Carbohydrate-Rich Foods in the Treatment of the Insulin Resistance Syndrome

Studies of the Importance of the Glycaemic Index and Dietary Fibre

BY

ANETTE JÄRVI
Dissertation for the Degree of Doctor of Philosophy (Faculty of Medicine) in Geriatrics - Clinical Nutrition presented at Uppsala University in 2001

**ABSTRACT**


The glycaemic responses to various carbohydrate-rich foods are partly dependent on the rate at which the carbohydrate is digested and absorbed. The glycaemic index (GI) is a way of ranking foods according to their glycaemic response and is recommended as a useful tool in identifying starch-rich foods that give the most favourable glycaemic response. This investigation was undertaken to determine whether carbohydrate-rich foods with a low GI and a high content of dietary fibre (DF) could have beneficial metabolic effects in the insulin resistance syndrome. This question was addressed both in single-meal studies and in randomised controlled clinical trials. Starch-rich foods with low GI values incorporated into composite meals resulted in lower postprandial responses of both glucose and insulin than foods with a high GI in meals with an identical macronutrient and DF composition, in subjects with type 2 diabetes. After three weeks on a diet including low GI starchy foods metabolic profile was improved in subjects with type 2 diabetes, compared with a corresponding high GI diet. The glucose and insulin responses throughout the day were lower, the total and low density lipoprotein cholesterol was decreased, and the fibrinolytic activity was normalised. In subjects with impaired insulin sensitivity and diabetes low GI foods rich in soluble DF for breakfast gave a more favourable metabolic profile, with smaller glucose fluctuations from baseline during the day, than a breakfast with high GI foods low in DF. A low GI breakfast high in DF also resulted in lower responses of insulin and C-peptide after breakfast and a lower triacylglycerol response after a standardised lunch. However, none of the tested breakfasts improved the glucose and insulin responses after lunch. Similar results were obtained in obese subjects after including a breakfast with a low GI high in soluble DF for a period of four weeks in comparison with a breakfast with a high GI and low content of DF.

These results support the therapeutic potential of a diet with a low GI in the treatment of diabetes and also in the treatment of several of the metabolic disturbances related to the insulin resistance syndrome.

**Key words:** Diabetes, insulin resistance, carbohydrates, starch, glycaemic index, dietary fibre, postprandial, second-meal, glucose, insulin, triacylglycerol.

Anette Järvi, Department of Public Health & Caring Sciences, Section for Geriatrics/Clinical Nutrition Research, Uppsala University, Box 609, SE-751 25 Uppsala, Sweden

© Anette Järvi 2001

ISSN 0282-7476
ISBN 91-554-5166-7

Printed in Sweden by Uppsala University, Tryck&Medier, Uppsala 2001
This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


IV. Järvi AE, Liljeberg Elmståhl HGM, Karlström BE, Björck IME & Vessby BOH. Lower postprandial triacylglycerol levels and more efficiently suppressed non-esterified fatty acids in serum after lunch following four weeks with a low glycaemic index-high dietary fibre breakfast. *Manuscript.*

* Reprints were made with the permission of the publishers.
Contents

Abbreviations ........................................ 6
Background ............................................. 7
   Introduction ...................................... 7
   Dietary carbohydrates in the treatment of diabetes ........... 8
   The glycaemic index .................................. 9
   Glycaemic load ...................................... 9
   Determination of glycaemic index ....................... 10
   Food factors influencing the glycaemic index .......... 10
   Glycaemic index, diabetes and hyperlipidaemia ........ 12
   Glycaemic index, satiety and weight management ...... 12
   Second-meal effects ................................ 13
   Clinical use if the glycaemic index concept .......... 13
Aims of the investigations .......................... 15
Subjects ............................................. 16
Study design ........................................ 18
Methods ............................................. 21
Results ............................................. 38
Discussion ......................................... 48
   Main findings ..................................... 48
   Glucose and insulin responses ....................... 49
   Insulin sensitivity ................................ 51
   Lipoproteins ...................................... 51
   Second-meal effects ................................ 53
   Effects of fermentation ............................ 55
   Effects on body weight ............................ 55
   Carbohydrate-rich foods in the optimal diet in the insulin resistance syndrome .......... 56
   What is considered to be a low glycaemic index? .... 58
   The glycaemic index concept revisited ............... 58
   Methodological concerns, strengths and limitations of the studies 59
Conclusions ........................................ 61
Future perspectives ................................. 62
Acknowledgements ................................ 64
References ......................................... 66
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo</td>
<td>Apolipoproteins</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BS</td>
<td>Blood sampling</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>C-peptide</td>
<td>Connecting peptide</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DF</td>
<td>Dietary fibre</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>DR</td>
<td>Dietary record</td>
</tr>
<tr>
<td>E%</td>
<td>Energy per cent</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>GI</td>
<td>Glycaemic index</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated haemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>kcal</td>
<td>Kilocalorie</td>
</tr>
<tr>
<td>kJ</td>
<td>Kilojoule</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>Lp</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>M/I</td>
<td>Insulin sensitivity index</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acids</td>
</tr>
<tr>
<td>M-value</td>
<td>Glucose disposal (measurement of insulin sensitivity)</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>OGGT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1 activity</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>SAFA</td>
<td>Saturated fatty acids</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>Waist to hip ratio</td>
</tr>
<tr>
<td>WWB</td>
<td>White wheat bread</td>
</tr>
</tbody>
</table>
Background

Introduction

Obesity, especially abdominal obesity, is a well known risk factor for type 2 diabetes, cardiovascular disease (CVD) and several of the metabolic disorders that are directly or indirectly related to the insulin resistance syndrome [1, 2]. As a consequence of a changing life-style, especially changes in diet and physical activity, the incidence of obesity is increasing epidemically. It is escalating worldwide and is estimated that by the year 2025 as many as 300 million people will be obese [3, 4]. The obesogenic environment, also known as a ‘pathoenvironment’, has been suggested as the driving force for the rising prevalence [5]. In other words, this means that obesity should be regarded as a normal response to an abnormal environment [6]. As a consequence of the drastically increasing incidence of obesity there is also a rapid rise in the incidence of diabetes. Diabetes develops in predisposed individuals as a consequence of impaired insulin sensitivity, in combination with inadequate glucose induced insulin secretion. The number of adults with diabetes is predicted to rise from 135 million in 1995 to about 300 million in the year 2025 [7]. The metabolic disorders that are seen in diabetes and which are part of the insulin resistance syndrome are associated with a high risk for CVD and related mortality and other secondary complications in diabetes.

