compared to dropouts ($p<0.01$, table 4.2). Results were similar when tested for the intervention group and control group separately.

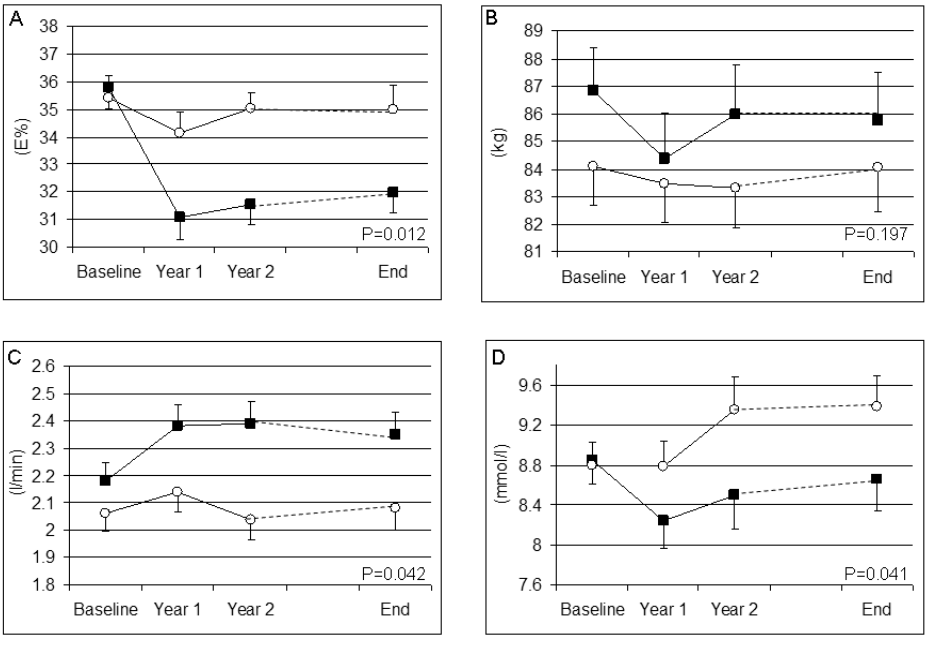

Figure 4.2 A-D. Changes in lifestyle parameters and 2-hr glucose concentrations, during a combined diet-and-exercise lifestyle intervention to prevent type 2 diabetes (SLIM study). The study was performed in the Netherlands between the year 2000 and 2006. Data are presented as mean ± SEM. Total fat intake (A), Body weight (B), VO2 max (C), and 2-hr plasma glucose levels (D) for the intervention group (black squares) and control group (open triangles) at baseline, after 1 year, 2 years and at the end of the lifestyle intervention (mean 4.1 years). Dashed lines between year 2 and endpoint represents estimate progression in between.

Table 4.2 Baseline characteristics of participants of the SLIM lifestyle intervention, which was held between the year 2000 and 2006 in the Netherlands. Participants who completed the full study protocol (completers) were compared to those who discontinued participation previous to the end of the study (dropouts).

		Completers	Dropouts	P
N		115	32	-
INT (male/female)		58 (30/28)	16 (7/9)	0.49
CON (male/female)		57 (31/26)	16 (6/10)	0.13
Age	(years)	56.2 ± 7.1	58.3 ± 6.7	0.16
Low Social Economic Status	N (% of total)	47 (41%)	23 (72%)	<0.01
Smoking status	N (% of total)	15 (13%)	3 (9%)	0.62
Weight	(kg)	85.1 ± 13.0	87.0 ± 11.4	0.46
BMI	(kg/m2)	29.4 ± 3.5	31.3 ± 4.4	<0.01
Waist	(cm)	102.8 ± 10.2	106.6 ± 11.3	0.07
Body fat percentage	(%)	38.1 ± 6.3	40.4 ± 6.4	0.07
Diastolic blood pressure	(mm Hg)	89.3 ± 8.4	88.2 ± 9.6	0.52
Systolic blood pressure	(mm Hg)	142.8 ± 15.7	145.1 ± 16.0	0.47
VO_2 max	(l/min)	2.2 ± 0.6	1.9 ± 0.6	0.04
Fasting glucose	(mmol/l)	5.9 ± 0.8	6.1 ± 0.7	0.23
2-hr Glucose	(mmol/l)	8.6 ± 1.7	9.8 ± 2.8	<0.01
HBA1c	(%)	5.9 ± 0.5	6.1 ± 0.5	0.15
Fasting insulin	(mU/l)	17.4 ± 6.6	18.8 ±11.5	0.94
2-hr Insulin	(mU/l)	99.12 ± 78.9	110.6 ± 72.9	0.42
HOMA-IR		4.6 ± 2.0	5.2 ± 3.8	0.79
Triglycerides	(mmol/l)	1.4 ± 0.7	1.4 ± 0.6	0.91
Total cholesterol	(mmol/l)	5.2 ± 0.8	5.3 ± 1.0	0.73
HDL cholesterol	(mmol/l)	1.1 ± 0.3	1.1 ± 0.3	0.95
LDL cholesterol	(mmol/l)	3.4 ± 0.7	3.5 ± 0.9	0.56
Total energy intake	(MJ/day)	8.8 ± 2.4	8.2 ± 2.1	0.18
Total fat intake	(En%)	35.6 ± 6.7	35.5 ± 6.4	0.94
Saturated fat intake	(En%)	13.6 ± 3.2	13.4 ± 3.2	0.74
Total carbohydrate intake	(En%)	42.2 ± 7.5	43.8 ± 6.0	0.31
Fiber intake	(mg/MJ)	2.7 ± 0.8	2.9 ± 0.9	0.17
Alcohol consumption	(En%)	5.8 ± 6.3	4.2 ± 7.6	0.24

Data are Mean ± SD.

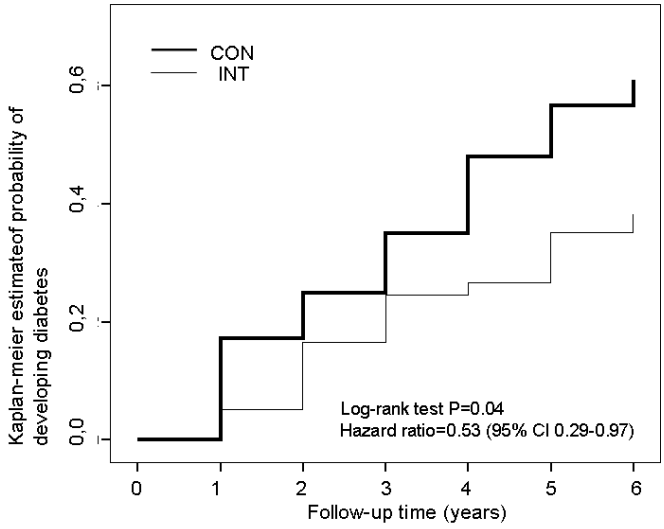

Figure 4.3 Proportion of subjects with diabetes during the SLIM lifestyle intervention in the Netherlands between the year 2000 and 2006. Cumulative diabetes incidence in the intervention group and control group. The relative risk of diabetes for subjects in the intervention group, as compared with those in the control group, was 0.53 (P=0.04 for the comparison between the groups).

Discussion

The present study demonstrates long-term effectiveness of lifestyle intervention in the Dutch setting as well as novel information on factors associated with dropout and intervention outcomes. Our findings add to the knowledge about identification, lifestyle effect and adherence in certain high-risk subgroups, and optimize implementation.

Effectiveness of the SLIM lifestyle intervention

In our lifestyle intervention the difference in 2-hr plasma glucose levels between groups remained as high as 0.72 mmol/l, which was associated with a diabetes risk reduction of 47%, despite no significant differences in body weight between groups. The diabetes risk reduction is similar to that found in the DPS (6, 7) and DPP (5). The control group showed a relatively small increase in 2-hr glucose levels and one may argue that the control group would not reflect the actual general population. However, at present, self-monitoring, self-tests, multimedia attention and increasing information availability and presentation of diabetes and diabetes-related complications may well increase awareness of the general population and induce small changes in their dietary and physical activity habits. The control group in our study may therefore be reflective of the informed general population at present.

Lifestyle-induced behavior change

At the end of the lifestyle intervention, subjects in the intervention group had a sustained higher aerobic capacity compared to the control group, and intervention subjects had increased their total number of physically active days per week. Unfortunately, we were not able to analyze whether attendance to the exercise program predicted the outcome of the intervention due to limited power. However, since approximately 70% of the intervention subjects also used other exercise facilities or physical activities, the total number of physically active days per week may be more indicative for the whole intervention group. Physical activity may independently reduce diabetes risk (9), also by sustaining weight loss (9). Even small sustained increases in physical activity, as was also observed in this study, seem beneficial in the long term. Surprisingly, changes in aerobic capacity were not correlated with changes in glucose tolerance, possibly due to lack of statistical power for aerobic capacity (n=48 for the intervention group).

Similar to the DPS, our lifestyle intervention did not have an effect on cholesterol, triglyceride levels and blood pressure, or on medication use, which could have masked the lifestyle effects (22). A high consumption of carbohydrates results in a rise in triglyceride concentrations (23). Body weight loss, mediated by both a low-fat or a high-fat diet may reduce triglyceride levels (24). It is possible that in the present lifestyle intervention, the increased carbohydrate intake and an increase in maximal aerobic capacity leveled out potential effects on plasma triglycerides.

Associations between changes in lifestyle behavior and changes in glucose tolerance

In agreement with previous results from the DPP (9), stepwise regression analyses revealed that variation in body weight loss and waist circumference was the most important determinant of the change glucose tolerance. It can therefore be assumed that adherence to a more rigid regime regarding weight loss, compared to what we have achieved in our study, may result in even greater improvements in glucose tolerance and diabetes risk reduction.

Factors associated with dropouts to the intervention program

A limitation in the present study is the seemingly high dropout rate, 21% in total (31 out of the 147 subjects). This percentage is higher compared to previous reports of the DPS (6) and the DPP (5). On the other hand, our dropout percentage is similar to that of other lifestyle interventions after 1-2 year follow-up (25, 26) and to that observed in the DREAM trial after a follow-up for a median of 3 years (27), with 29.3% (772/2635) dropout in the rosiglitazone group and 25.0% (658/2634) in the control group. Two explanations for the difference between the DPS, DPP and SLIM can be given. First, our participants were originally extracted from the general population and they may have had less internal motivation to participate in the study, as compared to subjects recruited via advertisements, as was done in the DPS (16). Second, no weight loss program was provided which may have led to dissatisfaction for participants. In our

study, subjects unable to participate until the end of the study had a lower social economic status, a lower VO_2 max and a higher BMI and 2-hr glucose levels at baseline, than those who completed the study. This clustering of factors is known from previous studies, which have found that a low educational background is associated with increased risk for obesity (28) and that a low occupational position in adulthood is associated with a higher prevalence of type 2 diabetes in men and women (29).

Subjects with a low maximal aerobic capacity were more likely to become a dropout. This is similar to a previous report in the U.S. showing that especially high-risk subjects do not engage in regular physical activity (30). Therefore, these subjects may be especially prone to dropout en stay in their non-active physical behavior. More studies are necessary that investigate why certain groups of people drop out and how this can be changed.

Conclusions

Our results underscore that prolonged feasible changes in diet and physical activity prevent deterioration of glucose tolerance and reduce diabetes risk by 47% over a mean of 4.1 years. Variation in body weight loss and waist circumference was most strongly associated with the improved glucose tolerance, exemplifying the importance of body weight and central body fat reduction. A low social economic status and a low maximal aerobic capacity are indicative of dropout to the program, suggesting that these subjects may need special attention to achieve beneficial changes in their lifestyle and metabolic profile.

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5

PERSONAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH ADHERENCE TO A LIFESTYLE INTERVENTION AMONG PERSONS WITH IMPAIRED GLUCOSE TOLERANCE

The SLIM study

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submitted

Abstract

Objective
To assess adherence to physical activity and nutrition advices in adults with impaired glucose tolerance (IGT) participating in a lifestyle intervention study, and to determine personal and environmental factors that may have affected adherence.

Method
59 participants in the intervention group completed a self-administered questionnaire measuring potential personal and environmental determinants, derived from a number of influential theoretical models, after a follow-up of 2 or 4 years. Measures of adherence were derived from food records and the SQUASH (Short QUestionnaire to ASsess Health enhancing physical activity) completed during one of the annual measurements of the trial.

Results
Adherence to the lifestyle advices of the SLIM intervention ranged from 22 to 70%. Participants self-rated their adherence to the physical activity and nutrition advices much more positive. Multiple regression analysis revealed that adherence to the physical activity advice was only positively associated with level of physical activity at baseline. Adherence to the nutrition advices was lower among participants who usually do the shopping for their household, participants who feel less susceptible for getting diabetes and younger participants. Improvement of dietary and physical activity behaviors compared to baseline was smallest among those with better baseline behaviors.

Conclusion
This study once more shows that inadequate adherence is a problem for intervention efficacy. Two possible ways to improve adherence in the present diabetes prevention intervention are to increase perceived susceptibility to getting diabetes and to decrease misconception about own adherence.

Introduction

The pandemic of type 2 diabetes is an enormous public health problem due to the high prevalence (1), the diabetes-associated morbidity (2) and premature mortality (3, 4) and the financial burden (5, 6). Lifestyle interventions in subjects at high risk for developing type 2 diabetes, i.e. those with impaired glucose tolerance (IGT), have proven to be cost-effective (7) and effective in reducing diabetes risk by affecting the risk factors for the development of type 2 diabetes (8-10). Important risk factors for the development of type 2 diabetes include a diet low in fiber, high in fat and especially saturated fat (11, 12), reduced physical activity, a high body mass index (BMI) (13) and an abdominal fat distribution (14, 15).

The Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM) is a 6 y randomized controlled trial to study the effect of a lifestyle intervention (healthy nutrition, increased physical activity) in adults with IGT in the age 40-65 years. This study has shown to be effective (10, 16, 17) and the results are comparable to other intervention trials (18). However, non-adherence to lifestyle interventions is a widespread problem (19) in need of clarification so that the success of lifestyle interventions involve the majority of the target group instead of a selected few. Adherence has been defined by WHO as "the extent to which a person's behavior – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider" (19). Estimates of non-adherence range from 0% to 100% , with 50% being an average, depending on the patient population, medical condition, form of treatment, and the definition of adherence (20, 21). Adherence tends to be low when the regimen is complex, must be followed for a long period of time, requires changes in the person's lifestyle and is designed to prevent rather than cure illness (21). The current SLIM regimen has all these characteristics.

The aims of the present study were to assess adherence to the physical activity and nutrition advices of the SLIM- intervention and to determine personal and environmental factors that may have affected adherence. Factors associated with adherence to nutrition and physical activity advices among IGT subjects have not been studied yet. For the present study potential influencing factors were derived from reviews of earlier studies in related populations and fields, and from a number of influential theoretical models that explain behavior and behavioral change (22-24), because no single model seems to be universally valid (23, 24) (see table 5.1). Attitudes, social influences, perceived behavioral control, barriers, perceived disease susceptibility and severity, past behavior, family foods rules and responsibilities, intervention characteristics, and participant characteristics were included as potential influencing factors. More insight in the association between these factors and adherence may help to develop targeted lifestyle implementation strategies for the prevention and treatment of diabetes and its related complications (table 5.1).

Table 5.1 Factors potentially influencing adherence to nutrition and physical activity, their source theory or model, and evidence as stated in reviews.