Coronary artery disease is the major cause of mortality in patients with diabetes. The greatest risk factors once diabetes has developed are increased concentrations of low density lipoprotein (LDL) cholesterol, decreased concentrations of high density lipoprotein (HDL) cholesterol, elevated blood pressure and hyperglycaemia [8]. A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes, and is more strongly associated with the risk for CVD than are fasting glucose levels [9-11]. Non-esterified fatty acid (NEFA) suppression is impaired in obese persons, persons with impaired glucose tolerance and those with type 2 diabetes. Failure to suppress the NEFA supply in a normal way in the postprandial period is likely to impair insulin action and may lead to sustained production of very-low density lipoprotein (VLDL) and impaired clearance of triacylglycerol (TAG)-rich lipoproteins in the postprandial period [12]. High postprandial TAG concentrations are associated with an increased risk of developing
atherosclerotic cardiovascular disease, probably because of reduced lipoprotein lipase (LPL) activity, causing a change in the composition of the lipoproteins towards more small dense atherogenic LDL particles [13, 14]. Intensified blood glucose control has been shown to lead to a substantial decrease in the risk of microvascular disease in type 2 diabetes [15]. However, the major complications of type 2 diabetes is macrovascular disease, which appears to be more effectively prevented by treatment of other cardiovascular risk factors such as dyslipidaemia [16]. Strategies for prevention and treatment of obesity and type 2 diabetes should mainly focus on life-style behaviour such as diet and physical activity [3]. Since most of the day is spent in a postprandial state, it is of great importance to find an effective dietary treatment that will improve the insulin economy and result in lower and sustained postprandial glucose and TAG responses with smaller postprandial peaks and intra-day excursions.

The purpose of the present work was to address the question whether carbohydrate-rich foods with a low glycaemic index (GI) and a high content of dietary fibre (DF) could have beneficial effects on components of the insulin resistance syndrome, on type 2 diabetes and on its risk factors for secondary complications.

**Dietary carbohydrates in the treatment of diabetes**

Diet is the cornerstone of all treatment in diabetes mellitus. As for all kinds of treatment in diabetes, the aim of the nutritional management is to optimise the glycaemic control and reduce risk factors for cardiovascular disease and nephropathy [17-19].

As early as in 1939 it was shown that carbohydrate-containing foods with the same macronutrient composition could differ in the glycaemic responses that they produced [20]. In 1973, Otto *et al* [21] classified carbohydrate-rich foods according to the glycaemic response. This classification of carbohydrate-rich foods was based on the size of the carbohydrate molecule, and starchy foods were classified as a homogeneous group. It was believed that all starches were digested similarly and that their digestion was slower than that of most low-molecular-weight carbohydrates or foods containing such carbohydrates, e.g. fruit or dairy products, resulting in smaller increases in blood glucose. Further studies showed differences, between starch-rich foods with similar macronutrient compositions [22-24], confirming that the glycaemic
response is strongly related to the rate of digestion of starchy foods [25]. A great deal of interest has been focused on the effects of DF on the glycaemic control in subjects with diabetes. High intakes of DF have been shown to improve the metabolic control in patients with diabetes [26-28]. Many studies have dealt with effects of soluble DF. Both in acute and long-term experiments it was shown that viscous DF tended to improve the blood glucose control and had favourable effects on the blood lipid status in subjects with diabetes [29-32].

**The glycaemic index**

The glycaemic response to different starchy foods can differ greatly, and one way of classifying food products according to their glycaemic response is by estimating their GI, a concept that was introduced by Jenkins in 1981 [33]. The GI value should be regarded as a supplement to information given in food tables.

"The glycaemic index is defined as the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrates from a standard food taken by the same subject."

**Definition given by the FAO/WHO Expert consultation, 1997 [34]**

Both the test food and the reference food are given in 50 g glycaemic carbohydrate portions (total carbohydrate minus DF) and either white bread or glucose can be used as a reference food. GI values obtained with white bread as a reference are about 1.4 times higher than those obtained with glucose as a reference [34, 35].

In this thesis, and in the included papers, all GI values are given with white bread as a reference food (white bread GI=100).

**Glycaemic load**

Not only the type of carbohydrate-rich food but also the total amount of carbohydrates has to be taken into consideration. The term glycaemic load represents both the quality and the quantity of the carbohydrates in a meal [36, 37] and is an indicator of the blood glucose response or of the insulin demand by the total carbohydrate intake. Each unit of dietary glycaemic
load represents the equivalent of 1 g of carbohydrate from white bread or pure glucose [38]. Foods with high GI values and a high content of carbohydrates have the highest glycaemic load.

**Determination of glycaemic index**

At least six subjects are required to determine the GI value of a food. The reference food should be given at least three times on different days in each subject, since blood glucose levels vary considerably from day to day within subjects. Up to four test foods can be investigated in the same series, on a total of seven test occasions in each subject, the foods being given on separate days. The GI determination should be performed in the morning after a 10-12-hour overnight fast, and a standardised drink of water, coffee or tea should be given with each meal. The area under the curve is calculated geometrically as the incremental area under the blood glucose response curve, ignoring the area beneath the fasting level, by applying the trapezoidal rule. Blood glucose is often measured in capillary whole blood, since this is easier to obtain and the rise in blood glucose is greater and less variable than in venous plasma glucose [34, 35]. It has been suggested that ideally the measurement should be continued until the blood glucose returns to the baseline levels, which means that blood samples should be taken for up to 120 minutes after the start of the test meal in healthy subjects and for up to 180 minutes for subjects with diabetes [35]. In the FAO/WHO expert consultation it is recommended that the duration of blood sampling should be 120 minutes after test meal ingestion in both healthy and diabetic subjects [34]. A number of methods have been used for GI determination and there is still some controversy regarding the length of blood sampling.

The insulin index can be determined in the same way as the GI. There is a positive correlation between the GI and the insulin response to starch-rich foods [39-41].