Name of concept/name of comparable concept, but from different theory or model (definition)	Theory or model	Reviews
Attitude (a person's general feeling of (un)favorableness toward the prescribed behavior) Attitudinal beliefs/outcome expectations/response effectiveness/costs and benefits (perceived positive and negative consequences of performing the prescribed behavior that, together, determine the overall attitude)	Theory of Planned Behavior (40) Social Cognitive Theory (41) Protection Motivation Theory (42) ASE Model (43)	e.g. (44-46)
Social influences -Subjective norm (perceived expectations of important others regarding the person's performance of the prescribed behavior) -Social support (perceived support for the prescribed behavior from other people) -Observed behavior of other people -Having family members with diabetes	Theory of Planned Behavior (40) Social Cognitive Theory (41) ASE Model (43)	e.g. (19, 44, 45, 47, 48)
Perceived behavioral control/self-efficacy (how much control a person thinks he or she has about performing the prescribed behavior)	Theory of Planned Behavior (40) Social Cognitive Theory (41) Protection Motivation Theory (42) ASE Model (43)	e.g. (19, 33, 44, 45)
Barriers/life situations (Actual or perceived barriers, personal or environmental, e.g. lack of time, physical problems, routine disrupting life events, long distance from program centre, transportation problems)	Theory of Planned Behavior (40) Health Belief Model (49) ANGELO Model (50)	e.g. (19, 44, 45, 47, 48)
Perceived disease susceptibility (perception of getting ill)	Health Belief Model (49) Protection Motivation Theory (42)	e.g. (51, 52)
Perceived disease severity	Health Belief Model (49) Protection Motivation Theory (42)	e.g. (51, 52)
Past behavior (e.g. exercise history)/ Behavior at the start of the intervention	Model of habit formation (53) Triandis Model (54)	e.g. (33, 44, 45)
Family food rules and responsibilities	ANGELO Model (50)	e.g. (48)
Intervention characteristics (e.g. whether it includes rewards, includes relapse prevention elements)	Self-management (55)	e.g. (56)
Participant characteristics (e.g. age, gender, main daily activities, marital status, personality traits)	In several models considered to be a more distal determinant; influencing the behavior through the other factors	e.g. (19, 44, 45, 48)

Methods

SLIM Intervention

The design of the study has been described before (25). Briefly, skilled dieticians conducted every three months one-hour individual counseling sessions with the participants regarding their dietary habits and physical activity at the research institute. Dietary advice was based on the Dutch guidelines for a healthy diet (26, 27). The participants were stimulated to consume less than 30-35% of energy intake as fat, less than 10% of energy intake as saturated fat, and at least 3g /MJ of fiber a day. In addition, a body weight loss of 5-10 kg was aimed at, depending on degree of obesity. Dietary advice was based on 3-day food records (2 weekdays, 1 weekend day) that participants kept before they visited the dietician.

With respect to physical activity, participants were stimulated to be moderate-intense physically active for at least 30 minutes on at least five days of the week (28, 29). Individual advice was given (e.g., walking, cycling, swimming) and individual goals were set and evaluated during every visit. Furthermore, participants were encouraged to participate in a Physical Activity Program (PAP) given 3 times a week, especially designed for the study. These free 1-hour PAP sessions consisted of aerobic exercise training and resistance training and were given 3 times a week. A skilled trainer supervised the exercise sessions.

Research population and measures

Participants were included in the SLIM study in either 1999/2000 or 2002. The study design has been described in detail previously (10, 30). Subjects with an increased risk for glucose intolerance were selected from a cohort in the area of Maastricht, and invited to undergo a capillary standard oral glucose tolerance test (OGTT). Those subjects with a 2-hr blood glucose concentration > 7.8 mmol/l were invited for a second venous OGTT. For inclusion, mean 2-hr glucose concentration of both OGTTs had to be between 7.8 and 12.5 mmol/l and fasting glucose concentration < 7.8 mM. Exclusion criteria were known diabetes, glucose concentrations outside the inclusion criteria, chronic illness, medication known to interfere with glucose tolerance, participation in a vigorous exercise and/or diet program.

Subjects were randomized with stratification for sex and mean 2-hr plasma glucose concentration to the intervention group or the control group. All 72 persons from the intervention group with a follow-up of four years (inclusion period 1999-2000) or two years (inclusion period 2002) were asked to participate in the present study, including the 23 persons that dropped out of the study meanwhile. For the present study, data were derived from the baseline SLIM measurements (time 1: baseline) and the annual measurement that took place between September 2003 and April 2004 (time 2 in the present study) during which the participants completed an adherence questionnaire.

Measures derived from the annual measurement

As a measure of adherence to the nutrition advices the daily energy percentage (E%) of total and saturated fat intake, and fiber intake (g) was derived from weighted 3-day food records. Nutrient intake was calculated using the Dutch food table (NEVO version 1996) (31). For each of the three nutrients an adherence score of one point was given if intake was in accordance with the dietary advice, i.e. below 35 E% for total fat, below 10 E% for saturated fat and more than 3 grams per day for fiber intake. An overall adherence score for the nutrition norms was made by summing the scores for the three nutrients (range from 0 to 3). Adherence to the physical activity advices was assessed with one item from the Short QUestionnaire to ASsess Health enhancing physical activity (SQUASH), i.e., on how many days a week are you physically active for at least 30 minutes (32). Finally, gender, baseline age, and time since inclusion (4 versus 2 years) were derived from the baseline measurement.

Adherence questionnaire

The adherence questionnaire was handed out during the time 2 annual measurements or mailed to the persons who dropped out. The participants were asked to complete the questionnaire at home. Participants that returned the questionnaire received a small present.

The adherence questionnaire included measures of potential influencing factors as well as self-rated adherence to the advices. Unless indicated, items were measured with bipolar five-point scales, e.g. completely agree to completely disagree, very easy to very difficult (scored from –2 to 2). Participants were asked to keep the last six months in mind in answering the questions. All measures were used in earlier studies and operationalized in accordance with theoretical guidelines (33), but most needed some adaptation to the intervention at hand.

To assess attitude, seven beliefs towards adherence to the physical activity advices were measured (e.g. is important, pleasant, helps to prevent diabetes). Similarly, eleven attitudinal beliefs towards the PAP, six beliefs towards adherence to the nutrition advices, and five beliefs towards the counseling sessions with the dietician were measured. The items were summed and divided by the number of items to obtain four overall attitude scores (Cronbach's α's ranged from 0.80 to 0.90).

Subjective norms were measured by questioning the perceived opinions of household members as well as other family members/friends about whether the study participant should adhere to the physical activity/nutrition advices. Furthermore, observed physical activity and nutrition behavior of household members and other family members/friends as well as their support in adhering to the advices was questioned.

Perceived behavioral control was assessed by asking how easy or difficult it is to adhere to the physical activity and nutrition advices, and to participate in the PAP. Perceived counseling characteristics was assessed with five items, i.e., the dieticians talks with me about the results of adherence, whether my expectations are met, reasons for non-adherence, solutions for better adherence, and gives compliments about the things I do well (Cronbach's $\alpha = 0.85$).

For eleven potential barriers it was asked how often the barrier had been a reason for not adhering to the physical activity or nutrition advices, e.g., illness of themselves or others, new job, holidays (Cronbach's α respectively 0.75 and 0.83).

As potential environmental barriers to participating in the PAP the participants were questioned about the distance from their home to the sport facilities, transportation issues, and convenience of the times.

Physical activity history was measured by asking how physically active participants were as a child, and as an adult before the start of the project.

Three items questioned prevalence (yes or no) of food rules at home, i.e. rules about eating times, rules that certain products (such as snacks or candy) should not be eaten too often, and rules that certain food products (such as fruit and vegetables) should be eaten regularly. To assess responsibilities in the household it was questioned who usually does the shopping and the cooking.

Diabetes related questions included whether they have family members or friends with diabetes, perceived seriousness of diabetes, and perceived susceptibility for developing diabetes.

As participant characteristics, educational level, household size, and main daily activities were measured.

Finally, participants were asked how often a week/month they participated in the PAP, and two items assessed self-rated adherence, i.e. whether they themselves thought they adhered to respectively the physical activity and nutrition advices well.

Analyses

The data were analyzed using the SPSS 13.0 (SPSS inc., Chicago, IL, USA). A positive score is believed to correspond with good adherence. To identify causes of dropout, multiple logistic regression of dropout (yes/no) on gender, time of inclusion and age was done.

To assess associations between adherence and potential influential factors, first univariate regression coefficients between the adherence measures and the potential influencing factors were calculated. Only factors with a regression coefficient of a significance level of less than 0.1 were included as independent variables in two backward multivariate linear regression analyses with respectively adherence to the physical activity advices and adherence to the nutrition advices as dependent variable. Similar analyses were conducted with relative measures of adherence as independent variables, i.e. change in number of days physically active for at least 30 minutes compared to baseline and a change in adherence score for the nutrition advices.

Results

Response rates, attrition and participant characteristics

Fifty-nine participants completed the questionnaire (82% response). The mean age of participants at baseline was 44.4 years (range 31-56; SD 5.7) and 54.2% were male.

Time since inclusion was two years for 27.1% and four years for 72.9% of the participants. Almost half (49.0%) had a low educational level (primary or basic vocational school), 32.1% a medium (secondary vocational or high school), and 18.9% a high educational level (college or university level). Most participants (84.9%) shared a household with one or more others. About half (47.5%) had a paid job.

The thirteen persons who did not complete the adherence questionnaire were all SLIM dropouts. Reasons for the 13 persons to dropout were medical problems, time restraints or lack of motivation. Gender, time of inclusion and age were not identified as causes of dropout. Dietary intake and physical activity data from the annual measurement at baseline and present were available for respectively 49 and 48 of the participants. Item non-response was low (0-6 missing values) for most items. For a few items (social influence items towards physical activity, the items about barriers and attitudinal beliefs towards participating in the PAP) non-response was higher (maximum 13 missing values). Scales were calculated accepting missing data for a minority of the items in that scale. Missing data were not substituted.

Adherence

Table 5.2 shows that two or four years after study inclusion about one third of the participants adhered to the norm on physical activity. Compared to baseline, the percentage participants that adhered to the norm increased by 6.2%. More than 60% self-rated to adhere to the physical activity advices at least most of the time.

With regard to the dietary advices, almost 70% of the participants adhered to the norm on total fat intake. The norm on fiber intake and saturated fat intake was reached by more than half and about one third of the participants, respectively. Compared to baseline, percentage adherence to the norm of all three dietary advices increased with more than 20%. Twenty-two percent of the participants adhered to all three advices, representing an increase of 18%. More than 60% self-rated to adhere to all the nutrition advices at least most of the time. Self-reported participation in the PAP was 60.3% never, 18.9% once a week, 13.8% twice a week and 6.9% three times a week (table 5.2).

Personal and environmental factors

Table 5.3 shows that overall participants had positive attitudes, meaning that participants had a general favorable feeling towards the prescribed advices (indicated as a positive value). Participants also had positive scores on perceived subjective norms towards the physical activity advices as well as the nutrition advices, and perceived characteristics of the counseling sessions. Participants had less positive scores on the observed physical activity as well as nutrition behavior of others, perceived support of others in adhering to the physical activity and nutrition advices, their exercise history, self-efficacy towards adhering to the physical activity and nutrition advices, and barriers to come to PAP. Participants indicated that barriers for adhering to the physical activity as well as nutrition advices did not occur very often.

Food rules were prevalent in most households. Most participants did not usually do the shopping, but about half usually did the cooking.

Regarding diabetes-related variables, getting diabetes was perceived to be very serious, but own susceptibility was rated lower. Most participants had a friend or family member with diabetes (table 5.3).

With regard to specific attitudinal beliefs towards adhering to the physical activity advices (not in table), participants were most positive about 'the importance' and 'pleasantness' and that 'it helps to feel more fit', and least positive about adherence to the physical activity advices as 'being a way to reduce medication'. 'Importance' and that 'it helps to prevent diabetes' were perceived to be the most positive aspects of adherence to the nutrition advices, while 'pleasantness' and 'results of adhering to the nutrition advices in comparison with initial expectations' were evaluated least positive.

With regard to participating in the PAP, the study participants were most positive about 'importance', 'PAP being a separate program' and 'the supervisors'. The least positive scores were given on items questioning whether the PAP is 'pleasant', 'boring', 'childish', 'though', and 'too long'.

With regard to the counseling sessions, the 'expertise of the dietician' and the 'pleasantness' were evaluated most positive. Participants were least positive about whether the sessions 'help to adhere to the advices' and 'are adapted to their daily life'.

Table 5.2 Dietary intake and adherence to the nutrition advices (n=49), and adherence to the physical activity advices (n=48): mean score (SD) or percentage.

	Baseline	Two/four years after inclusion	Change
Fat			
E% from fat	35.89 (6.79)	31.88 (5.93)	-4.00 (7.28)
% less than 35 en% from fat	46.9	69.4	22.5
Saturated fat			
E% from saturated fat	13.59 (2.98)	11.00 (2.88)	-2.60 (3.73)
% less than 10 en% from saturated fat	6.1	30.6	24.5
Fiber			
fiber (grams)	2.68 (0.77)	3.12 (0.82)	0.45 (0.87)
% at least 3 gram	28.6	57.1	28.5
Adherence to nutrition advices			
score (range 0-3)	0.82 (0.88)	1.57 (1.02)	0.76 (1.18)
% all nutrition advices	4.1	22.4	18.3
% self-rated adherence		62.7	
Adherence to physical activity advices			
number of days physically active	2.95 (2.35)	3.11 (2.68)	0.17 (2.81)
% at least 5 days	29.2	35.4	6.2
% self-rated adherence		61.4	

Table 5.3 Factors associated with adherence to SLIM advices: mean score (SD) or percentage (n=59).

Variables	Score
Attitude (scales -2 to 2)	
-physical activity advices	1.02 (0.56)
-nutrition advices	0.96 (0.53)
-PAP	0.97 (0.57)
-counseling sessions	1.09 (0.51)
Subjective norm household members (–2 to 2)	
-physical activity advices	1.49 (0.73)
-nutrition advices	1.45 (0.80)
Subjective norm other family members and friends (–2 to 2)	
-physical activity advices	1.19 (0.84)
-nutrition advices	1.12 (0.83)
Behavior household members (-2 to 2)	
-Physical activity	0.04 (0.85)
-Nutrition	0.89 (0.60)
Behavior other family members and friends (-2 to 2)	
-physical activity	0.04 (0.77)
-nutrition	0.21 (0.65)
Support household members (–2 to 2)	
-physical activity advices	0.63 (0.91)
-nutrition advices	0.61 (0.92)
Support other family and friends (-2 to 2)	
-physical activity advices	0.09 (0.89)
-nutrition advices	-0.07 (0.74)
Perceived behavioral control (-2 to 2)	
-physical activity advices	0.31 (1.03)
-PAP	0.29 (1.06)
-nutrition advices	0.04 (0.89)
Counseling characteristics (scale -2 to 2)	1.21 (0.62)
Past exercise (-2 to 2)	
-as a child	-0.28 (1.08)
-as an adult	-0.30 (0.94)
Occurrence of barriers for adhering to advices (scales 1-5)	
-physical activity advices	4.08 (0.53)
-nutrition advices	4.19 (0.61)
Barriers for participating in PAP	
-Distance (mean km)	10.85 (20.20)
-A lot of time for transportation (-2 to 2)	0.38 (1.12)
-Problems with transportation (-2 to 2)	0.78 (1.12)
-Convenience of times (-2 to 2)	0.18 (1.25)
Food rules (% yes)	
-when to eat	63.8
-products that should be eaten met mate	50.0
-product that should be eaten regularly	67.2
Household responsibilities (% yes)	
-Usually do the shopping	28.0
-Usually do the cooking	52.8
Diabetes related variables	
-Family or friends with diabetes (% yes)	72.9
-Seriousness of getting diabetes (0-3)	2.41 (0.62)
-Susceptibility for getting diabetes (-2 to 2)	0.38 (0.78)

A positive score indicates a score positive to adherence.

Factors associated with adherence to the physical activity and nutrition advices

Table 5.4 shows that only adherence to the physical activity advices at baseline was positively associated with present adherence to the physical activity advices. Number of active days at baseline was negatively associated with change in the number of days that participants were physically active for at least 30 min compared to baseline. This means that participants who were most active at baseline showed the least increase in number of days physically active.

Usually do the shopping and susceptibility for getting diabetes were negatively associated with adherence to the nutrition advices. Age was positively associated with adherence to the nutrition advices. Usually do the shopping and baseline adherence were negatively associated with change in the adherence score for the nutrition advices compared to baseline (table 5.4).

Table 5.4 Multivariate associations between adherence and personal and environmental factors.

	Standardized β	P
Adherence to physical activity advices		
Adherence at baseline	0.40	0.01
Change in adherence	-0.45	0.00
Number of active days at baseline		
Adherence to nutrition advices		
Usually do the shopping	-0.31	0.03
Susceptibility for getting diabetes	-0.49	0.00
Age	0.38	0.01
Change in adherence	-0.33	0.01
Usually do the shopping	-0.47	0.00
Adherence at baseline		

Discussion

Adherence to the lifestyle advices of the SLIM intervention ranged from 22 to 70%, which is in line with the international literature where estimates of non-adherence have often been found to be quite suboptimal, especially for lifestyle interventions (20, 21).