**Food factors influencing the glycaemic index**

The glycaemic response to foods cannot be predicted simply from their chemical composition, and despite an apparent lack of difference in their macronutrient and DF compositions, different carbohydrate-rich foods can produce very divergent glycaemic responses. The GI of a food is
influenced by the rate of digestion and absorption of the carbohydrates [42]. Various food properties, other than the molecular size of the carbohydrate component, have been shown to be important determinants of the glycaemic response [43-45]. These food factors are related to the choice of raw material and to the processing of foods [41]. One of the major factors appears to be the food structure, and processes that reduce particle size will increase the enzymatic availability of starch and therefore the glycaemic response [46]. Usually, the more processed a food is, the higher the glycaemic response it will produce [43, 47-51]. Decreasing the particle size by grinding increases the surface area, which in turn will increase the rate of digestion and absorption and hence the insulin secretion. The nature of the starch has been shown to influence the glycaemic response, with a lower response after ingestion of starch-rich foods with a high amylose content [52-55]. The degree of gelatinisation that occurs during heat treatment and retrogradation of gelatinised starch also have a major impact on the GI in food [47].

The glycaemic response is also influenced by the content and type of DF in the food, but high DF foods are not necessarily synonymous with low GI foods [33, 56, 57]. Different types of DF have different effects. A high content of purified soluble DF, such as guar and psyllium, improves the glycaemic response [58]. However, viscous naturally occurring DF in commonly eaten cereal products only has marginal reducing effects on the GI [41]. The presence of certain organic acids, produced during sourdough fermentation and when vinegar is added to starch-rich meals [59-61], and antinutrients, such as lectins, phytate and polyphenols present in leguminous food, have also been shown to lower the glucose response [62, 63]. Addition of fat and protein to a meal reduces the glycaemic response [33, 46, 64, 65]. Addition of fat decreases the postprandial blood glucose response without altering the insulin response [66, 67]. It has been argued that differences in GI between foods diminish when the foods are incorporated into normal meals, as the effects of protein and fat may influence insulin secretion and gastric emptying. However, these effects are not seen unless relatively large amounts of about 25 g of fat or protein per 50 g of carbohydrates are added, which is not likely to occur in normal meals [68, 69]. Although addition of fat and protein to a meal containing carbohydrates may result in a lower glucose response, the relative difference in response between starch-rich foods with different GIs remains [39] Furthermore, not only the amount but also the type of fat seems to be related to the postprandial glycaemic response [70-72]. However, it should be stressed that low GI foods high in fat, particularly of saturated
origin, should not usually be recommended. In the case of some foods, several factors can be responsible for their low GI values. Pulse vegetables, for example, have a high content of soluble DF and a high amylose content. Besides, their cell structure remains intact and after boiling, and they have a high content of antinutrients.

**Glycaemic index, diabetes and hyperlipidaemia**

Several experimental studies have shown that a diet with a low GI has favourable metabolic effects in subjects with type 2 diabetes [73-77] and hyperlipidaemia [78-80]. Also, in type 1 diabetes a low GI diet has been shown to improve the glycaemic control [81-83] and to lower the insulin requirements [84], as well as to reduce the number of hypoglycaemic events [85]. In a meta-analysis of 11 medium- to long-term studies specifically dealing with the GI approach, that were carried out between 1987 and 1992 in subjects with diabetes or hyperlipidaemia and in healthy subjects, a low GI diet was shown to lead to an average decrease in fasting blood glucose by 5 %, average blood glucose by 6 %, glycosylated haemoglobin A1c (HbA1c) by 9 %, fructosamine by 8 %, urinary C-peptide by 20 %, total cholesterol by 6 %, and TAG by 9 %. Three of the four studies concerning glucose tolerance showed improvements [76]. In all but one study improvements in the carbohydrate and lipid metabolism were observed. The lack of effect in one study might have been due to the small difference in GI values between the compared diets. The GI value of the diets investigated was on average reduced from 91 to 75 (white bread =100). However, in many of the previous evaluations of the GI concept in mixed meals there have also been differences in the type and amount of DF, differences in the macronutrient composition, and differences in the types of food tested, making it difficult to interpret the results. Other factors that can make it hard to distinguish between effects of differences in GI between tested diets are changes in body weight during the studies and alterations in the treatment of diabetes and other, concomitant diseases.

**Glycaemic index, satiety and weight management**

The results obtained in the study by Jenkins et al in 1988 [86], who investigated the effect of low GI starchy foods in the diabetic diet, indicated that use low GI starchy foods may enhance weight loss. Low GI foods have been shown to be associated with a greater feeling of satiety and
an inverse relationship has been found between peak satiety scores and both the GI and the insulin index [87, 88]. The particle size of cereal foods influences not only the glucose and insulin response but also the degree of satiety after a meal [88]. A meal with a high GI induces hormonal and metabolic changes that promote excessive food intake in obese subjects, which suggests an advantageous effect of a low GI diet in the treatment of obesity [89]. In a retrospective cohort of obese children a 4.3-month intervention period with a low GI diet significantly decreased the body weight and body mass index (BMI), compared with a reduced fat diet [90]. These results are promising, but additional trials are needed to investigate the long-term weight loss and capability of weight maintenance.

**Second-meal effects**

Single-meal studies in both healthy and diabetic subjects have shown that the GI of carbohydrate-containing foods not only influences the immediate postprandial metabolic response, but also modifies the postprandial response after an ensuing standardised meal, the so-called “second-meal” effect [84, 91-95]. The rate of delivery of carbohydrates, and consequent insulin secretion after the first meal, may be of importance for the metabolic response to a subsequent meal. A “second-meal” effect has been demonstrated from breakfast to lunch [84, 92, 93], and from an evening meal to the succeeding breakfast [91, 96]. Different fibre supplements, such as guar and psyllium in relatively large amounts, have shown a beneficial second-meal effect in both healthy and subjects with diabetes [97-99]. However, it has not been established whether a change in the breakfast composition only, in the direction of a low GI and a high content of soluble DF, can improve the metabolic response later during the day in the longer term also.