Adherence to the physical activity advice (being moderately active at least 30 minutes on at least five days a week) was only positively associated with the number of active days at baseline. This is in line with the results of some earlier studies (34). However, increase in number of active days was negatively associated with number of active days at baseline. This might be due to the statistical analyses in which the change of a variable is strongly inversely associated with the baseline value (regression to the mean). Another explanation is a ceiling effect or because of social comparison, i.e. those who are already quite active see that they are more active than other participants, and therefore already quite satisfied with their present behavior. This finding means that treatment efficacy of the physical activity part of the intervention is less in participants with a relatively high initial level of physical activity.

Adherence to the nutrition advices was lower among participants who usually do the shopping for their household, participants who feel less susceptible for getting diabetes and younger participants. Participants who usually do the shopping also improved less compared to baseline than other participants. There were no differences between men and women with regard the people who usually do the shopping. Similar to the physical activity advices, improvement of dietary habits compared to baseline was smallest among those with better baseline dietary habits. These results indicate that it is important that the dieticians discuss susceptibility with participants and aim to achieve realistic perceptions of the risk of getting diabetes among all participants. This potential fear arousing information should however be balanced with information and strategies to help coping and increase perceived effectiveness of the behavioral changes (35).

Age is not a behavioral determinant that can be changed, but it needs to be realized that adherence to the dietary advices seems to be especially difficult for younger participants. This also applies to those who are responsible for the household shopping. Further research should give insight into why these subgroups have lower adherence rates. Those who do the shopping might for instance face more treats.

An interesting finding is that participants self-rated their adherence to the physical activity advice as well as the nutrition advices much more positive than the more objective measure. Misconceptions of own dietary intake and exercise behavior have been found before in earlier studies, and are a barrier towards desirable behavioral changes (36-38). Aside from reasons of giving socially desirable answers, a possible cause for misconceptions about own dietary intake is that the dietary advice was quite complicated, and people might have needed more personal feedback on whether they achieved the recommendations. It is, however, considered less obvious that people grossly misinterpret the advice to be active at least 30 minutes a day for at least five days a week. Another explanation for misconception might be that people do not compare their behavior with an objective norm, but with other people who are doing worse (39). People need to be made aware of this tendency.

Several factors that were found to be associated with adherence to nutrition and physical activity advices in earlier studies (see table 5.1) were not found to be significant in this study. This might be due to some methodological problems of our study. Due to the small sample size, only a few independent variables could be included in the multivariate tests. We dealt with this by conducting univariate analyses first. Subsequently only a few variables with the highest univariate associations were included in the multivariate test. Nonetheless, the analyses did not have much power.
Another issue is that the sample consisted of two subsamples, i.e., participants who were included two years ago and participants who were included four years ago. The small sample size did not allow subgroup analyses. However, we included time of inclusion as potential determinant in the univariate analyses, but no significant associations were found (data not shown).

We consider the broad conceptual framework including both personal and environmental factors to be a strong point of the study. Many earlier adherence studies tended to be patient blaming by a strong focus on patient-related factors. There has been a relative neglect of environmental factors (19). However, we also had to select out of the many factors that have been suggested in the literature as possible predic-

tors of adherence. We selected concepts from the most influential theories and models. Reviews of studies into factors associated with adherence to nutrition and physical activity advices in related field and populations were studied for empirical evidence. Our approach is in line with recommendations in the field of health promotion to combine insights from different theories (24, 40). However, using concepts from different theories also has a drawback, because the relations between the concepts are no longer taken into account, and no distinction is made between more distal or more proximal determinants. It is because of this that our study cannot provide an explanation why younger participants and participants who are responsible for the household shopping are less adherent.

The descriptive data of the potential influential factors revealed several opportunities for improvement of the intervention, for instance with regard to support from family and friends, perceived behavioral control, and certain beliefs about consequences of adherence. However, aside from the susceptibility and misconception issue, we did not find evidence in the present study that improvements in the SLIM intervention, such as achievement of more social support or more positive attitudinal beliefs would also improve adherence.

Implications

This study once more shows that inadequate adherence is a problem for intervention efficacy. Two possible ways to improve adherence in the present diabetes prevention intervention are to increase perceived susceptibility to getting diabetes and to decrease misconception about own adherence.

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6

LIFESTYLE INTERVENTION, 1-YEAR CHANGES IN GLUCOSE TOLERANCE AND INFLAMMATORY AND IMMUNE MARKERS

The SLIM study

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Abstract

Objective
To evaluate whether changes in glucose tolerance and insulin resistance are related to changes in inflammation and immune markers in Dutch subjects with impaired glucose tolerance (IGT).

Method
In the Study of Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM), 147 IGT subjects were randomized to a lifestyle intervention, directed towards diet and physical activity, or a control group. From 104 subjects at baseline and after 1y, glucose tolerance, body weight, anthropometric measurements, maximal aerobic capacity, C-reactive protein (CRP), interleukin-6 (IL-6), complement factor 3 (C3) and 4 (C4), and plasminogen activator inhibitor-1 (PAI-1) concentrations were determined. Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR).

Results
Δ2-hr Glucose was positively related with ΔCRP, ΔIL-6, ΔC3, ΔC4, independent of Δbody weight, Δsaturated fat intake, Δmaximal aerobic capacity, age, medication use, smoking status. The relation between ΔIL-6 and 2-hr glucose was independent of the other markers. ΔHOMA-IR was related to ΔPAI-1 and ΔC3, but this relation disappeared after correction for Δbody weight.

Conclusion
The 1-year lifestyle intervention effectively reduced body weight and 2-hr glucose levels, but did not change CRP, IL-6, C3 and C4 levels. These latter inflammation factors were related to reductions in 2-hr glucose levels, not affected by lifestyle changes. Change in IL-6 was the only marker related with the change in 2-hr glucose, independent of the other markers, supporting the concept that IL-6 may be considered as a 'nontraditional' risk marker in the etiology of glucose intolerance.

Introduction

With global rates of type 2 diabetes mellitus rising, it has become essential to understand its etiology in order to obtain clues for a more effective prevention or treatment. Lifestyle interventions have already proven to be effective in the prevention of type 2 diabetes in subjects with impaired glucose tolerance (IGT) (1). The Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM) has shown that changes in nutritional intake and physical activity according to general guidelines results in a sustained improvement in 2-hr glucose values in subjects at high risk for type 2 diabetes (2, 3). Furthermore, sustained lifestyle changes and a reduction in diabetes incidence are observed after a lifestyle intervention, even after discontinuation of active counseling (4). Information on the underlying mechanisms, responsible for the positive lifestyle effect and on determinants of intervention success may provide information for a more personalized intervention and a more targeted implementation into the health care system.

There is increasing evidence that markers of low-grade inflammation and fibrinolysis, i.e. interleukin-6 (IL-6), C-reactive protein (CRP) and plasminogen activator inhibitor 1 (PAI-1), are related to the insulin resistant state (5) and predict the development of type 2 diabetes (6-9). Increased IL-6 levels seem to predict future risk of type 2 diabetes, although CRP may be a stronger predictor than IL-6 (7, 10, 11). Also, high PAI-1 levels are associated with diabetes incidence, which is mediated by body weight and insulin resistance (12). In addition, the complement system may be involved in the development of type 2 diabetes, since complement factor 3 (C3) was associated with diabetes development in middle-aged men (13). Additionally, C3 and complement factor 4 (C4) are expressed in adipose tissue with a high expression in intra-abdominal adipose tissue of obese men, suggesting that (visceral) adipose tissue may mediate the relationship between C3 and diabetes risk (14).

Until now, several studies have shown that lifestyle interventions can reduce CRP (15, 16), IL-6 (15, 17) and PAI-1 levels (18, 19). To the best of our knowledge, only mechanistic and population-based cohort studies have provided evidence for a relationship between elevated levels of C3 and diabetes development, whereas the effect of lifestyle interventions on C3 are still unknown. The aim of this study was to evaluate the 1-yr effect of a lifestyle intervention based on general public health recommendation towards a healthy diet and physical activity level and to evaluate if the individual outcome of the intervention may be partly reflected in the plasma profile of immunological and inflammatory markers. Therefore, the present study investigated the effect of the lifestyle intervention on CRP, IL-6, C3, C4 and PAI-1 and investigated to what extent these markers are related to observed changes in glucose tolerance and insulin resistance.

Methods

The SLIM study (Study on Lifestyle Intervention and Impaired Glucose Tolerance Maastricht) is a randomized controlled trial, designed to study whether a 6-yr combined dietary and physical activity intervention program can improve glucose tolerance

in IGT subjects. This paper will present data from the 104 subjects who completed the 1-year intervention and on whom complete data of inflammation markers was available. In addition, changes in body composition, fasting and 2-hr insulin and plasma glucose concentrations, free fatty acid levels and maximal aerobic capacity were determined. The Medical Ethical Review Committee of Maastricht University approved the study protocol and all subjects gave their written informed consent before the start of the study.

Study design and subjects

The study design has been described in detail previously (20). Briefly, subjects with an increased risk for glucose intolerance, e.g. family history of diabetes, age >40 years, BMI > 25 kg/m2, were selected from a cohort in the area of Maastricht, and invited to undergo a capillary standard Oral Glucose Tolerance Test (OGTT) (response rate 46.2%, see reference (20)). Those subjects with a 2-hr blood glucose concentration > 7.8 mM were told their status and invited for a second venous OGTT after and overnight fast. For inclusion, mean 2-hr glucose concentration of both OGTTs had to be between 7.8 and 12.5 mM and fasting glucose concentration < 7.8 mM. Data obtained during the second (venous) OGTT were used as baseline values. Exclusion criteria were known diabetes, glucose concentrations outside the inclusion criteria, chronic illness known to interfere with glucose tolerance or that makes participation of a lifestyle intervention and/or 5-year survival improbable, medication known to interfere with glucose tolerance, and participation in a vigorous exercise and/or diet program. The study population consisted of 147 subjects. Subjects were randomized with stratification for sex and mean 2-hr plasma glucose concentration to the intervention group (INT: 74 subjects; 38 male, 36 female) or the control group (CON: 73 subjects; 37 male, 36 female). It was calculated, according to the preliminary results after 1 year of the Finnish DPS (21), that 50-60 subjects per group would be sufficient to detect a 1.0 mmol/l difference in 2-hr glucose concentration between groups. Data analyses of these 1-year results include 104 subjects: 51 INT subjects and 53 CON subjects on whom complete data were available. Data of 43 subjects were not included in the analyses due to incomplete data for regression analysis (n=27) and dropout (n=16, 10 INT: 3M/7F, 6 CON: 5M/1F). The latter subjects did not complete the first year due to lack of time in 6 cases, medical reasons in 5 cases, lack of motivation in 2 cases, dissatisfaction in 2 cases and lack of mobility in 1 case. Between excluded and included subjects, i.e. between dropouts and completers, there were no differences in age, BMI, 2-hr glucose levels, homeostasis model assessment for insulin resistance (HOMA-IR, calculation based on fasting glucose and insulin levels), saturated fat intake, CRP, IL-6, C3, C4 and PAI-1. Dropouts had a lower maximal aerobic capacity at baseline compared to completers (p=0.01).

Lifestyle Intervention

The intervention program consisted of a dietary and physical activity part. Dietary recommendations were based on the Dutch guidelines for a healthy diet (Dutch Nutri-

tion Council) and consisted of a carbohydrate intake of at least 55 energy% (E%), a total fat intake of 30-35 E%, with a saturated fat intake below 10 E%. A skilled dietician gave personal dietary advice during a one-hour counseling session every 3 months, after consideration of a 3-day food record (two weekdays, one weekend day). In addition, subjects received individual advice on how to increase their level of physical activity to at least 30 minutes a day for at least 5 days a week (22). Furthermore, subjects were encouraged to participate in a free, supervised combined aerobic- and resistance exercise program, especially designed for this study. Three times a year subjects were asked to participate in the exercise program using heart rate monitoring and continuous registration to validate the exercise intensity, which was approximately 70% of their maximal peak oxygen consumption (VO_2max). Control subjects were only briefly informed about the beneficial effects of a healthy diet and physical activity, whereas no individual advice was provided.

Measurements and Biochemical analyses

Body weight was measured with an electronical scale to the nearest 0.1 kg. Waist was measured to the nearest 0.5 cm, with the subject in standing position at the level midway between the lowest rib and the iliacal crest. Blood pressure was measured with a Maxi Stabil 3 pressostabil (CE0047, Speidel en Keller, Jungingen, Germany) in duplo with the subject in supine position, after 10 minutes of rest. The 3-day food records were analyzed for nutrient intake using the Dutch food table (NEVO version 1996). An incremental exhaustive exercise test was performed on an electronically braked bicycle ergometer to determine maximal peak oxygen consumption (VO_2max).
Changes in glucose tolerance were studied using an OGTT, as explained above. Plasma glucose and free fatty acids (FFA) were measured in the fasting state and after 2 hours, using a standard enzymatic technique, automated on a Cobas Fara centrifugal analyzer (intra assay coefficient variation for glucose was 1.50%, the inter-assay coefficient variation was 3.66%). Plasma insulin concentration was measured with a Radio Immuno Assay (Cat. #HI-14K, Linco Research, St. Charles, MO, USA) that shows no cross-reactivity with pro-insulin. The HOMA-IR index for insulin resistance was calculated as described by Matthews et al.(23). Glycated haemoglobin (HBA1c) was determined in a fasting serum sample with high-performance liquid chromatography (reference values for our laboratory 4.4-6.2%). hsCRP, C3 and C4 were measured in duplicate in fasting serum using an autoanalyser (Hitachi 912, Roche Diagnostics, Almere, The Netherlands). IL-6 was measured in duplicate in fasting EDTA plasma with a high-sensitive ELISA assay (R&D systems, Abingdon, United Kingdom). PAI-1 was measured in duplicate in fasting EDTA plasma with an ELISA assay (Zymutest, Hyphen Biomed, NEUVILLE-SUR-OISE, France).

Statistical analysis

SPSS for Windows (version 14.1) was used for statistical analysis. Insulin, FFA concentrations and CRP, IL-6, C3, C4 were not normally distributed and were therefore ln-transformed. Data are presented as mean ± SD; non-linear variables are expressed as

median (25^{th}-75^{th} percentile). Differences between groups were tested with a Student's t test for independent samples or by a χ^2 test when applicable. ANOVA was used to assess the effect of group, time and group x time interaction. Adjustment for dichotomous variables in the linear regression analyses was done using dummy variables. Adjustment for lifestyle changes in the multiple linear regression analyses was done by inclusion of three different variables representing changes in body weight, dietary pattern and physical fitness. A p-value of less than 0.05 was considered statistically significant. All tests were two-sided.

Results

General lifestyle effects

At baseline, no differences were seen in subject' characteristics apart from age, which was higher in the control group compared to the intervention group (p=0.02) (table 6.1). After 1 year, body weight decreased in intervention subjects from 87.7 kg to 84.1 kg, whereas a smaller decrease was observed in the control subjects (p=0.004, time x group interaction, table 6.1). 2-Hour plasma glucose levels decreased in the intervention group from 8.9 ± 2.2 mM at baseline to 8.4 ± 1.7 mM after 1 year. In the control group an increase was seen from 8.1 ± 2.1 mM at baseline to 8.8 ± 2.3 mM after 1 year (p= 0.006 time x group interaction). 2-hr Insulin levels showed a significant reduction in the intervention group compared to the control group (p=0.004). No differences between groups were observed in fasting plasma glucose, HBA1c, fasting insulin and HOMA-IR (table 6.1). No subjects developed type 2 diabetes (fasting glucose > 7.0 mmol/l and/or 2-hr glucose > 11.1 mmol/l) during the first year of study.

In line with the reduction in body weight, total fat intake and saturated fat intake decreased significantly more in the intervention subjects and carbohydrate intake and fiber intake increased more in the intervention subjects, compared to the control subjects (p_{all}<0.01, table 6.2).

Changes in CRP, IL-6, C3, C4 and PAI-1 did not differ between study groups (p_{all}>0.05, table 6.1).

Inflammation and immune markers and glucose tolerance

We evaluated if the individual outcome of the intervention, i.e. changes in 2-hr glucose levels and changes in HOMA-IR, are reflected in the plasma profile of immunological and inflammatory markers. Since the randomization, i.e. lifestyle intervention or control, did not affect the levels of CRP, IL-6, C3 and C4 and PAI-1, study groups were combined (total group, n=104). In the total group, 1-year changes in CRP, IL-6, C3 (p_{all}<0.01) and to a lesser extend C4 (p<0.05) were significantly positively related with changes in 2-hr glucose levels (table 6.3, crude analysis). The associations between ΔIL-6 and ΔC3 and Δ2-hr glucose were similar when tested in the intervention group separately (data not shown). When these analyses were adjusted for three major aspects of the lifestyle intervention, i.e. changes in body weight, saturated fat intake

Table 6.1 Participant characteristics at baseline and after 1 year of the Dutch SLIM lifestyle intervention.