**Clinical use of the glycaemic index concept**

GI values obtained from GI tables [100] can be used for calculation of an expected glycaemic response after a mixed meal [34, 101], with a good correlation between the calculated meal GI and the observed glycaemic responses to meals with equal nutrient compositions. When comparing GI values of different foods, with the purpose of predicting their glycaemic responses, it is important to make comparisons between foods within the same food group that are eaten in roughly the same amounts. It is also
important to point out that the GI concept is most useful in comparisons of starch-rich foods. The clinical usefulness of the GI concept is controversial; one point of the criticism being that it may not be applicable to mixed meals. Also, the long-term effects have been questioned [102, 103]. From some studies it has been concluded that the GI can be predicted from the GI of the different carbohydrate-rich foods included [40, 104, 105] whereas other authors have concluded that differences in GI between foods are considerably diminished when the foods are incorporated into composite meals [102, 103]. However, the most relevant question is whether a low GI diet improves the glycaemic control in the long term.

In spite of the number of studies showing that replacement of foods with a high GI by those with a low GI results in improved glycaemic control and reduced fasting serum lipids, there is still no international consensus as to the clinical usefulness of the GI concept in the dietary management of diabetes. In the nutritional recommendations for people with diabetes from the Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes in 1988, the attitude towards low GI foods was cautious. A carbohydrate intake of 50-60 E % was encouraged and carbohydrate-containing foods rich in soluble DF were especially advocated, and low GI foods such as pulses, oats and barley were regarded as potentially beneficial foods. In the dietary recommendations from the DNSG published in 1995, and revised in 1999 [17, 106, 107], a clear position for an increase in carbohydrate-rich foods with a low GI was taken. In the healthy eating guidelines for people with diabetes in Australia and New Zealand the GI concept is promoted [108]. The FAO/WHO [34] also recommends a diet with a low GI, not only for people with diabetes but also for those with in hyperlipidaemia or obesity, and for healthy subjects. On the other hand, in the American Diabetes Association’s “Nutrition Recommendations and Principles for People with Diabetes Mellitus” [19, 109], it is stressed that the first priority should be given to the total amount of carbohydrate consumed, rather than to the source of carbohydrate. One of their stated reasons for not recommending a low GI diet is that it would severely limit the food choice [18].
**Aims of the investigations**

The overall aim of these investigations was to evaluate the effects of carbohydrate-rich foods in the treatment of the insulin resistance syndrome with special reference to the importance of the GI and DF.

The specific aims were:

- to evaluate the effect of differences in GI, achieved by varying the botanical and physical structure of the starchy foods, on the postprandial blood glucose and insulin responses in subjects with type 2 diabetes, in order to establish whether the GI concept is applicable to composite meals (paper I);

- to evaluate the effects of pronounced differences in GI, mainly achieved by varying the botanical structure of the starchy foods, on the metabolic control in subjects with type 2 diabetes, in order to establish whether the GI approach is useful in a realistic diabetic diet for a relatively long period of time (paper II);

- to evaluate the metabolic response postprandially and throughout the day, with special emphasis on the second-meal response, after breakfast meals with different GI values and amounts and types of DF in subjects with impaired insulin sensitivity (paper III);

- to evaluate metabolic effects of a period with either a low GI and high DF breakfast, or a high GI and low DF breakfast, in an otherwise unchanged diet in overweight men with special reference to the second-meal effect after a subsequent standardised lunch (paper IV).
Subjects

Paper I

Study I (paper I) was divided into two parts with ten subjects with type 2 diabetes in each part. The first part comprised five women and five men, with a mean age of 57 years (range 36-72 years). Body weight was $78.2 \pm 14.6$ kg (mean ± SD), with a body mass index (BMI) of $26.7 \pm 3.7$ kg/m$^2$. Six of the subjects were being treated with sulphonylurea, three of them in combination with metformin; the other four were treated with diet only. In the second part eight women and two men with a mean age of 61 years (range 36-77 years) were studied. Their mean body weight was $73.4 \pm 8.2$ kg, with a BMI of $27.0 \pm 2.9$ kg/m$^2$ (Table 1). Five of these ten subjects were treated with sulphonylurea and five with diet only. The duration of diabetes varied between 0.5 and 28 years in the subjects of the first part and between 0.5 and 10 years in those of the second part, respectively. The subjects were recruited from local primary health care centres in Uppsala.

Paper II

Twenty patients with type 2 diabetes mellitus participated in study II (paper II). Five of them were women and 15 were men with a mean age of 66 years (range 50-77 years). Their mean body weight on admission was $76.3 \pm 7.5$ kg, with a BMI of $25.3 \pm 2.7$ kg/m$^2$. The average HbA$_{1c}$ was $7.2 \pm 1.4$ % (Table 1). Four of the patients were treated with diet only and the other 16 were dietary treated in combination with oral antidiabetic drugs. One was being treated with metformin only and the other 15 were taking sulphonylurea, nine in combination with metformin. The duration of diabetes varied between 0.5 and 17 years. The subjects were recruited from local primary health care centres in Uppsala.

Paper III

Twelve overweight men with a mean age of 73.4 ± 1.2 years (range 71-75 years) with impaired insulin sensitivity participated in study III (paper III). Their mean body weight was $81.1 \pm 8.7$ kg, with a BMI of $27.3 \pm 1.9$
kg/m². Eight had type 2 diabetes mellitus. The mean fasting plasma glucose was $6.2 \pm 0.8$ mmol/l (Table 1). None were being treated with oral antidiabetic drugs or insulin. All subjects were non-smokers. The subjects were recruited from an ongoing health survey of 70-year-old men.

**Paper IV**

Study IV (paper IV) initially comprised 15 overweight or obese men with a mean age of $49.1 \pm 7.8$ years (range 33-61 years). Two of them did not complete the study. The mean body weight of the remaining 13 subjects was $99.6 \pm 7.2$ kg, with a BMI of $30.6 \pm 2.8$ kg/m² (Table 1) and a waist circumference of $103.3 \pm 5.8$ cm. At screening the mean fasting plasma glucose was $5.3 \pm 0.4$ mmol/l, fasting serum cholesterol $5.6 \pm 1.0$ mmol/l, fasting LDL-cholesterol $3.4 \pm 0.8$ mmol/l and fasting serum triacylglycerol $2.1 \pm 1.2$ mmol/l. None of the subjects were being treated with any medication that could have influenced the glucose or lipid metabolism. The subjects were recruited by advertisement in the local newspaper.