		Intervention group		Control group		P		
		Baseline	After 1 year	Baseline	After 1 year	Group	Time	Group x Time
Men/women (n)		31/20(51)	31/20 (51)	31/22 (53)	31/22 (53)	0.81	-	-
Smokers	(%)	14	12	13	23	0.49	0.51	0.02
Medication use for blood pressure	(%)	27	33	23	25	0.55	0.06	0.35
Medication use for blood lipids	(%)	6	10	8	11	0.78	0.10	0.99
Age	(years)	55.6 ± 6.0	-	58.7 ± 1.0*	-	0.02	-	-
Body weight	(kg)	87.7 ± 13.1	85.1 ± 12.7	84.1 ± 13.1	83.5 ±12.1	0.30	0.00	<0.01
BMI	(kg/m^2)	29.7 ± 3.5	28.8 ± 3.4	29.5 ± 3.6	29.3 ± 3.2	0.82	<0.01	0.01
Waist	(cm)	103.8 ± 9.9	100.3 ± 10.2	103.3 ± 10.0	101.8 ± 10.2	0.78	<0.02	0.05
VO2 max	(l/min)	2.3 ± 0.6	2.4 ± 0.6	2.1 ± 0.5	2.1 ± 0.6	0.06	0.00	0.02
Fasting glucose	(mmol/l)	6.1 ± 0.9	6.0 ± 0.9	5.8 ± 0.6	5.9 ± 0.6	0.21	0.77	0.06
2-hr Glucose	(mmol/l)	8.9 ± 2.2	8.1 ± 2.1	8.4 ± 1.7	8.8 ± 2.3	0.75	0.34	<0.01
HBA1c	(%)	6.0 ± 0.5	5.8 ± 0.4	5.9 ± 0.5	5.7 ± 0.5	0.35	<0.01	0.24
Fasting insulin	(mU/l)	17.8 (11.4; 21.8)	15.3 (9.9; 20.8)	16.7 (11.4; 22.7)	16.0 (10.3; 22.6)	0.55	0.13	0.12
2-hr Insulin	(mU/l)	88.5 (60.0; 120.6)	66.9 (47.2; 101.4)	82.0 (61.3; 122.7)	95.1 (59.5; 139.7)	0.09	0.96	<0.01
HOMA-IR		4.8 (3.1; 6.0)	4.0 (2.6; 5.9)	4.3 (2.8; 5.9)	4.2 (2.5; 5.8)	0.80	0.27	0.08
FFA	(µmol/l)	562 (459; 682)	435 (366; 572)	549 (421; 663)	483 (386; 585)	0.99	<0.01	0.13
2-hr FFA	(µmol/l)	100 (79; 126)	72 (60; 89)	100 (74; 121)	78 (60; 108)	0.65	<0.01	<0.01
CRP	(mg/l)	2.6 (1.3; 4.9)	2.5 (0.8; 4.9)	3.1 (1.3; 4.8)	2.5 (1.7; 4.8)	0.13	0.39	0.11
IL-6	(pg/ml)	1.5 (1.1; 2.0)	1.4 (1.0; 1.9)	1.6 (1.2; 2.6)	1.8 (1.2; 2.3)	0.06	0.27	0.34
C3	(g/l)	1.3 (1.1; 1.4)	1.3 (1.1; 1.4)	1.3 (1.1; 1.4)	1.3 (1.1; 1.4)	0.93	0.69	0.21
C4	(g/l)	0.3 (0.2; 0.3)	0.3 (0.2; 0.3)	0.3 (0.3; 0.3)	0.3 (0.3; 0.3)	0.95	0.47	0.21
PAI-1	(ng/ml)	46.0 (38.5; 60.3)	48.4 (39.3; 57.3)	46.7 (33.1; 53.0)	48.8 (40.5; 55.0)	0.55	0.33	0.12

Data are Mean ± SD, n= 104. Fasting insulin, 2-hr insulin, HOMA-IR, FFAs, CRP, IL-6, C3, C4 and PAI-1 are expressed as median (25th percentile; 75th percentile.

and maximal aerobic capacity, as well as age, medication use and smoking status, all aforementioned associations remained significant (table 6.3, adjusted). Replacement of body weight by BMI and inclusion of gender in these models did not affect the outcomes significantly. To estimate which of these markers had an independent contribution on the change in 2-hr glucose levels, we carried out stepwise linear regression analysis in a model that included ΔCRP, ΔIL-6, ΔC3, ΔC4, age, smoking status, mean value 2-hr glucose of concentrations, Δbodyweight, Δsaturated fat intake and ΔVO_2max. Of all the inflammation markers, only ΔIL-6 was independently associated with Δ2-hr glucose. Delta IL-6 explained 6.7% of the variation in glucose tolerance. Also in the intervention group separately, IL-6 was the only marker significantly associated with Δ2-hr glucose when adjusting for the aforementioned lifestyle changes (p=0.04, data not shown).

CRP levels above 10 mg/ml may be indicative of infection. To investigate whether the association between CRP and 2-hr glucose was not mediated by the acute-phase response, 10 subjects (7 INT: 5F/2M, 3 CON: 1F/2M) were excluded with CRP levels above 10 mg/ml at baseline or at year 1. Exclusion of these subjects abolished the significant correlation between ΔCRP and Δ2-hr glucose (β=0.131 mmol/l, p=0.19).

Table 6.2 Energy intake, based on 3-day food records, of participants of the SLIM lifestyle intervention at baseline and after 1 year.

			Baseline	Year 1	P Group	P Time	P Group X Time
Energy	(MJ/day)	INT	9.1 ± 2.3	8.1 ± 2.1	0.68	0.01	0.06
		CON	8.5 ± 2.3	8.4 ± 2.3			
Total fat	(E%)	INT	35.8 ± 5.4	30.8 ± 6.7	0.10	<0.01	<0.01
		CON	35.5 ± 7.2	34.5 ± 6.3			
Saturated fat	(E%)	INT	13.6 ± 2.4	11.3 ± 2.9	0.04	<0.01	<0.01
		CON	13.8 ± 3.4	13.3 ± 3.5			
Carbohydrates	(E%)	INT	41.9 ± 7.5	46.9 ± 7.7	0.52	<0.01	<0.01
		CON	43.0 ± 7.4	44.1 ± 7.6			
Fiber	(g/MJ)	INT	2.7 ± 0.8	3.2 ± 1.0	0.08	<0.01	<0.01
		CON	2.7 ± 1.0	2.7 ± 0.8			
Alcohol	(E%)	INT	6.1 ± 7	4.9 ± 6.2	0.87	0.26	0.36
		CON	5.4 ± 5.9	5.3 ± 5.6			

Data are mean ± SD.

Inflammation and immune markers in relation to insulin resistance

The 1-year changes in PAI-1 and C3 were positively associated with changes in estimated insulin resistance (table 6.3, crude). However, these associations were to a large extent explained by Δbody weight (after adjustment p>0.5).

Table 6.3 Linear regression analysis of the 1y changes during the SLIM lifestyle intervention in 2-hr glucose levels on changes in markers of inflammation, fibrinolysis and the immune system crude and after adjustment.

			Crude			Adjusted		
Dependent	Independent		Standardized β	P	Adjusted R^2	Standardized β	P	Adjusted R^2
Δ 2-hr Glucose (mmol/l)	Δ CRP	(mg/l)	0.28	<0.001	0.13	0.25	<0.001	0.22
	Δ IL-6	(pg/ml)	0.26	<0.001	0.11	0.32	0.001	0.25
	Δ C3	(g/l)	0.37	<0.001	0.17	0.27	0.01	0.22
	Δ C4	(g/l)	0.23	0.02	0.10	0.22	0.03	0.20
	Δ PAI-1	(ng/ml)	0.06	0.53	0.05	-0.08	0.45	0.17
Δ HOMA-IR	Δ CRP	(mg/l)	0.04	0.73	-0.01	-0.09	0.25	0.47
	Δ IL-6	(pg/ml)	-0.03	0.78	-0.01	-0.07	0.37	0.46
	Δ C3	(g/l)	0.35	0.001	0.11	0.07	0.44	0.47
	Δ C4	(g/l)	0.08	0.43	0.01	-0.02	0.81	0.47
	Δ PAI-1	(ng/ml)	0.23	0.02	0.05	0.04	0.61	0.48

Regression model adjusted for age, study group, mean value of the dependent and independent (crude) and in addition medication use, smoking status, and the change in body weight, saturated fat intake and maximal aerobic capacity (adjusted).

Discussion

The lifestyle intervention used in the SLIM study is effective in preventing deterioration in glucose tolerance (3), but does not affect CRP, IL-6, C3, C4 or PAI-1 levels. However, the first four of these markers are significantly associated with 1-year changes in 2-hr glucose levels and these associations are not affected by lifestyle changes, i.e. changes in body weight, saturated fat intake and maximal aerobic capacity. IL-6 was the strongest marker associated with 2-hr glucose, independent of the other markers studied, suggesting that these markers and especially IL-6 may be linked to the etiology of glucose intolerance.

Inflammation and immune markers and glucose tolerance

The change in IL-6 levels was the only marker related with Δ2-hr glucose, independent of the other markers and lifestyle changes, suggesting that IL-6 has an essential role in the changes of glucose tolerance, compared to the other markers investigated. The relationship between 2-hr glucose and IL-6 has been established before in cross-sectional studies (24, 25) and is in agreement with previous findings showing a plasma

IL-6 increase in response to acute hyperglycemic spikes (26) and increased IL-6 production from muscle in the postprandial phase of IGT men (27). In agreement with our results, IGT was recently found to be associated with IL-6 levels in a well-functioning older population, independent of obesity, fat distribution and inflammation-related conditions (28).

The association between IL-6 and 2-hr glucose, independent of the other markers investigated, suggests an effect of IL-6 independent of the acute-phase response. IL-6 may negatively affect insulin sensitivity via a stimulatory action on adipose tissue lipolysis (29) or by direct effects on skeletal muscle affecting insulin-stimulated glucose disposal (30). However, in the present study we could not demonstrate a relationship between IL-6 and markers of insulin metabolism (fasting insulin, HOMA-IR or 2-hr insulin). The strong relationship between IL-6 and 2-hr glucose may be explained by an inhibitory effect of IL-6 on insulin signaling in the liver (31), i.e. by decreasing insulin-mediated suppression of endogenous glucose production (EGP) (32), observed in animal studies. Human studies to verify this hypothesis are scarce. One study showed no effect of IL-6 on EGP (30), but this study was done in healthy individuals and not in subjects with a disturbed glucose metabolism. IL-6 may also increase insulin secretion promoting hyperinsulinemia, possibly via a Ca^{2+}-dependent mechanism (33), although limited in vivo studies are available at present.

High CRP levels are indicative of infection. Exclusion of 10 subjects with high CRP levels attenuated the relation between 2-hr glucose and CRP. Therefore, we cannot exclude involvement of the acute-phase response mediating the association between CRP and 2-hr glucose. The 10 subjects were more often current smokers, suggesting that they may be especially vulnerable for progression towards type 2 diabetes.

The mechanism behind the positive association between IL-6 and C3 and C4 may be due to hepatic synthesis of C3 and C4, which is regulated by proinflammatory cytokines, such as IL-6 (34). Up to now, there is limited information regarding the relationship between inflammation factors and the complement system in the development of type 2 diabetes. Recently, CRP, C3 and C4 were all associated with body fat in healthy adolescents, while no statement was made concerning the mutual relationships (35).

Body weight did not explain the relationship between ΔC3 and Δ2-hr glucose, suggesting that C3 may have effects on the glucose metabolism, not mediated by body weight (36). C3 has been shown previously to be related to diabetes development (13), independently of the main indexes of abdominal and general obesity and has shown to be strongly related to 2-hr glucose in non-diabetic Pima Indians (37). These data suggest a detrimental effect of C3 on the development of type 2 diabetes, while the mechanism is still unclear.

Inflammation and immune markers and insulin resistance

Changes in PAI-1 were associated with HOMA-IR, which was largely explained by changes in body weight. In the Diabetes Prevention Program a similar association was found with tissue plasminogen activator (tPA), which is used to estimate the level of antifibrinolytic activity, and HOMA-IR, independent of measures of demographics, adiposity, insulin and glucose (38). Also, Festa et al. (2006) found the relation of PAI-1

to incident diabetes to be independent of insulin resistance and/or bodyweight (12). Lifestyle interventions have shown to be able to reduce PAI-1 levels (18, 19), but more mechanistic studies are necessary to clarify the role of adipose tissue in the relationship between PAI-1 and insulin resistance.

Body weight also explained the positive relation between changes in C3 and ΔHOMA-IR, suggesting that adipose tissue may mediate this relationship. These data suggest that besides the liver, adipose tissue may be a proinflammatory source of C3 production which seems consistent with findings of a high C3 expression in (omental) adipose tissue (14).

Our study has several limitations that need to be addressed. First, the sample size was relatively small. However, the original power calculation suggest that these numbers are sufficient to detect a 1.0 mmol/l difference in glucose tolerance. Also, our study shows strong associations ($p<0.001$), despite the relatively low sample size. In addition, characterization of immunological and inflammatory markers was more comprehensive than in previous studies. Second, associations were performed combining the two study groups together, whereas the control group was not given personalized lifestyle advice. However, as can be observed in the one-year decrease in body weight, subjects in the control group had their own 'mini intervention'. Several reasons may have attributed to the change in body weight e.g. increasing awareness of the advantages of a healthy lifestyle. In addition, regression analyses were adjusted for study group and the main results regarding IL-6 and C3 were similar in the intervention group separately as well.

In summary, the changes in 2-hr glucose observed in our lifestyle intervention study were associated with changes in IL-6, C3 and C4, independent of study group, age, medication use, smoking status and lifestyle changes. Change in IL-6 was the only marker related with the change in 2-hr glucose independently of the other markers, suggesting that IL-6 is an independent mediator of glucose tolerance and may be considered as a 'nontraditional' risk marker in the etiology of type 2 diabetes mellitus.

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7

INFLAMMATORY FACTORS AND ADIPOKINES AND LIFESTYLE INTERVENTION OUTCOME AFTER 3 YEARS IN HIGH-RISK SUBJECTS FOR TYPE 2 DIABETES

The SLIM study

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Submitted

Abstract

Objective
To investigate the effect of lifestyle intervention according to general recommendations on circulating adiponectin, resistin, leptin, plasminogen-activator inhibitor-1 (PAI-1), C-reactive protein (CRP), Complement factor 3 (C3) and Interleukin-6 (IL-6) in subjects at risk for diabetes as well as the potential of these inflammation factors as biomarkers of changes in glucose metabolism and insulin resistance.

Method
In the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM), 147 subjects with impaired glucose tolerance were randomized to either a combined diet-and-exercise intervention or a control program. At baseline and after 1, 2 and 3 years, an oral glucose tolerance test, an exercise test, and anthropometric measurements were performed. After 3 years, complete data of 102 subjects (50 intervention and 52 control) were obtained.

Results
Over the 3 years, leptin levels increased less in the intervention group, compared to the control group ($p_{all}<0.05$). High baseline leptin levels were predictive of improvements in glucose tolerance, partly independent of body fat mass, suggesting a role of leptin mediating glucose tolerance. Independent of changes in body weight, saturated fat intake and maximal aerobic capacity, 3-yr changes in PAI-1 levels were significantly positively associated with changes in HOMA-IR.

Conclusion
The 3-yr SLIM lifestyle intervention blunts the increase in leptin levels in the intervention group compared to the control group. Baseline leptin levels are reflective of baseline body weight and are indicative of beneficial lifestyle-induced changes in 2-hr glucose levels.

Introduction

There is increasing evidence for a relationship between inflammatory factors and the progression towards type 2 diabetes (1-3). C-reactive protein (CRP) and IL-6 concentrations are increased in obesity and have been reported to be significantly elevated in impaired glucose tolerance subjects (IGT) compared with normoglycemic subjects, as shown in the KORA Survey 2000 (4). In the DPS lifestyle intervention study, CRP was the best predictor for progression towards type 2 diabetes in the control group after a mean of 3.9 years (1). Plasminogen activator inhibitor 1 (PAI-1), a factor known to be involved in blood clotting, has also been able to predict the development of type 2 diabetes, an effect independent of body weight (5). Also, baseline complement factor 3 (C3), mainly produced by the liver, has recently been shown to be predictive of diabetes development after 6.1 years in non-diabetic healthy men (6).

Adipo(cyto)kines, endocrine factors that are produced by adipose tissue, may also be associated with the development of type 2 diabetes. Among others, adipose tissue secretes adiponectin, leptin and resistin. Based on animal and in vitro data, resistin was originally thought to be a hormone linking obesity with insulin resistance, possibly by having antagonistic effects compared to those of insulin (7). However, recent human studies provide conflicting data showing no association between resistin and insulin resistance (8; 9). Adiponectin is solely produced by adipose tissue and hypoadiponectinemia is associated with Impaired Glucose Tolerance (IGT) in non-diabetic men (10) and has shown to be predictive of diabetes development in Pima Indians (2) as well as in a Caucasian IGT population (3). In these studies, the relationship between low adiponectin levels and progression towards type 2 diabetes was not completely explained by fat mass, suggesting adiponectin as a marker for metabolic status, e.g. adipocyte dysfunction. Both adiponectin and leptin are suggested to be associated with diabetes development and cardiovascular disease via pathways involving fat metabolism, inflammation and vascular function (10; 11).

Lifestyle interventions have been shown to reduce CRP, IL-6 (12), PAI-1 (13) after 1 year as well as leptin after periods of 1-2 years (14), together with improvements in glucose tolerance and insulin sensitivity. Currently, limited lifestyle intervention studies have reported a potential effect on adipokines and inflammation factors that extends periods of 1-2 years. Furthermore, besides the effect of lifestyle intervention on adipokines and inflammation factors, more information is also warranted on the relationship between inflammation factors and lifestyle intervention outcome. The objective of this study was to investigate the effect of a 3-yr lifestyle intervention on adipokines and inflammation markers in subjects with impaired glucose tolerance. In addition, we established the potential of inflammatory factors as biomarkers for lifestyle induced improvements in glucose metabolism and insulin resistance.

Methods

Screening

The Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM) is a randomized controlled trial, designed to study whether a 6-yr combined dietary and physical activity intervention program can improve glucose tolerance in subjects with impaired glucose tolerance (IGT). Study design, inclusion and exclusion criteria, and the diet and exercise program of SLIM have previously been described in detail (15; 16). Briefly, subjects with an increased risk for glucose intolerance, e.g. family history of diabetes, age >40 years, BMI > 25 kg/m2, were selected from a cohort in the area of Maastricht, the Netherlands and invited to undergo a capillary standard oral glucose tolerance test (OGTT), response rate 46.2%, see reference (15). Those subjects with a 2-h blood glucose concentration > 7.8 mmol/l were invited for a second venous OGTT. For inclusion, mean 2-h glucose concentration of both OGTTs had to be between 7.8 and 12.5 mmol/l and fasting glucose concentration < 7.0 mmol/l. Data obtained during the second (venous) OGTT were used as baseline values. Exclusion criteria were known diabetes, chronic illness, medication known to interfere with glucose tolerance and participation in a vigorous exercise and/or diet program.

Inclusion

Screening and inclusion of subjects for the SLIM study occurred between March 1999 and May 2000 and in 2002. A total of 147 subjects were included in our study. Subjects were randomized with stratification for sex and mean 2-hr plasma glucose concentration to the intervention group (INT: 74 subjects; 38 male, 36 female) or the control group (CON: 73 subjects; 37 male, 36 female). From the preliminary 1-yr results of the Finnish DPS (17), we calculated that 50–60 subjects per group would be sufficient to detect a 1.0 mmol/l difference in 2-h glucose concentration between groups. Of the 147 subjects enrolled, 106 completed the first 3 years. For regression analysis, four subjects were excluded because of missing values for adipokine or inflammation factors (data analyses in n =102: 50 INT subjects and 52 CON subjects). Reasons for dropout have been reported previously (18). The study protocol was approved by the local medical ethical committee of the Maastricht University. All subjects gave written informed consent.

Intervention

The intervention program consisted of a dietary and physical activity part. Dietary recommendations were based on the Dutch guidelines for a healthy diet (Dutch Nutrition Council) and consisted of a carbohydrate intake of at least 55 energy% (E%), a total fat intake of 30-35 E%, with a saturated fat intake below 10 E%. A skilled dietician gave personal dietary advice during a one-hour counseling session every 3 months, after consideration of a 3-day food record (two weekdays, one weekend day). In addition, subjects received individual advice on how to increase their level of physical

activity to at least 30 minutes a day for at least 5 days a week (19). Furthermore, subjects were encouraged to participate in a free, supervised combined aerobic- and resistance exercise program, especially designed for this study, which was offered to them 4 times a week Participation in the exercise program was monitored and 17 subjects participated at least once a week during the second and third year. Intensity of the exercise program was determined using heart rate monitoring which was approximately 70% of their maximal peak oxygen consumption (VO_2max). Control subjects were only briefly informed about the beneficial effects of a healthy diet and physical activity, whereas no individual advice was given.

Measurements

In both groups, several measurements were performed annually including an OGTT as a measurement of glucose tolerance, insulin levels, VO_2max, body weight, waist circumference, body fat percentage, blood pressure, HBA1c, cholesterol and HDL. Body weight was measured with an electronical scale to the nearest 0.1 kg. Waist was measured to the nearest 0.5 cm, with the subject in standing position at the level midway between the lowest rib and the iliacal crest. Blood pressure was measured with a Maxi Stabil 3 sphygmomanometer (CE0047, Speidel en Keller, Jungingen, Germany) in duplo with the subject in supine position, after 10 minutes of rest. The 3-day food records were analyzed for nutrient intake using the Dutch food table (NEVO version 1996). An incremental exhaustive exercise test was performed on an electronically braked bicycle ergometer to determine maximal peak oxygen consumption (VO_2max). The HOMA-IR index for insulin resistance was calculated as described by Matthews et al.(20). The incidence of DM2 was determined according to WHO criteria of 1999 (21). Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula (22).

During the OGTT, plasma glucose, free fatty acids (FFA) and insulin were measured in the fasting state and after 2 hours, using a standard enzymatic technique, automated on a Cobas Fara centrifugal analyzer (intra assay coefficient variation for glucose was 1.50%, the inter-assay coefficient variation was 3.66%). Plasma insulin concentration was measured with a Radio Immuno Assay (Cat. #HI-14K, Linco Research, St. Charles, MO, USA) that shows no cross-reactivity with pro-insulin. Glycated haemoglobin (HBA1c) was determined in a fasting serum sample with high-performance liquid chromatography (reference values for our laboratory 4.4-6.2%). In addition, adipokines and inflammation markers are determined. Stored plasma samples were analyzed for levels of fasting plasma adiponectin (total adiponectin: full length and globular, coefficient of variation (CV) 5.8%), resistin (homodimeric, CV 3.7%), leptin (CV 3.7%), PAI-1 (CV 2.3%) with enzyme-linked immunosorbent assays (Biovendor, Heidelberg, Germany). Samples were analyzed with paired samples from each subject run in the same batch. hsCRP, C3 and C4 were measured in duplicate in fasting serum using an autoanalyser (Hitachi 912, Roche Diagnostics, Almere, The Netherlands). IL-6 was measured in duplicate in fasting EDTA plasma with a high-sensitive ELISA assay (R&D systems, Abingdon, United Kingdom).

Statistics

Data analysis was conducted using SPSS for Windows (version 14.1). Data are presented as mean ± SD or, if not normally distributed (insulin, leptin, adiponectin, resistin, CRP, IL-6), as median (25^{th}-75^{th} percentile). Repeated-measures ANOVA were used for differences between groups over time. A two-tailed P value of 0.05 was considered statistically significant. Multiple linear regression models in the total group (intervention + control) were used to assess whether baseline or 3-yr change of inflammatory markers were predictive of the change in 2-hr glucose levels and HOMA-IR index. The models used for multiple linear regression analyses are based on the standard model (model 1) which is adjusted for inclusion time, study group, age, medication use and smoking status and in addition for mean of the dependent and independent variables when investigating the changes in inflammatory markers. Lifestyle variables, like body weight, maximal aerobic fitness and saturated fat intake were added sequentially to each model to assess to what extent the associations were an indirect effect of lifestyle factors.

Results

Baseline characteristics

At baseline, inflammation markers were not different between study groups. During the 3-yr lifestyle intervention, the intervention group decreased in body weight (-1.1 kg) and in HOMA-IR (-0.2), whilst the control group increased in these parameters ($p<0.05$, table 7.1). VO_{2max} improved in the intervention group (+0.2 l/min) compared to a stable VO_{2max} in the control group ($p=0.02$). In the intervention group, 2-hr glucose levels returned to baseline values whereas 2-hr glucose levels in the control group deteriorated ($p=0.02$). In the intervention group, saturated fat intake decreased more compared to the control group ($p<0.01$).

Leptin

Table 7.1 shows that during the 3-yr lifestyle intervention leptin levels increased more in the control group (+4.4 ng/ml, 95% CI: 2.3 to 6.6) compared to the intervention group (+1.0 ng/ml, 95% CI -0.8 to 2.8; $p<0.01$). In the total group (intervention and control group together), baseline leptin levels were related to changes in 2-hr glucose levels, even after adjustment for baseline body weight (table 7.2, model 2). Baseline leptin levels were not related to changes in HOMA-IR, when adjusted for baseline body weight, VO_2max and saturated fat intake (table 7.2, model 3). In the standard model, Δleptin was related to ΔHOMA-IR, but this relationship was abolished when adjusted for Δbody weight ($p=0.10$, table 7.3, model 2).

Table 7.1 Levels of inflammation factors at baseline and after 3 years of follow-up in the SLIM study, according to study group. Data are mean±SD or median (25th-75th percentile)

		Baseline		Year 3				
		Intervention	Control	Intervention	Control	Group	Time	Group X Time
n (male/female)	102	50 (26/24)	52 (27/25)	50 (26/24)	52 (27/25)	-	-	-
Body weight	(kg)	87.5 ± 13.7	82.9 ± 11.7	86.4 ± 13.7	83.1 ± 11.1	0.23	<0.01	0.01
VO_2max	(l/min)	2.2 ± 0.6	2.1 ± 0.5	2.4 ± 0.6	2.1 ± 0.7	0.02	<0.01	0.02
Saturated fat intake	(E%)	13.6 ± 2.9	13.6 ± 3.5	10.7 ± 3.0	12.9 ± 3.2	<0.01	<0.01	<0.01
2-hr glucose	(mmol/l)	8.6 ± 1.6	8.5 ± 1.8	8.5 ± 2.3	9.3 ± 2.5	0.09	0.09	0.02
HOMA-IR		4.8 ± 2.0	4.6 ± 2.1	4.6 ± 2.1	5.0 ± 2.1	0.81	<0.01	0.04
Leptin	(ng/ml)	19.2 (15.7-22.7)	16.2 (13.1-19.4)	20.2 (16.3-24.2)	20.7 (17.0-24.5)	0.57	<0.01	<0.01
C3	(g/l)	1.3±0.2	1.3±0.2	1.5±0.3	1.5±0.3	0.78	<0.01	0.92
PAI-1	(ng/ml)	49.2±16.9	44.0±13.3	29.4±9.7	30.4±9.6	0.94	<0.01	0.43
Resistin	(ng/ml)	3.6 (3.1-4.0)	3.6 (3.2-4.0)	2.8 (2.5-3.1)	2.7 (2.5-2.9)	0.76	<0.01	0.58
CRP	(mg/l)	3.9 (2.9-4.9)	4.1 (2.2-6.1)	3.5 (2.6-4.5)	3.9 (1.9-6.0)	0.71	0.27	0.77
IL-6	(pg/ml)	2.2 (1.0-3.4)	2.3 (1.6-3.0)	2.2 (1.0-3.4)	2.8 (1.6-4.1)	0.18	0.11	0.95
Adiponectin	(ug/ml)	13.4 (11.5-15.2)	14.2 (12.4-15.9)	9.1 (8.0-10.2)	8.9 (8.0-9.8)	0.27	<0.01	0.66

C3

The lifestyle intervention did not affect C3 levels (table 7.1). In the total group, baseline C3 levels were not associated with changes in 2-hr glucose levels or HOMA-IR (table 7.2). ΔC3 and ΔHOMA-IR were positively related in the standard model but this relationship disappeared after adjustment for body weight (table 7.3, model 2). The positive relation between ΔC3 and 2-hr glucose concentration in the standard model became non-significant after additional adjustment for changes in body weight, saturated fat intake and VO_2max (p=0.07, table 7.3, model 3).

PAI-1

The lifestyle intervention did not affect PAI-1 levels. Baseline PAI-1 was not associated with change in HOMA-IR or 2-h glucose (table 7.2). In the total group, ΔPAI-1 was positively associated with ΔHOMA-IR, even after adjustment for change in body weight, VO_2 max and saturated fat intake (p=0.02, table 7.3, model 3).

Table 7.2 Multivariable associations between baseline levels and changes in HOMA-IR and 2-hr glucose levels during the 3-yr SLIM lifestyle intervention. Models are built with increasing complexity.

		Model 1		Model 2		Model 3	
		Standardized β	P	Standardized β	P	Standardized β	P
Δ HOMA-IR	Baseline Leptin	-0.20	0.13	-0.34	0.03	-0.22	0.25
N=93	Baseline C3	0.05	0.61	0.04	0.74	-0.07	0.55
	Baseline PAI-1	-0.04	0.68	-0.06	0.54	-0.17	0.16
	Baseline Resistin	0.02	0.84	0.02	0.87	0.05	0.68
	Baseline CRP	0.10	0.33	0.09	0.39	0.09	0.46
	Baseline IL-6	0.11	0.29	0.11	0.31	0.04	0.75
	Baseline Adiponectin	-0.14	0.26	-0.14	0.25	-0.16	0.20
Δ 2-hr Glucose	Baseline Leptin	-0.28	0.03	-0.37	0.02	-0.17	0.38
N=102	Baseline C3	-0.15	0.13	-0.16	0.15	-0.22	0.06
	Baseline PAI-1	-0.04	0.67	-0.02	0.83	-0.11	0.32
	Baseline Resistin	0.15	0.26	0.16	0.25	0.08	0.59
	Baseline CRP	0.04	0.68	0.05	0.62	0.03	0.79
	Baseline IL-6	-0.06	0.53	-0.05	0.61	-0.14	0.22
	Baseline Adiponectin	-0.05	0.64	-0.05	0.66	-0.48	0.70

Model 1: Adjusted for, inclusion time, study group, age, medication use and smoking status.
Model 2: Model 1 + adjustment for baseline body weight.
Model 3: Model 1 + adjustment for baseline body weight, maximal aerobic fitness and saturated fat intake

Resistin

The lifestyle intervention did not affect resistin levels and baseline resistin levels showed no association with ΔHOMA-IR or Δ2-hr glucose (table 7.2). However, Δresistin was inversely associated with ΔHOMA-IR (table 7.3, model 1). Adjustment for changes in body weight did not change this association (table 7.3, model2). The relationship became borderline significant (p=0.07) after additional adjustment for changes in saturated fat intake and VO_2max.

CRP

The lifestyle intervention did not affect CRP levels. Also, (the change in) CRP levels did not show any relationship with changes in HOMA-IR or 2-hr glucose (table 7.2 and table 7.3).

IL-6 and Adiponectin

The lifestyle intervention did not alter the levels of IL-6 and adiponectin. Nor baseline, neither change in these adipokines was related to (change in) HOMA-IR or 2-hr glucose (table 7.2 and table 7.3).

Table 7.3 Multivariable associations between changes inflammation factors and changes in HOMA-IR and 2-hr glucose during the 3-yr SLIM lifestyle intervention. Models are built with increasing complexity.