**Table 1.** Characteristics of the subjects in studies I – IV before entry into the studies.

<table>
<thead>
<tr>
<th></th>
<th>Study I Part 1</th>
<th>Study I Part 2</th>
<th>Study II</th>
<th>Study III</th>
<th>IR &amp; Type 2 DM</th>
<th>Overweight or obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, total</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Female/Male</td>
<td>5/5</td>
<td>8/2</td>
<td>5/15</td>
<td>0/12</td>
<td>0/13</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 (36-72)</td>
<td>61 (36-77)</td>
<td>66 (50-77)</td>
<td>73.4 (71-75)</td>
<td>99.6 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78.2 ± 14.6</td>
<td>73.4 ± 8.2</td>
<td>76.3 ± 7.5</td>
<td>81.1 ± 8.7</td>
<td>5.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 3.7</td>
<td>27.0 ± 2.9</td>
<td>25.3 ± 2.7</td>
<td>27.3 ± 1.9</td>
<td>30.6 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>P-glucose (mmol/L)</td>
<td>-</td>
<td>-</td>
<td>10.3 ± 3.2</td>
<td>6.2 ± 0.8</td>
<td>4.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-</td>
<td>-</td>
<td>7.2 ± 1.4</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD, (range). DM, diabetes mellitus; IR, insulin resistance
Study design

Paper I

This study was performed as a randomised crossover study. Each part of the study consisted of a comparison between two test meals with different GIs. The test meals were served at lunch after a standardised breakfast, and were given in randomised order with one week in between. The meals were composed in accordance with the dietary recommendations for people with diabetes [110-112] and had an identical macronutrient and dietary fibre composition but relatively large differences in GI. Blood glucose and plasma insulin were measured before the test meal and 30, 60, 90, 120, 180 and 240 minutes after the meal for calculation of the area under the curve (AUC). Plasma C-peptide was measured before and 60 and 180 minutes after the meals. Serum cholesterol, serum TAG and HDL concentrations were also measured before and 60 and 240 minutes after the meals.

Paper II

In this randomised crossover study the subjects were given two diets with either a low or a high GI during two consecutive 3.5-week periods (Figure I). Both diets were composed in accordance with dietary recommendations for people with diabetes [110-112]. The two diets differed considerably in GI, but did not differ in their macronutrient and dietary fibre composition. At the entry and at the end of each dietary period the insulin sensitivity was evaluated by the euglycaemic-hyperinsulinaemic clamp technique, and blood samples were taken in the fasting state for determination of plasma glucose, HbA1c, serum fructosamine, plasma insulin, plasma C-peptide, serum lipoproteins, serum apolipoproteins, NEFA, fatty acid composition of the serum cholesterol esters, serum tocopherols and plasminogen activator inhibitor-1 (PAI-1) activity. In addition, blood samples were taken throughout the day (profile days) for calculation of the AUC for glucose, insulin, C-peptide and NEFAs. For AUC calculations of glucose and insulin, blood samples were taken before and 60, 120, 180, 240, 300, 360, 480 and 540 minutes after breakfast. For AUC calculations of C-peptide, serum lipoproteins and NEFA, blood samples were taken 120 and 300 minutes after breakfast. During profile days the subjects followed the study diets, with breakfast at 08.00, lunch at 12.15, dinner at 16.15 and snacks at
10.00 and 14.00. Before entering the study the participants kept a 2-day estimated dietary record, using household measures.

**Figure 1.** Design of study II. GI, glycaemic index; BS, blood sampling.

**Paper III**

In this randomised trial five bread- or cereal-based breakfasts, with different GIs and with a varied type and amount of DF, were given to each participant in varying order, followed by a standardised lunch after four hours. Apart from the difference in GI and in the type and amount of DF, the breakfast menus were matched for their contents of protein and fat, fat quality and available carbohydrates. The study design made it possible to address the question of whether other dietary factors, irrespective of the GI, such the type and/or amount of DF, contributed to the second-meal phenomenon. Blood samples were taken before and 30, 60, 90, 120, 180 and 240 minutes after each meal for measurement of glucose and insulin in plasma and of cholesterol, TAG and NEFAs in serum.
In a randomised crossover design, subjects were given breakfast meals with either a low GI and a high content of soluble DF (Low GI-High DF) or with a high GI and a low content of DF (High GI-Low DF), as part of an ordinary diet, for four weeks. Dietary periods were separated by a 3-week washout period (Figure 2). Before and after each dietary period, and also after the washout period, blood samples were taken in the fasting state for measurement of blood glucose, HbA1c, plasma concentrations of fructosamine, insulin and C-peptide, serum concentrations of TAG, NEFAs, cholesterol, LDL-cholesterol and HDL-cholesterol, and PAI-1 activity, after a 12-hour overnight fast. At baseline and after each dietary period, blood samples were taken for calculation of the AUC during the day for glucose, insulin, C-peptide, TAG and NEFAs. During test days the breakfasts were followed by a standardised lunch meal four hours later. During both dietary periods, as well as during the washout period, the subjects kept a 3-day weighed dietary food record.

**Figure 2.** Design of study IV. BS, blood sampling; DR, dietary record
Methods

Test meals and study diets

Paper I

Composite meals were created with starch-rich key foods with differences in GI values as a basis. Differences in GI were mainly achieved by altering the botanical and physical structures of the starchy foods. The starch-rich key foods contributed with 50 g of the carbohydrates in all meals. Except for the ingredients responsible for the differences in GI values, all remaining ingredients in the two meals in each part of the study were identical.

In the first part of the study, a meal containing pasta (Low GI) was compared with a meal containing a white wheat bread (High GI). The estimated GI values in these meals were 62 and 81 units, respectively. This GI estimation was based on 94 per cent of the carbohydrates in the meals. The calculated energy content was 650 kcal (2700 kJ), with an energy percentage of 57, 18, and 25 for carbohydrates, protein and fat, respectively. The nutrient compositions from chemical analyses are shown in Table 2.