		Model 1		Model 2		Model 3	
		Standardized β	P	Standardized β	P	Standardized β	P
Δ HOMA-IR	Δ Leptin	0.49	<0.01	0.20	0.10	0.07	0.65
N=93	Δ C3	0.29	0.01	-0.05	0.65	-0.09	0.49
	Δ PAI-1	0.32	0.01	0.24	0.02	0.28	0.02
	Δ Resistin	-0.37	0.04	-0.38	0.01	-0.36	0.07
	Δ CRP	0.11	0.31	-0.03	0.77	-0.04	0.70
	Δ IL-6	0.01	0.97	-0.03	0.79	-0.01	0.95
	Δ Adiponectin	-0.01	0.95	0.01	0.95	-0.02	0.90
Δ 2-hr Glucose	Δ Leptin	0.12	0.23	-0.04	0.77	-0.16	0.35
N=102	Δ C3	0.26	<0.01	0.18	0.11	0.24	0.07
	Δ PAI-1	0.18	0.08	0.14	0.17	0.05	0.71
	Δ Resistin	-0.27	0.09	-0.26	0.09	-0.10	0.63
	Δ CRP	0.07	0.48	0.20	0.03	0.17	0.14
	Δ IL-6	0.11	0.26	0.10	0.30	0.14	0.26
	Δ Adiponectin	0.01	0.96	0.00	1.00	-0.19	0.27

Model 1: Adjusted for mean of the dependent and independent, inclusion time, study group, age, medication use and smoking status.
Model 2: Model 1 + adjustment for change in body weight
Model 3: Model 1 + adjustment for change in body weight, maximal aerobic fitness and saturated fat intake

Discussion

After adjustment for baseline body weight, maximal aerobic capacity and saturated fat intake, none of the baseline parameters measured were related to changes in estimated insulin resistance. At baseline, high leptin levels were predictive of an improvement of glucose tolerance and this association was independent of baseline body weight. The association was also independent of baseline 2-hr glucose levels. It would go beyond the scope of this study to discuss the mechanism behind this association. Although speculative, high baseline leptin levels in our subjects may reflect leptin resistance, similar to insulin resistance, with increasing leptin concentrations target cells become resistant to its actions (23). It is also possible that a high secretion of leptin per unit fat mass may stimulate fatty acid oxidation and improve the metabolic profile (24).

During this study, leptin levels increased more in the control compared to the intervention group and there was a significant relationship between change in leptin levels and change in HOMA-IR, an effect that disappeared after correction for change in body weight. Exercise is also know to reduce circulating leptin levels (25), and the 1-yr results of this study (14) have suggested an association between changes in leptin levels and HOMA-IR partly independent of changes in body composition, possibly reflecting a change in leptin sensitivity. However, it can be speculated that after 3 years, the impact of body weight changes seem to predominate the possible exercise-induced effect on leptin sensitivity.

The 3-yr changes in PAI-1 levels were related to changes in HOMA-IR, independent of changes in body weight, maximal aerobic capacity and saturated fat intake. This is in agreement with previous results in non-diabetic healthy subjects who found an association between the changes in PAI-1 and the changes in HOMA-IR after a follow-up of 5.2 years (26). The finding that the relationship between PAI-1 and HOMA-IR persisted after correction for changes in body weight indicates that changes in adipocyte function rather than fat mass per se may contribute to the improved metabolic profile. Multiple factors seem to influence PAI-1 expression (27) and diabetes incidence, e.g. triglycerides, free fatty acids, glucose and subclinical inflammation, suggesting that changes in PAI-1 may be a marker of the complex mechanism underlying obesity and diabetes development.

The 3-yr changes in C3 levels were positively associated with changes in 2-hr glucose levels, which was for a large part explained by change in body weight. Previous studies have shown a relationship between C3 and 2-hr glucose in non-diabetic Pima Indians (28) and with diabetes development in men aged 38–50 years (6), independently of abdominal and general obesity. The attenuation of the relationship between changes in C3 and 2-hr glucose may be due to the relatively small sample size. Although C3 is mainly produced in the liver, the finding that body weight attenuated the relationship indicates that C3 may also be produced by other tissues like adipose tissue. However, an indirect effect of adipose tissue mass (29) (i.e. FFA inducing inflammation (30)) on liver C3 production also offers a plausible explanation. Another possible explanation may be that C3 levels reflect ASP, the proteolytic fragment of C3, which stimulates glucose uptake and lipid storage in adipose tissue (31; 32). A reduced uptake of glucose and fatty acids in adipose tissue of IGT subjects could be related to a blunted response to activation of the C3-ASP system.

Based on animal and in vitro data, resistin was originally thought to be a hormone linking obesity with insulin resistance (7). In contrast with other human studies (8; 9) we did find a relationship between changes in resistin and changes in insulin resistance , which was independent of changes in body weight. Further studies have to elucidate the exact role of resistin in insulin resistance

In conclusion, the 3-yr SLIM lifestyle intervention blunts the increase in leptin levels, compared to the control group. At baseline, high leptin levels are predictive of improvements of glucose tolerance, independent of baseline body weight, suggesting a role of leptin mediating glucose tolerance, partly independent of body fat mass. A trend was observed for a relationship between 3-year changes in resistin levels and changes in HOMA-IR and between changes in C3 levels and changes in 2-hr glucose levels. Lifestyle intervention did not affect PAI-1 levels, despite a positive association

between the changes in PAI-1 levels and changes in estimated insulin resistance. Baseline leptin levels are reflective of baseline body weight and are indicative of beneficial lifestyle-induced changes in 2-hr glucose levels.

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8

CHANGES IN TRANSFERRIN ARE RELATED TO CHANGES IN INSULIN RESISTANCE

The SLIM study

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Abstract

Objective
To evaluate the effect of a lifestyle intervention on serum transferrin and ferritin levels and the relationship between changes in transferrin and ferritin and changes in glucose tolerance and insulin resistance.

Method
Randomized controlled lifestyle intervention directed at a healthy diet and increased physical activity in subjects with IGT.

Results
After 1 year, ferritin levels tended to decrease in the intervention group as compared to the control group (p=0.06). Transferrin change was independently related to the change in HOMA-IR and ferritin change was related to the change in 2-hr FFA.

Conclusions
Changes in insulin sensitivity and postprandial lipid metabolism are related to changes in iron metabolism.

Introduction

High serum transferrin and ferritin levels have been associated with the onset of hyperglycemia, insulin resistance and type 2 diabetes in several studies (1, 2), indicating that the iron status may play a role in the etiology of type 2 diabetes. The novelty of the present study is that it aims to evaluate the effect of lifestyle changes on transferrin and ferritin levels and the relationship between changes in transferrin and ferritin concentration and glucose tolerance and insulin resistance.

Methods

The SLIM study (Study on Lifestyle Intervention and Impaired Glucose Tolerance Maastricht) is a randomized controlled trial investigating a 6-yr dietary and physical activity intervention on glucose tolerance in subjects with impaired glucose tolerance (IGT). The local Medical Ethical Review Committee approved the study protocol. The study design has been described before (3). Participants gave their informed consent before the start of the study.

Study design and subjects

The study is described previously (3). For inclusion, mean 2-hr glucose concentration of 2 oral glucose tolerance tests had to be between 7.8 and 12.5 mmol/l and fasting glucose concentration < 7.8 mmol/l. Originally, 147 subjects were randomized to a intervention (INT) or control group (CON), with stratification for 2-hr glucose levels and sex. The one-year examination included 129 subjects (see table 8.1).

Lifestyle intervention

Intervention subjects were individually guided (every 3 months visits) to achieve a healthy diet of 55 Energy% carbohydrates, 35 Energy% fat, <10 Energy% saturated fat and more than 3 g/MJ fiber each day, and to increase physical activity to at least 30 minutes a day for at least 5 days a week. Subjects were encouraged to participate in a free aerobic- and resistance exercise program. Control subjects received annually general information about the beneficial effects of a healthy diet and increased physical activity.

Laboratory and clinical measurements

Body weight was measured with an electronical scale to the nearest 0.1 kg. Waist was measured to the nearest 0.5 cm, with the subject in standing position at the level midway between the lowest rib and the iliacal crest. Plasma glucose, free fatty acids (FFA) were measured after an overnight fast and 2 hours after the use of a 75 gram glucose drink (OGTT), with standard enzymatic techniques, automated on a Cobas Fara centrifugal analyzer. Plasma insulin concentration was measured before and during the

OGTT with a Radio Immuno Assay (Cat. #HI-14K, Linco Research) that shows no cross-reactivity with pro-insulin. Transferrin, ferritin and high-sensitive C-reactive protein (CRP) were measured after an overnight fast in serum using an autoanalyser (Hitachi 912, Roche Diagnostics, Almere, The Netherlands). The HOMA-IR index for insulin resistance was calculated as described by Matthews et al. (1985) (4).

Statistical analysis

Insulin, FFA, ferritin, CRP were not normally distributed and were ln-transformed. Subjects with CRP (n=12) levels above 10 mg/l were excluded from analysis. Changes over time between groups were assessed using ANOVA (SPSS for Windows 14.0). Multiple linear regressions were used to evaluate the relationship between the individual changes in transferrin and ferritin and the individual changes in glucose levels and insulin resistance.

Results

Table 8.1 Subjects characteristics at baseline and after 1 year.

		Baseline		Year 1		Time	Group	Group x Time
		INT	CON	INT	CON	P	P	P
	N	64 (35m/29f)	65 (34m/31f)	64 (35m/29f)	65 (34m/31f)			
Body weight	(kg)	86.7±1.60	84.1±1.59	84.4±1.53	83.5±1.53	<0.001	0.46	0.01
BMI	(kg/m^2)	29.62±0.45	29.60±0.45	28.83±0.44	29.41± 0.44	<0.001	0.66	0.01
Waist	(cm)	102.95±1.25	103.22±1.24	100.11±1.31	101.60± 1.30	<0.001	0.62	0.15
Vo2 max	(l/min)	2.28±0.08	2.10±0.08	2.38±0.08	2.11±0.08	0.01	0.06	0.03
Fasting glucose	(mmol/l)	6.05±0.10	5.89±0.10	5.95±0.10	5.94±0.10	0.61	0.52	0.12
2-hr glucose	(mmol/l)	8.82±0.25	8.55±0.24	8.2±0.27	8.79±0.27	0.28	0.62	0.01
Fasting insulin	(mU/l)	17.78±1.01	17.45±1.00	16.03±0.99	17.68±0.97	0.26	0.64	0.32
2-hr insulin	(mU/l)	97.79±10.38	100.19±10.47	90.31±9.71	104.45±9.80	0.96	0.48	0.05
HOMA-IR		4.88±0.32	4.64±0.32	4.32±0.30	4.74±0.30	0.25	0.81	0.22
Transferrin	(g/l)	2.56±0.05	2.44±0.05	2.55±0.05	2.44 ±0.05	0.82	0.08	0.72
Ferritin	(µg/l)	164.10±21.54	188.64±21.20	143.80±18.77	165.22±18.48	<0.001	0.41	0.06
Fasting FFA	(µmol/l)	602.61±25.58	567.00±25.38	476.04±18.08	480.44±17.94	<0.001	0.69	0.12
2-hr FFA	(µmol/l)	114.30±7.68	100.75±7.61	80.57±4.67	86.51±4.63	<0.001	0.90	0.02
CRP	(mg/l)	2.94±0.30	3.02±0.28	2.49±0.31	3.07±0.29	0.04	0.25	0.16

Data are mean ± SEM. (n=129). P was measured by ANOVA repeated measures. For all skewed variables (fasting insulin, 2-hr insulin, HOMA-IR, transferrin, ferritin) statistics were performed on ln-transformed values.

Baseline characteristics

At baseline, INT subjects were younger than CON subjects (54.9±0.83 yr versus 58.4±0.87 yr, p=0.004). 18 Subjects (6 male, 12 female; 10 INT, 8 CON) discontinued

the study and were excluded. They had a lower VO_2 max, higher 2-hr glucose levels ($p_{all}<0.05$) and a higher BMI (p=0.06) at baseline than those who continued participation.

Results of 1-yr intervention

INT subjects decreased more in body weight and BMI versus CON (p<0.05). Mean 2-hr glucose, 2-hr insulin, 2-hr FFA decreased more in INT ($p_{all}<0.05$) and ferritin tended to decrease compared to the CON group (table 8.1). VO_2 max increased in INT with no changes in CON. Change in transferrin level was similar across groups.

Table 8.2 Standardized regression coefficients of 1-yr changes in transferrin and ferritin regressed 1-yr changes in metabolic parameters.

Change in		Transferrin Unadjusted	P	Transferrin Adjusted	P	Ferritin Unadjusted	P	Ferritin Adjusted	P
Change in BMI	(kg/m2)	0.217	0.018	0.221	0.019	0.042	0.638	0.010	0.912
Change in Waist	(cm)	0.118	0.193	0.172	0.064	-0.007	0.937	-0.035	0.705
Change in Fasting Glucose	(mmol/l)	0.146	0.139	0.185	0.064	-0.006	0.954	-0.032	0.744
Change in 2-hr Glucose	(mmol/l)	0.133	0.174	0.132	0.184	0.119	0.210	0.080	0.405
Change in Fasting Insulin	(mU/l)	0.299	0.001	0.292	0.002	0.001	0.987	-0.024	0.799
Change in 2-hr Insulin	(mU/l)	0.188	0.063	0.153	0.148	0.050	0.613	0.016	0.875
Change in HOMA		0.295	0.002	0.305	0.001	-0.002	0.982	-0.032	0.736
Change in Fasting FFA	(µmol/l)	0.214	0.038	0.199	0.066	0.090	0.366	0.074	0.482
Change in 2-hr FFA	(µmol/l)	-0.037	0.725	<0.001	0.998	0.224	0.020	0.225	0.030
Change in CRP	(mg/l)	-0.002	0.983	-0.003	0.974	0.174	0.067	0.157	0.105

Unadjusted: regression analysis adjusted for the baseline value of the dependent and independent variables. Adjusted: regression analysis adjusted for age, gender, random assignment and the baseline value of the dependent and independent variables. Data are expressed as standardized betas. For all skewed variables (fasting insulin, 2-hr insulin, HOMA-IR, fasting FFA, 2-hr FFA, CRP, transferrin, ferritin) statistics were performed on ln-transformed values.

Regression analyses

Changes in transferrin and ferritin were inversely associated (r= -0.49; p<0.01). Change in transferrin was higher in men than in women, was positively associated with change in BMI, fasting insulin, HOMA-IR and fasting FFA ($p_{all}<0.05$), and tended to be associated with change in 2-hr insulin (table 8.2). ΔTransferrin explained 7.5% of the change in ΔHOMA-IR (p=0.02). Results were comparable when analysis was performed on INT and CON separately (data not shown). After adjustment for age, gender and study group, change in transferrin remained independently associated with change in BMI, fasting insulin and HOMA-IR. In addition, the association between change in transferrin

and change in HOMA-IR was also independent of changes in BMI and fasting FFA (β=0.285 g/l, p=0.02). When replacing HOMA-IR with fasting insulin, results were essentially similar (β=0.252 mU/l, p=0.04).

For serum ferritin, no association with changes in glucose tolerance or insulin sensitivity were observed. Change in serum ferritin was significantly positively associated with change in Δ2-hr FFA, independent of age, gender, study group and change in CRP (β=0.247 μg/l, p=0.02).

Discussion

Epidemiological studies have demonstrated that the iron metabolism may be involved in the etiology of type 2 diabetes (1, 2, 5). In our observational study, changes in transferrin levels were positively associated with changes in estimated insulin resistance in subjects at high risk for developing type 2 diabetes, i.e. those with impaired glucose tolerance. Although these data do not imply causality, they are compatible with the results of the DESIR study (1), which showed that transferrin predicted changes in fasting insulin. The mechanism behind the relationship between transferrin and insulin resistance is still unclear. This study and the DESIR findings merit further investigation to clarify causality between transferrin, insulin resistance and the development of type 2 diabetes.

Our intervention program did not affect transferrin, possibly due to the variation in response to the lifestyle intervention within study groups. Our intervention program tended to reduce ferritin, which has been associated with type 2 diabetes onset (2), fasting insulin and fasting glucose (1). In other studies, ferritin and other inflammation markers such as C-reactive protein have also been associated with the metabolic syndrome (6, 7). In our study, ferritin and 2-hr FFA levels were positively associated, independent of CRP as an indicator of the acute phase response, suggesting involvement of the iron status itself in the regulation of 2-hr FFA levels. The mechanism by which iron may stimulate lipolysis is unclear, but ferritin may catalyze hydroxyl radicals (8) and contribute to insulin resistance.

In conclusion, lifestyle-induced changes in transferrin levels were related to changes in fasting insulin, whilst change in ferritin was related to change in postprandial fatty acids. These data have shown an association between changes in insulin sensitivity and postprandial lipid metabolism with changes in iron metabolism.

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9

GENERAL DISCUSSION

Over decades type 2 diabetes has changed from a chronic metabolic disease in elderly into a major health problem affecting an increasing number of people worldwide (1). Diabetes-related complications are considerable and prevention and treatment of diabetes are of utmost importance to increase quality of life and reduce the increasing health care costs. Pharmacological treatment has shown to be effective in reducing diabetes risk, but lifestyle interventions have shown to be even more effective than pharmacological treatment (2; 3). Even more, the effect of lifestyle interventions is sustained over more than a decade after active counseling is discontinued (4; 5). However, non-adherence to lifestyle interventions is a widespread problem (6) and tends to be high with increasing time and complexity and when designed to prevent rather than cure illness (7). In this thesis, we focused on the potential benefits of a combined nutritional and exercise intervention strategy to stimulate a healthy lifestyle and, as such, improve glucose tolerance, insulin sensitivity and prevent/postpone the development of type 2 diabetes in a group of subjects at risk. In addition, this thesis describes potential behavioral as well as metabolic factors related to lifestyle outcome and adherence, in light of future implementation strategies.

9.1 Can lifestyle intervention prevent or postpone the development of type 2 diabetes?

It has been known for several years that lifestyle interventions targeting changes in dietary intake and composition and changes in physical activity frequency and intensity reduce diabetes risk in IGT subjects (2; 8; 9) (as reviewed in chapter 2). Based on estimates from the Diabetes Prevention Program (DPP), lifestyle interventions seem to postpone diabetes onset with approximately 11 years, and prevent diabetes onset with 20% (10). In recent years, more has become known about long-term effectiveness (3-5) and cost-effectiveness (10-12).

In chapter 3, we showed that the SLIM lifestyle intervention, following general public health guidelines, is effective in reducing body weight (-1.1 kg) and improving maximal aerobic capacity (VO_{2max}) (+0.05 l/min) (13). After 3 years, the 0.8 mmol/l difference in 2-hr glucose levels between study groups was associated with a 58% reduction in diabetes incidence. This is consistent with the diabetes risk reductions found in the major lifestyle intervention studies in the USA and Finland, i.e. the DPP (2) and DPS (8).The course of body weight and 2-hr glucose levels is similar to that found in the Diabetes Prevention Study (DPS). They showed a weight reduction of −4.5 kg and a 2-hr glucose reduction of -0.9 mmol/l during the first year and a regain of approximately 1 kg and 0.4 mmol/l after 3 years, respectively (14).

At the end of the SLIM study, after minimal 3 years and maximal 6 years of intervention (mean 4.1 years), a significant beneficial effect remained on VO_2max (0.3 l/min difference between intervention and control group). 2-hr Glucose (0.7 mmol/l difference between intervention and control group) improved and dietary composition improved (reduced total and saturated fat, increased fiber and carbohydrate intake), despite loss of effect on body weight (chapter 4). Including all available observations, diabetes risk reduction was 47% for the intervention group relative to the control group. Most lifestyle interventions show a beneficial lifestyle effect on diabetes risk,

while the effect on 2-hr glucose levels have been far less consistent (chapter 2). Interestingly, in the DPS a borderline effect on 2-hr glucose (-0.1 mmol/l, p=0.07) is still associated with a diabetes risk reduction of 58% (14). Part of the risk reduction may be mediated via other pathways than 2 hr-glucose, e.g. on fasting glucose levels, via subclinical inflammatory factors (15) or restoration of β-cell function (16).

Lifestyle interventions, i.e. SLIM, have shown to be more cost-effective than metformin treatment (10; 11) and have shown to be effective resulting in sustained beneficial effects on 2-hr glucose levels and diabetes risk.

9.2 Lifestyle intervention and the metabolic syndrome

As mentioned above, current evidence clearly indicates the effect of lifestyle interventions in reducing diabetes incidence as clinical endpoint. However, do lifestyle interventions also reduce metabolic syndrome features?

In recent years, the metabolic syndrome has evolved as a term for a clustering of metabolic disorders associated with diabetes and cardiovascular risk, including most often criteria for hypertension, (abdominal) obesity, a disturbed blood lipid spectrum and an impaired glucose metabolism. Several definitions of the metabolic syndrome exist (17), and besides the debate regarding whether obesity or insulin resistance plays a central role in the in the underlying pathophysiology, the clinical relevance of the syndrome has not been established yet. In chapter 3, we did not observe a lifestyle intervention-induced effect on the prevalence the metabolic syndrome or the individual components waist circumference, fasting trigyceride levels, HDL cholesterol levels, blood pressure or fasting glucose levels, despite a marked improvement in 2-hr glucose. The minor changes in body weight and waist circumference may be responsible for the lack of effect on the metabolic syndrome. In the DPP (18) and DPS (19), a lifestyle intervention reduced the incidence of metabolic syndrome by 38-41%. In both studies, the reduction in metabolic syndrome appeared strongly related to a reduction in waist circumference, while in SLIM no significant changes in waist were observed after 3 years. It seems that sustained body weight loss, and primarily reduced abdominal fat, is necessary to reduce the metabolic syndrome. A recent 15-week low-calorie diet showed that body weight loss of nearly 10% reduces the metabolic syndrome by 61%, in obese, primarily female subjects (20).

9.3 Dropout and adherence

Lifestyle interventions face the challenge of keeping participants in the program and sustaining adherence to the program, especially when implemented in the health care system. Dropout to the SLIM intervention was 28% after 3 years (chapter 3), compared to a dropout rate of 8% and 7.5% in the DPS and DPP, respectively. It is not entirely clear why the dropout rate in SLIM is higher compared to the DPS and DPP. However, subjects in SLIM had less overweight compared to the subjects in the DPS and DPP. Therefore, our SLIM subjects may have had more difficulties to loose weight and be satisfied about it. In addition, we observed that half of the SLIM participants had a low

socio-economic status, which was a strong determinant of dropout (chapter 4). In Europe, mortality rates and poorer self-assessments of health, are higher in groups of lower socioeconomic status compared to the higher-status counterparts (21). Recent Dutch research has also shown that higher educated people not only live longer, but also spend a longer period of their lives in good health (22). In the SLIM study, approximately 50% of the subjects had a low socio-economic status, which is higher than the general proportion in Limburg in 2006, ranging from 33-38% (23). Although the SLIM population may not be a representative sample of the general population, it may well represent the vulnerable group at high risk for diabetes and other metabolic disturbances. The high dropout rate in the SLIM study is worrisome and exemplifies the difficulty to reach and sustain lifestyle changes in this vulnerable group and, as such, increase the quality of life. The dropout rate of SLIM is not specific for lifestyle interventions. Also non-lifestyle studies have reported high drop-out rates, i.e. the DREAM trial (24), with 29.3% dropout in the rosiglitazone group.

In chapter 5, we showed that a low perceived susceptibility to getting diabetes and misconception about own adherence are associated with non-adherence in participants. Increasing awareness could be a way to improve adherence in participants as well as clinicians, since awareness and perception of advantages (25; 26) have been shown to play a critical role in adherence of a certain behavior. Perceived susceptibility, as well as awareness and motivation have also been found important mediators of participation (26). Also, restricting the travel time towards the exercise facility could have improved adherence, although our study did not confirm this (27). Both sufficient participation and adherence to the lifestyle changes are necessary for cost-effective implementation in the general population (28). Increasing awareness and changing perceived susceptibility to one that is more likely to be true may reduce refusal and dropout to the lifestyle program, although scientific studies are warranted that study these approaches. Other factors that were associated with decreased adherence like doing the shopping and cooking or being male are more difficult to explain and need further investigation.

9.4 Lifestyle approach versus pharmacological approach

Not all subjects that initially participate in lifestyle interventions comply with the regime, respond metabolically and are able to continue adherence. For subjects that are unable to adhere or do not show a beneficial metabolic response, a pharmacological approach, with or without lifestyle changes, may be considered. In the prevention and treatment of diabetes, pharmacological approaches have also been used and with success (3; 24). Unfortunately, the effect by medication is only present when adhering to the prescription and is not sustained over several years after discontinuation, as is the case for lifestyle interventions (4; 5).

In the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) trial, subjects with IGT, IFG, or both were randomly assigned to receive rosiglitazone or placebo for a median of 3 years (24). Rosiglitazone substantially reduced incident type 2 diabetes (hazard ratio 0.36 for isolated IGT) and a larger number of subjects receiving rosiglitazone progressed towards normoglycemia (hazard ratio

1.7) than did individuals receiving placebo. However, the occurrence of heart failure was higher in the rosiglitazone group (hazard ratio 7.0), as tended the overall cardiovascular events (p=0.08). In light of prevention of major cardiovascular events, a recent study from the USA and Canada showed that compared to standard drug therapy, intensive drug therapy to target normal glycated hemoglobin levels for 3.5 years, using several medications, increased mortality and did not significantly reduce cardiovascular events (29). New pharmacological approaches on diabetes incidence and cardiovascular outcome are currently investigated in subjects with IGT, like the effect of valsartan, a selective angiotensin II antagonist acting on the AT_1 receptor subtype (30). Lifestyle interventions can have multiple beneficial metabolic effects beyond glycemic control and should therefore remain the cornerstone in the prevention and treatment of type 2 diabetes. Pharmacological treatment targeting glycemic control is a plausible treatment for subjects with incident type 2 diabetes when lifestyle interventions have not reached a beneficial effect.

9.5 Factors associated with the lifestyle-induced effect on glucose tolerance, insulin resistance and diabetes risk

The total effect of lifestyle interventions depends on its components namely the effects on body weight, body composition, dietary intake and composition and physical activity. Genetic susceptibility and gender can mediate the lifestyle-induced effect.

9.5.1 Body weight

In the Netherlands, approximately 46% of the Dutch adults were overweight in 2007 (31), which seems to be the most important risk factor for type 2 diabetes (32-36). In the SLIM study, body weight loss and reduced waist circumference were the strongest predictors of changes in 2-hr glucose levels, as discussed in chapter 4. Although body weight is the strongest predictor of 2-hr glucose in SLIM, at the end of the study a beneficial effect on 2-hr glucose levels was sustained, most likely due to sustained changes in dietary composition and VO_2max. In the SLIM study, 1-year changes in insulin sensitivity were partly related to changes in fatty acid profile of serum cholesteryl esters (which partly reflect dietary fatty acid intake), and in particular to changes in desaturase activities (37). This suggests that the shift in fatty acid composition of the diet (reduced saturated fat) may have explained part of the positive lifestyle effects.

Not all adipose tissue depots seem to have an equal contribution to diabetes risk. A study has shown that a minor loss of body weight, accompanied by a major reduction in visceral and liver fat, was associated with improved insulin sensitivity (38; 39). Reductions in visceral fat depot may depend on the initial fat size (40-42). It is still unclear if there is an inverse dose-response relationship between a reduction in visceral fat and insulin sensitivity, or if the visceral fat depot has to decrease below a specific threshold to have a metabolic benefit (38). To date, the results of SLIM once more reinforce that reductions in body weight and especially waist circumference are most strongly associated with a reduced diabetes risk. Future studies have yet to determine to what extent body fat distribution and changes in adipose tissue depots

add to the metabolic benefits of traditional lifestyle factors, i.e. body weight reduction, improved dietary composition and increased physical activity.

9.5.2 Dietary composition

Dietary intake and dietary composition have attributed to the obesity and diabetes epidemic. Therefore, it is rational to realign our eating habits with our physiological needs (43). The term 'dietary pattern' comprises multiple components and this section will highlight several of them, including fat, fiber, carbohydrate intake, and glycemic index.

Total fat, and especially saturated fat intake is an important diabetes risk factor (44; 45). Reduced-fat diets without energy restriction have been shown to reduce weight with 3-5 kilograms (46; 47), sustain weight loss and reduce diabetes incidence (45). In chapter 3 and chapter 4, we confirmed that changes in dietary composition towards a reduction in total and saturated fat, and an increase in carbohydrate and fiber intake, was associated with a beneficial effect on 2-hr glucose levels and a reduction in diabetes incidence. A reduction in total fat intake may affect diabetes development by a decrease in lipid overflow and triglyceride storage in other tissues than adipose tissue. Although the type of fatty acids may also play a role in the development of type 2 diabetes (37; 48; 49), we did not investigate this in this thesis.

Dietary fibers can reduce the rate of glucose absorption in the intestine, thereby lowering postprandial glycemic, insulinemic responses (50) and improving insulin sensitivity (51). In SLIM, fiber intake increased more in the intervention group, as compared to the control group (chapter 3) and a recent meta-analysis once more established the importance of dietary fibers in reducing diabetes risk (52). In the DPS (45), high fiber was a independent contributor to a reduced diabetes risk. High glycemic-index, low-fiber diets, independently increase the risk of type 2 diabetes and cardiovascular disease (53; 54). A reduced glycemic response diet seems to improve β-cell function in IGT subjects (55). In addition, a recent meta-analysis and review has shown that a reduced glycemic response diet in combination with unavailable carbohydrate intake is followed by favorable changes in, among others, insulin sensitivity (56).

Proteins of high biological value, i.e. whey protein, seem to have a high potential of reducing postprandial glucose levels as well as to increase satiety (57). Another potential dietary factor is alcohol use. Light to moderate alcohol use (defined as 0.5 to 1 drink daily for women, and 1 to 2 drinks daily for men) is associated with increased insulin sensitivity, lower postprandial glucose levels and better health (58; 59). With regard to the relationship between alcohol abuse and diabetes development, little information has been published. In SLIM, we did not observe a significant change in alcohol use and total protein intake (chapter 3).

The SLIM study shows that an intervention consisting of four meetings a year with a trained dietician with advice for a healthy diet is effective in changing the dietary composition towards reduced fat and increased carbohydrates and fiber intake after 3-6 years. Although these changes may help to reduce diabetes risk, body weight reduction seems the most important factor.

9.5.3 Physical activity

In the Netherlands and across most European countries less than half of the population is sufficiently active. The Eurobarometer study in 2006 showed that the prevalence of sufficient physical activity for health (5 times a week 30 minutes moderately active or 3 times a week vigorously active) across the Netherlands was 44%, ranging from 40% in women to 48% in men (60). Among European countries, the Netherlands had the highest prevalence of people with sufficient physical activity, while in contrast, it also inhabited the second-highest prevalence of people who sit at least 6 hours per day (60). More studies regarding sedentary time should be performed, but there seems to be a positive correlation with waist circumference, independent of moderate-to-vigorous physical activity (61).

In SLIM, we showed that the lifestyle intervention was effective in increasing VO_2max after 3-6 years (chapter 3 and chapter 4) and in the intervention group this was accompanied by an increased number of days that subjects were at least 30 min physically active (bicycling, gardening or doing sports). In addition, we showed that VO_2max is higher in subjects that continue participation of the lifestyle intervention until the end of the study.

Current physical activity recommendations to maintain health and prevent disease are at least 30 minutes of moderate-intensity physical activity on most, preferably all days of the week (62; 63), which seem an effective and safe way to improve insulin sensitivity and insulin secretion (64) and prevent type 2 diabetes in all populations (65). However, recent studies investigating interval training (with brief high intensities ~95% VO_2max) suggests that this type of interval training may be more powerful than training at 70% VO_2max in eliciting changes in the metabolic disturbances associated with IGT and heart failure patients (66-71). If interval training is at least as effective as moderate intensity training, than more exercise options can be provided, i.e. those that limit time use (71). The SLIM study lacks the statistical power to draw conclusions about the efficacy of exercise or leisure time physical activity in increasing aerobic capacity. In conclusion, it seems that high intensity exercise may be more effective for beneficial metabolic changes, while leisure-time physical activity may be easier to put into practice. In addition, low-intensity physical activity and physical activity during leisure time and work should also be encouraged since even moderate levels of usual physical activity are associated with significantly reduced 2-hr glucose levels (72) and a reduced risk of mortality and cardiovascular disease in men and women (73; 74).

9.5.4 Genetics

In the SLIM study, genetic variation has not yet been analyzed while the interaction of genes with the environment may play a role in the metabolic response to a lifestyle intervention. Several gene-environment interactions have been found, e.g. the X/Ala genotype of the PPARγ-2 Pro12Ala SNP seems associated with bad lifestyle habits, but subjects with this genotype may also profit more from the lifestyle intervention (75). Also, in the Tübinger Lifestyle Intervention Program (TULIP) (76), which followed the DPS lifestyle protocol, showed that the minor G allele of SNP rs2267668 in PPARD and the minor serine-encoding allele of the common Gly482Ser SNP in PPARGC1A were

independently associated with less increase in individual anaerobic threshold (77), indicating that these alleles impair the effectiveness of aerobic training.