The differences in GI in the test meals were mainly due to differences in the physical structure between pasta and white wheat bread but also to some extent to differences in botanical structure between a whole and a crushed apple. The durum wheat spaghetti in the Low GI meal was industrially made (Storhushälls-spaghetti, Kungsörnen AB, Järna, Sweden). The corresponding white wheat bread was made from the same type of durum wheat and the same ingredients as were included in the spaghetti and was baked in a home baking machine (Panasonic SD-BT 2P, Matushiba Electric Trading, Osaka, Japan). The ingredients in the bread were: 300 g durum wheat flour, 200 g water, 7.5 g monoglycerides, 3 g salt and 3 g dry yeast. All ingredients were mixed, kneaded and fermented in four steps and baked in the machine. The whole procedure took four hours. After baking, the bread was allowed to cool to room temperature until the next day. The bread and spaghetti were served with a sauce made from margarine, wheat flour, low fat milk, smoked ham, mushrooms and leeks, together with a salad made from white cabbage, peas, corn and red pepper.
and a vinaigrette made from corn oil and vinegar (for amounts see paper I, Table 2, Page 838 [113]). The spaghetti was boiled for 10 minutes in a large amount of water. Immediately after boiling, the water was drained through a colander. Before ingestion, both the spaghetti and the white bread were baked in the oven together with the sauce and cheese.

In the second part of the study, a meal with parboiled rice, red kidney beans and bread made from whole-wheat grain (Low GI), was compared with a meal containing sticky rice, ground red kidney beans and bread made from ground wheat (High GI). The GI values based on 95 per cent of the carbohydrates in the meals were 58 and 80, respectively. The calculated energy content was 670 kcal (2800 kJ) with an energy percentage of 53, 18, and 29 for carbohydrates, protein and fat, respectively. The nutrient compositions of the diets, obtained from chemical analyses, are shown in Table 2.

The differences in GI values between these two meals were due to differences in the chemical starch structure attributable to differences in the amounts of amylose and amylopectin in the parboiled and sticky rice, and differences in the botanical starch structure between the whole and the ground red kidney beans and between bread made from whole wheat and that made from ground wheat. The whole-wheat grain and the kidney beans in the High GI meal were finely ground (Cemotec 1090 Tecator AB, Höganäs, Sweden) to flour before preparation of the bread or meals. The whole and the finely ground wheat grains was made into bread with the following ingredients: 370 g whole or finely ground wheat, 520 g water, 93 g white wheat flour and 50 g yeast. The whole-wheat grain bread in the high GI meal was made as follows: the whole-wheat grain was boiled in 370 g of water for 20 minutes and allowed to cool to room temperature. The remaining ingredients were then added. The dough was left to rise for 45 minutes and then put in a baking-tin, and proved for 45 minutes. The bread was baked at 200 °C for 45 minutes. The ground wheat grain bread was made as follows: hot water (80 °C) was poured over the milled grain and then cooled. The other ingredients were then added and the rising, proving and baking procedure were the same as for the whole-wheat grain. After baking, the bread was allowed to cool to room temperature and was then frozen in slices until used. Before being mixed with the other ingredients, the whole kidney beans were boiled for 1 hour and the milled kidney beans were boiled for 10 minutes. The kidney beans were made into a wok together with olive oil, beef, garlic, corn, haricots verts, cauliflower, leeks, tomato juice, soy sauce, and beef bouillon (for amounts see paper I,
Table 3, Page 839 [113]). This dish was served together with rice and bread with full-fat spread. Both varieties of rice were boiled for 20 minutes.

The standardised breakfast meal in both parts of the study consisted of 200 g low-fat yoghurt (0.5 % fat), 35 g home-made muesli (made from a mixture of 15 g rye-flakes, 15 g oats and 5 g rye crisp bread), 60 g raspberries, 24 g rye crisp bread, 6 g full-fat spread (80 % fat, 25 % linoleic acid), 30 g liver paste (23 % fat) and 20 g fresh cucumber. This breakfast had a calculated energy content of 450 kcal (1890 kJ) and the proportions of energy derived from carbohydrates, protein, and fat were 55, 16, and 29 per cent respectively, and the DF content was 9 g.

**Paper II**

The diets in study II were planned with starch-rich key foods with differences in GI values as a basis (for GI values of the starch-rich foods included, see paper II, Table 2, Page 11 [114]). Dishes including these foods were created and, in turn, were put together into a one-week menu for each diet. Differences in GI were mainly achieved by altering the botanical and physical structures of the starchy foods and to some extent also by changing the chemical starch structure, in order to exclude potential effects of differences in DF or other dietary components. Except for differences in the starch structure, all dietary constituents in both diets were kept identical and were included in the same amounts.

The GIs of the Low and High GI diets were 57 and 83, respectively. On average, GI was calculated on the basis of data for 94 per cent of the carbohydrates in the diets. In order to get the subject’s weight stable, the estimated energy level was calculated on the basis of their initial body weight. Female subjects received 30 kcal (125 kJ) and male subjects 35 kcal (145 kJ) per kg body weight. All food was prepared individually for each subject. The body weight was measured on all occasions when the subjects collected their food, and if necessary the energy level was adjusted. Four different energy levels with the same relative contents of nutrients were used. The menus were calculated on the 1600 kcal level, and in order to achieve the other energy levels used, namely 2000 kcal (8400 kJ), 2400 kcal (10 100 kJ), and 2800 kcal (11 800 kJ), the ingredients of the 1600 kcal menu were multiplied by 1.25, 1.50 and 1.75, respectively. The calculated proportions of energy derived from carbohydrates, protein, and
fat, were 55, 16 and 28 per cent, respectively. The nutrient compositions of the diets obtained from chemical analyses, are shown in **Table 2**.

The starch-rich foods included in the low GI diet that were responsible for the differences in GI were: whole barley, durum pasta, parboiled rice, whole dried lentils and beans, and high-amylose maize starch. The corresponding starchy foods in the high GI diet were ground barley, white wheat bread, sticky rice, ground lentils and beans, and ordinary maize starch. The whole and ground barley was made into bread and porridge. The pasta and white wheat bread were used as components for composite meals. The rice varieties were used as components in composite meals and in porridge and desserts. The lentils and beans were included in composite meals and the varieties of maize flour were used for cakes and dessert. For barley, lentils and beans the differences in GI were due to differences in the botanical starch structure. In the High GI diet the structure was disrupted by grinding before preparation (Messerschmidt Kornmeister 55000, Messerschmidt GmbH, Mönchweiler, Germany). For pasta and white wheat bread the differences in GI were due to differences in the physical structure. For rice, maize starch and maize flour the differences were due to differences in the chemical starch structure attributable to differences in the amounts of amylose and amylpectin.

There were three types of breakfast meals in the low and high GI menus. The low GI diet included pasta porridge made from boiled spaghetti, which was mixed in a food-processor, porridge made from whole barley kernels and porridge made from parboiled rice, and the corresponding breakfast meals in the high GI diet included white wheat bread, porridge made from barley flour and porridge made from sticky rice.

The type of pasta used was the same as that described in study I. The white wheat bread was made from the same ingredients in the same amounts and in the same way as the white wheat bread in study I. The beans and the bean flour were prepared in the same way as in study I before being added to dishes or composite meals.

The whole-grain barley bread included in the Low GI diet was made as follows: 3073 g whole barley grain was boiled in 3073 g of water for 20 minutes and allowed to cool to room temperature. Thereafter the remaining ingredients, namely 1500 g water, 627 g wheat flour, 200 g yeast and 10 g salt, were added. The dough was left to rise for 30 minutes and then put in a baking-tin, and proved for 35 minutes. The bread was baked at 200 °C for
45 minutes. The wholemeal barley bread was made from 3073 g barley flour, 3200 g water, 627 g wheat flour, 200 g yeast and 10 g salt. Hot water (40°C) was poured over a mixture of part of the barley flour mixed with the other ingredients. The remaining ground barley was then added and the dough was kneaded. The dough was allowed to rise for 30 minutes and then put in a baking-tin and proved for 45 minutes. The bread was baked at 200°C for 27 minutes. After baking, the bread was allowed to rest for one day and was then cut into slices and frozen until used.

The subjects were provided with all food throughout the study period, and breakfast, lunch and dinner were prepared in ready-to-eat dishes. The subjects received instructions about how to prepare the ready-to-eat dishes from a dietitian before entering the study. In a written menu they were informed about which dishes they should eat for each meal and each day. They were requested to eat all the food they received and no other food was allowed, except for water, mineral water, coffee and tea. If for some reason a participant was unable to eat all of the food received, he or she was instructed to bring it back to us.
Table 2. GI values and nutrient composition of test meals and test diets in studies I – IV.

<table>
<thead>
<tr>
<th>Study</th>
<th>GI</th>
<th>Energy</th>
<th>CHO (E%)</th>
<th>DF</th>
<th>Soluble DF</th>
<th>Insoluble DF</th>
<th>Protein (E%)</th>
<th>Fat (E%)</th>
<th>SAFA (E%)</th>
<th>MUFA (E%)</th>
<th>PUFA (E%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I - Part 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low GI meal</td>
<td>62</td>
<td>602 (2500)</td>
<td>84.8 (56)</td>
<td>11.5</td>
<td>2.7</td>
<td>8.8</td>
<td>29.9 (20)</td>
<td>15.9 (24)</td>
<td>5.8 (9)</td>
<td>4.9 (7)</td>
<td>5.2 (8)</td>
</tr>
<tr>
<td>High GI meal</td>
<td>81</td>
<td>640 (2700)</td>
<td>89.7 (56)</td>
<td>11.0</td>
<td>2.9</td>
<td>8.1</td>
<td>32.5 (20)</td>
<td>16.8 (24)</td>
<td>6.1 (9)</td>
<td>5.0 (7)</td>
<td>5.7 (8)</td>
</tr>
<tr>
<td>Study I - Part 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low GI meal</td>
<td>58</td>
<td>601 (2500)</td>
<td>84.5 (56)</td>
<td>14.0</td>
<td>4.3</td>
<td>9.7</td>
<td>28.4 (19)</td>
<td>16.6 (25)</td>
<td>3.1 (5)</td>
<td>10.2 (15)</td>
<td>3.3 (5)</td>
</tr>
<tr>
<td>High GI meal</td>
<td>80</td>
<td>606 (2500)</td>
<td>83.6 (55)</td>
<td>17.2</td>
<td>4.4</td>
<td>12.8</td>
<td>26.8 (18)</td>
<td>18.3 (27)</td>
<td>3.4 (5)</td>
<td>11.2 (17)</td>
<td>3.7 (5)</td>
</tr>
<tr>
<td>Study II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low GI diet</td>
<td>57</td>
<td>1880 (7900)</td>
<td>255 (55)</td>
<td>38</td>
<td>*</td>
<td>*</td>
<td>84 (18)</td>
<td>57 (27)</td>
<td>19 (9)</td>
<td>25 (12)</td>
<td>14 (7)</td>
</tr>
<tr>
<td>High GI diet</td>
<td>83</td>
<td>1820 (7600)</td>
<td>239 (54)</td>
<td>34</td>
<td>*</td>
<td>*</td>
<td>78 (18)</td>
<td>60 (29)</td>
<td>18 (9)</td>
<td>26 (12)</td>
<td>15 (7)</td>
</tr>
<tr>
<td>Study III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWB reference</td>
<td>78</td>
<td>464 (1900)</td>
<td>47.3 (41)</td>
<td>1.9</td>
<td>*</td>
<td>*</td>
<td>20.9 (18)</td>
<td>21.4 (42)</td>
<td>13.3 (26)</td>
<td>5.3 (10)</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>High GI-Low DF</td>
<td>78</td>
<td>460 (1900)</td>
<td>47.1 (41)</td>
<td>5.7</td>
<td>*</td>
<td>*</td>
<td>21.1 (18)</td>
<td>21.9 (43)</td>
<td>13.4 (26)</td>
<td>5.4 (10)</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>Low GI-Low DF</td>
<td>44</td>
<td>463 (1900)</td>
<td>46.5 (40)</td>
<td>5.9</td>
<td>*</td>
<td>*</td>
<td>21.0 (18)</td>
<td>21.2 (41)</td>
<td>12.6 (24)</td>
<td>5.0 (10)</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>Low GI-High sol DF</td>
<td>54</td>
<td>463 (1900)</td>
<td>46.3 (40)</td>
<td>24.1</td>
<td>*</td>
<td>*</td>
<td>21.0 (18)</td>
<td>21.8 (42)</td>
<td>11.1 (22)</td>
<td>4.4 (8)</td>
<td>0.5 (1)</td>
</tr>
<tr>
<td>Low GI-High insol DF</td>
<td>44</td>
<td>465 (1900)</td>
<td>46.5 (40)</td>
<td>24.2</td>
<td>*</td>
<td>*</td>
<td>21.1 (18)</td>
<td>21.3 (41)</td>
<td>12.2 (24)</td>
<td>4.9 (9)</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>Study IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low GI-High DF</td>
<td>53</td>
<td>670 (2800)</td>
<td>88 (53)</td>
<td>33.5</td>
<td>*</td>
<td>*</td>
<td>30 (18)</td>
<td>21 (28)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>High GI-Low DF</td>
<td>96</td>
<td>660 (2800)</td>
<td>87 (53)</td>
<td>3.5</td>
<td>*</td>
<td>*</td>
<td>30 (18)</td>
<td>22 (29)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