To conclude, genetic variation can play a role in the response to a lifestyle intervention. Studies involving interactions between genes and lifestyle intervention response are still limited and need confirmation in large cohorts. When more conclusive evidence is provided, the effectiveness of tailored lifestyle programs should be tested in subgroups with genotypes that are associated with adequate and impaired metabolic response to a lifestyle intervention (78).

9.5.5 Gender

Across the general population, lifestyle changes may differ between men and women. In the SLIM lifestyle intervention group, men had higher fasting glucose, blood pressure, caloric intake, and lower HDL cholesterol levels at baseline, similar to recent results from the DPP (79), presumably making their diabetes risk higher. After 1 year in SLIM, lifestyle-induced changes in 2-hr glucose were similar across both sexes and after 3 years similar changes were observed, except for waist–hip ratio and percentage of body fat which increased in women but remained relatively stable in men (chapter 3). After 1 year in the DPP, men showed a greater decrease in 2-hr glucose levels when losing >3% body weight (79), while diabetes incidence was not lower in men, compared to women. The greater decrease in 2-hr glucose levels may not have leveled out the contribution of the high fasting glucose levels at baseline in men. Although it is plausible that sex differences mediate metabolic and cardiovascular risk, sex per se does not seem to be a predictor of coronary heart disease mortality in subjects with type 2 diabetes (80). Studies powered to examine sex-specific consequences of different prevention strategies would be useful.

9.6 Adipokines and Inflammation factors

Work over the past decades has revealed that adipose tissue is metabolically active in controlling glucose homeostasis and insulin sensitivity. With increasing adipose tissue mass and reduced adipose tissue function, the lipid buffering capacity of adipose tissue decreases, resulting in an increased flux of lipids to non-adipose tissues like muscle and liver (lipid overflow). Secondly, the function of adipose tissue to secrete multiple biologically active proteins may be impaired (81). Adipocyte dysfunction is characterized by hypertrophied adipocytes with an altered secretion of adipokines and high secretion of monocyte chemoattractant protein-1 (MCP-1) perhaps enhancing macrophage infiltration and the pro-inflammatory state (82; 83). Results from chapter 6 and 7 will be discussed in light of these hypotheses.

In the SLIM study, we observed a relation between change in complement factor 3 (C3) and change in 2-hr glucose, after 1 and borderline after 3 years of intervention, independent of changes in body weight (chapter 6 and chapter 7). Our results were similar to those found in non-diabetic Pima Indians (84), suggesting a relation between C3 and diabetes development at least partly independent of obesity (85; 86). The mechanisms underlying these associations are unclear. Acylation stimulating protein

(ASP), a peptide hormone which is formed by the protolytic cleavage of C3, may stimulate the uptake of glucose and fatty acids in adipose tissue (87). Hypothetically, a blunted response to the C3–ASP system could be related to increased levels of C3, glucose and lipids (88). C3 is mainly produced in the liver (89) and cytokines may stimulate C3 production as well as the production of lipids, thereby reducing insulin resistance. In contrast to the relationship with 2-hr glucose, in SLIM, changes in C3 and changes in HOMA-IR were explained by changes in body weight (chapter 6 and chapter 7). Besides the liver, adipose tissue may be a proinflammatory source of C3 production (90), by activated macrophages (91) and adipocytes (92). In agreement with another study (93), C3 was a stronger inflammatory marker than CRP, both of which may reflect the hepatic inflammatory contribution to diabetes development.

In the SLIM 1-year results (chapter 6), we observed an independent relationship between changes in 2-hr glucose and changes in IL-6. In a prospective study with 3075 men and women aged 70-79 years, IGT has shown to be associated with IL-6 (94). Also in cross-sectional studies IGT has been associated with increased IL-6 levels (95; 96). These findings are in agreement with previous results showing an increased IL-6 production from muscle in the postprandial phase of IGT men (97). The strong relationship between IL-6 and 2-hr glucose may possibly be explained by an inhibitory effect of IL-6 on insulin signaling in the liver (98), i.e. by decreasing insulin-mediated suppression of endogenous glucose production (EGP) (99), as observed in animal studies. Human studies to verify this hypothesis are scarce. One study showed no effect of IL-6 on EGP (100), but this study was done in healthy individuals and not in subjects with a disturbed glucose metabolism. Surprisingly, after 3 years, IL-6 was no longer associated with 2-hr glucose levels (chapter 7). The mechanism explaining this result is not clear, IL-6 may only have a short-term effect on 2-hr glucose levels. On the long term, IL-6 does not seem to play an important role. IL-6 seems to play a physiological role when increased during relatively short periods, while it seems to have a pathological role when elevated chronically. The rapid rate at which IL-6 is cleared after infusion (101; 102) or exercise (103) suggests that chronic elevation of IL-6 is not desirable (104).

Lifestyle interventions may have beneficial effects on inflammation factors (105; 106), although we were unable to confirm this directly in the SLIM study. We did observe that beneficial changes in glucose tolerance in the intervention and control group together were associated with changes in CRP, IL-6 and C3 after 1 year and borderline with C3 after 3 years. This suggests that beneficial changes in glucose tolerance may be partly explained through changes in these inflammatory markers. However, the magnitude of these factors in contributing to the etiology of type 2 diabetes and vascular abnormalities remains to be determined.

In the SLIM study, leptin levels increased significantly less in the intervention group compared to the control group (chapter 7). Changes in leptin were associated with changes in HOMA-IR after 1 year (107) and after 3 years (chapter 7). Changes in body weight appeared the primary factor explaining this relationship. Similarly, in previous studies in men but in not women, baseline leptin levels were related to incident diabetes (108;109), independent of markers of body fat. The higher levels of leptin observed in women, compared to men, may be an explanation, suggesting a

non-linear relationship in women between leptin levels and insulin resistance/ diabetes development.

After 3 years of lifestyle intervention, changes in PAI-1 were associated with changes in HOMA-IR, independent of changes in body weight, saturated fat intake and VO_2max (chapter 7). After 1 year however, body weight loss abolished the relationship between changes in PAI-1 and changes in HOMA-IR, similar with previous findings (106; 110; 111) (chapter 6). The results after 3 years are similar to those found in the DPP (112). Multiple factors seem to influence PAI-1 expression (113) and diabetes incidence, e.g. triglycerides, free fatty acids, glucose and subclinical inflammation, suggesting that in accordance with our findings, changes in adipocyte function rather than fat mass per se may contribute to the improved metabolic profile. The beneficial changes in adipocyte function may become apparent when they are not overshadowed anymore by beneficial changes in adipocyte mass. Also, genetic variation in PAI-1 may influence diabetes incidence, since genetically obese and diabetic (ob/ob) mice lacking the PAI-1 gene have reduced adiposity and amelioration of the diabetic phenotype (114). Changes in PAI-1 may be a marker of the complex mechanism underlying obesity and diabetes development.

In this lifestyle intervention study, the 1-year results (107) and the 3-yr results (chapter 7) have shown not to affect adiponectin levels. Some other lifestyle interventions did find an increase adiponectin levels, which seemed mediated by reductions in fat mass (115; 116) while others did not in diabetic or obese subjects (117; 118). Adiponectin, seems to be modestly affected by lifestyle changes while baseline adiponectin seems a strong predictor of incident diabetes in the DPP (119). Physical activity does not seem to influence adiponectin levels, while considerable weight loss restores high adiponectin levels (120; 121).

Lifestyle interventions according to general recommendations for a healthy dietary intake and composition as well as a healthy physical activity level seem to have little effect on adipokine levels. However, adipokines seem to play a role in the deterioration of glucose tolerance and insulin sensitivity, as shown in the SLIM study for leptin. Although more research is necessary to verify the role of adipokines in the development of type 2 diabetes, adipokines seem to reflect processes that influence metabolic status, e.g. adipocyte dysfunction, and do not seem to play an important role in lifestyle-induced improvements in glucose tolerance and insulin resistance during a long-term follow-up.

9.7 Iron metabolism

Already in the ancient world, Erasistratus of Chios (304 BC- 250 BC), a Greek anatomist and physician, theorized that many diseases were caused by plethoras, or overabundances, in the blood, and advised that these plethoras be treated, by initially, exercise, sweating, reduced food intake, and vomiting. Herophilus advocated bloodletting. Nowadays, increasing exercise and reducing food intake remain the initial advice in the prevention and treatment of type 2 diabetes, while bloodletting has almost vanished as a therapeutic remedy. A possible therapeutic treatment of blood letting can be found in type 2 diabetic patients with high ferritin concentrations, in which blood

letting still seems effective in improving insulin sensitivity (122) and vascular dysfunction (123). Also in carriers of the hemochromatosis gene, blood letting, and subsequent iron depletion, ameliorates insulin secretion and sensitivity (124). Improvement of peripheral insulin sensitivity after blood letting may be due to decreased liver iron storage and decreased liver insulin resistance although more studies are warranted.

Markers of the iron metabolism, i.e. ferritin and transferrin, have been associated with the development of hyperglycemia and type 2 diabetes (122; 125; 126), while the mechanism behind these relationships are still unclear. Iron seems to have lipolytic properties (127) and is a catalyst in the formation of hydroxyl radicals, which are powerful prooxidants that attack cellular membrane lipids, proteins, and nucleic acids (128). Both processes may be related to insulin resistance. In SLIM, we observed a relationship between the 1-yr change in ferritin, the main intracellular iron storage protein, and the change in 2-hr FFA levels (chapter 8). This association suggests that ferritin may be involved in the stimulation of lipolysis, subsequent lipid overflow towards liver and skeletal muscle, and the development of insulin resistance (129). Transferrin, the blood plasma protein for iron ion delivery, was independently positively associated with changes in insulin resistance (chapter 8). Besides that transferrin may also have lipolytic properties giving lead to increased circulating free fatty acids concentrations and thereby promoting insulin resistance, we cannot exclude a bidirectional relationship. Therefore, insulin may upregulate transferrin production, as has been shown in human hepatocytes (130) and may stimulate iron uptake by fat cells (131). Overall, these findings merit further investigation to clarify causality and the mechanism behind the iron metabolism, insulin resistance and the development of type 2 diabetes.

9.8 Implementation strategies

Currently, subjects at risk for developing type 2 diabetes are detected by measuring fasting glucose levels or HbA1c levels in the general practitioners office. The measurement of 2-hr glucose levels in general practice is time consuming and therefore may not be an even match for fasting glucose, which is easily performed. However, by not measuring 2-hr glucose levels, a considerable group at risk will be missed for prevention purposes. A keen solution may be to incorporate a 2-hr glucose measurement in the increasingly popular health checks, allowing the subject at risk to take the glucose load at home, before coming to the general practitioners office. In any case, general practitioners should be aware and convinced of the clinical significance of IFG and IGT as risk factor for developing type 2 diabetes and the beneficial effects that lifestyle interventions can have on that process. Besides 2-hr glucose levels, risk scores have become an appealing tool to predict diabetes risk and several questionnaires have been proposed as a simple, practical, non-invasive and inexpensive way of identification (62; 132; 133). These questionnaires include criteria of obesity, family history, age cardiovascular history, gestational history, drug history (62) and criteria of physical activity, fruit and vegetable intake (132; 133). The Finnish Diabetes risk score (FINDRISC) seems to better predict diabetes incidence than metabolic syndrome (17) and the German Diabetes Risk Score (134). In the Netherlands, the Finnish questionnaire has been shown to be a reasonably good predictor of diabetes incidence (135). Smok-

ing status, alcohol use, and physical activity do not seem to add to the predictive value of the variables age, BMI, waist circumference, family history of diabetes, history of hypertension, history of high blood glucose, all of which were used in the FINDRISC score (134). Until easy and time-limiting methods have been developed and proven to screen high-risk subjects more accurately than fasting glucose, fasting glucose levels remains the primary factor for screening, identification and treating at risk subjects for type 2 diabetes.

For successful implementation of lifestyle interventions, efforts are necessary from the individual and the health care sector as a whole. The private sector and the government can help by stimulating initiatives that stimulate a healthy behavior, i.e. by stimulating stair use and providing healthy food in the canteen. The combined efforts can help to create a stimulating environment for achieving a healthy lifestyle among most individuals (62). In popular words, diabetes prevention will prove effective when we are supportive of the saying: 'All for one and one for all'.

9.9 Recommendations for future research

1. Subjects with a low socio-economic status are at risk for dropout to a lifestyle intervention, impaired quality of life and premature death, compared to subjects with a moderate-to-high socio-economic status. Therefore, future studies should investigate how subjects with low socio-economic status can be reached and motivated to change their lifestyle. In addition, future studies should evaluate the relevance and effect of changes in the direct environment to encourage a healthy lifestyle, such as cheap and easy transportation to sports locations.
2. Inadequate adherence is a problem for lifestyle intervention efficacy. The SLIM study shows that perceived susceptibility to getting diabetes and misconception about own adherence are associated with adherence to nutrition and physical activity advices, although other factors like environment and social support may also play a role. Future studies should investigate how susceptibility of disease, conception of adherence and possible other behavioral factors can be influenced so that the person at risk has a truthful perception of their individual situation in which considerations are made. It may be helpful to stimulate high-risk individuals to discuss their (difficulties in) adherence to get a more truthful image of their susceptibility of disease and adherence.
3. Questionnaires and prediction algorithms based on biomarkers have been developed as a tool to predict diabetes incidence and may be used to predict lifestyle success. In recent years, molecular profiling has gained interest since biomarkers for the development of type 2 diabetes can be discovered, as a large number of genes and proteins can be analyzed by high throughput technologies (136). Biomarkers may help to classify high-risk subjects, personalize the preventive actions and increase the success of lifestyle interventions. Although biomarkers may help to facilitate high-risk screening, prediction and prevention, much work remains before these biomarkers can be used in clinical practice. In general practice, fasting glucose levels remains the method for screening and treatment of high blood glucose levels. Future studies are necessary to evaluate how short questionnaires and

possibly biomarkers can be effectively implemented in the general public health system for the benefit of both the subject at risk as well as the general practitioner.

4. Current physical activity guidelines recommend at least 30 minutes of moderate-intensity physical activity on most, preferably all days of the week. However, recent data suggest that high-intensity interval training may be more effective in improving metabolic disturbances associated with IGT. The effectiveness, efficacy and safety of this exercise method should be tested in large randomized trials with populations differing in age and co-morbidities.
5. Inflammation factors, immune markers and adipokines may help to predict intervention success or diabetes development more accurately. These factors may also be used to identify subgroups at risk and subgroups more or less responsive to lifestyle interventions. However, future research should reveal the additional value of identification of subgroups. Like in breast cancer screening, identification may not lead to a better outcome, i.e. less mortality.

9.10 What does it mean: the way of progress?

The acknowledgement starts with a statement of Marie Curie: 'The way of progress is neither swift nor easy'. Along with this statement, it should be emphasized that progress as a whole is not a direct consequence of developments in time. As time passes, old problems are solved whereas new ones become known. Therefore, 'progress' should only be used as a term referring to a specific topic in a certain time frame, acknowledging the relative contribution of man to 'progress' and acknowledging problems as exciting part of our passing lives.

Type 2 diabetes mellitus is one of those exciting problems, due to the metabolic complications, the worldwide scale and the multiple components involved in the pathophysiology. In this thesis, we show that lifestyle interventions according to general public health recommendations are effective after 3-6 years and that body weight and waist circumference seem the most important predictors for a reduction in diabetes risk. Lifestyle-induced beneficial changes in dietary composition and maximal aerobic capacity can be sustained with 3-6 years intervention. These factors have independent effects on the reduction of diabetes risk and are of utmost importance in sustaining weight reduction. However, this thesis also brings about new problems, or reinforces old ones. It is still unknown which manner is best to identify subjects at risk and measure their risk, although questionnaires may be promising. In addition, drop-out to lifestyle intervention is a serious threat for effectiveness and implementation in the community. Low aerobic capacity, a low social economic status, time constraints and behavioral factors like lack of awareness, motivation and susceptibility may all be important for dropout, making adherence a complex problem. Even more complex is implementation of lifestyle programs. Health care at present is still primarily focused on the treatment of disease, rather than preventing them, exemplifying that a switch will be necessary towards prevention of disease. For this switch to take place, efforts will be necessary from all layers in the population, which amplify, not counteract each other's efforts and thát may be the greatest challenge of all, given the diverse ideas about the challenge: type 2 diabetes mellitus.

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