GI, glycaemic index; CHO, carbohydrates; DF, dietary fibre; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. GI values calculated on the whole meal or the whole diet. In studies I and II, the energy values and the energy per cent were calculated on the basis of chemical analysis of carbohydrates, protein and fat. In study I, the chemical analysis were performed on portions calculated to have a mean energy content of 650 kcal (2720 kJ). In study II, the chemical analyses were performed on a menu calculated to contain 2000 kcal (8370 kJ). In studies III and IV, the energy values and energy per cent were calculated on the basis of a combination of values from chemical analyses of carbohydrates, protein and fat and values obtained from food tables. * Not analysed.
The breakfast meals in this study were planned to achieve large differences both in GI and in the DF content, as well as differences in the type of DF. The key foods responsible for these differences were bread and cereals. The energy content was 460 kcal (1900 kJ) with an energy percentage of 41, 18, and 41 per cent, for carbohydrates, protein and fat, respectively. The contribution of carbohydrates from the starch-rich key foods was 25 g in each test meal. The carbohydrate-rich foods included in the different breakfasts were 1) high in GI (a white-wheat reference bread); 2) high in GI and low in DF (a wholemeal wheat bread); 3) low in GI and low in DF (minimally processed wheat flakes); 4) low in GI and high in soluble DF (steamed barley flakes made from a β-glucan-rich genotype); 5) low in GI and high in insoluble DF (minimally processed wheat flakes and wheat bran) (Table 3). The nutrient compositions are shown in Table 2.

**Table 3.** GI values and DF content of the different bread and cereal products included in the breakfast meals in study III.

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>GI</th>
<th>DF Soluble/insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) White wheat reference bread (WWB reference)</td>
<td>100</td>
<td>0.4/0.8</td>
</tr>
<tr>
<td>2) Whole-meal wheat bread (High GI-Low DF)</td>
<td>100</td>
<td>0.7/4.3</td>
</tr>
<tr>
<td>3) Wheat flakes (Low GI-Low DF)</td>
<td>37</td>
<td>0.6/4.6</td>
</tr>
<tr>
<td>4) Barley flakes (Low GI-High soluble DF)</td>
<td>54</td>
<td>12.5/10.9</td>
</tr>
<tr>
<td>5) Wheat flakes + wheat bran (Low GI-High insoluble DF)</td>
<td>37</td>
<td>1.1/22.5</td>
</tr>
</tbody>
</table>

GI, glycaemic index. DF, dietary fibre. GI values based on 50 g of available carbohydrates for each food. DF for the amount of bread and cereals included in the breakfasts (25 g of available carbohydrates). GI value estimated for breakfast number 2 and adapted from Granfeldt, Eliasson & Björck (unpublished data) for breakfast 3 & 4.

The bread and cereals were served together with butter, full-fat milk (3 % fat), cream (40 % fat), strawberries, sugar (sucrose) and cheese. In order to balance the fat and protein contents the amounts and types of milk, cheese and cream differed between the breakfast meals.

The white wheat bread in the WWB reference breakfast was made from ordinary wheat flour, but otherwise the bread was made from the same ingredients and in the same way as the white wheat bread in study I. After baking, the bread was allowed to cool to room temperature and was then...
frozen in slices. Before ingestion the crust was discarded. The wholemeal wheat bread included in the High GI-Low DF breakfast was made from whole kernels of wheat, which were ground to a fine, wholemeal flour. Except for the wholemeal wheat flour, both the ingredients and the baking procedure were exactly the same as for the WWB. The minimally processed wheat flakes included in the Low GI-Low DF breakfast were prepared as follows: wheat grains were soaked in cold water until soft and then flaked between rollers to a thickness of 0.5 mm in a full-scale plant. The wheat flakes were thus not subjected to heat treatment. The barley flakes included in the Low GI-High soluble DF breakfast, were made from a β-glucan-rich barley genotype (Prowashonupana, 17.5 % β-glucans by dry weight; Con Agra, Omaha, USA). The barley flakes were prepared as follows: pearled barley was steamed and rolled to flakes with a thickness of 0.5 mm in a full-scale plant. The Low GI-High insoluble DF breakfast consisted of the same wheat flakes as in the Low GI-Low DF breakfast, mixed with wheat bran (Fiberform®, Tricum AB, Höganäs, Sweden).

The standardised lunch meal was composed in accordance with results from Swedish food surveys [115], and consisted of: meatballs with brown sauce, boiled potatoes, green peas, carrots, lingonberry jam and crisp bread with low-fat spread. The calculated energy content was 500 kcal (2090 kJ) and the proportions of energy derived from carbohydrates, protein and fat were 41, 21 and 38 per cent, respectively; the content of DF was 6 g. The participants were not allowed to eat anything else but were allowed 1.5 dl water together with both the breakfast and lunch meal, and the same amount of water 120 minutes after both meals.

**Paper IV**

In this study the two breakfast meals were planned so as to achieve large differences in both GI and the type and amount of DF. The key foods responsible for these differences were bread and cereals. The two breakfast meals had the same macronutrient composition and an identical total energy content. The Low GI-High DF breakfast had a GI of 53 and a DF content of 33.5 g, while the High GI-Low DF breakfast had a GI of 96 and a DF content of 3.5 g. The GI estimation was based on 99 per cent of the carbohydrates in the meals. The breakfast meals were planned to give approximately 25 per cent of the total daily energy intake recommended for men between 31 and 60 years of age. The energy contents were 660 and 670 kcal (2800 kJ) with an energy percentage of 53, 18, and 29 for
carbohydrates, protein and fat, respectively. The bread and breakfast cereals contributed 52-55 g of carbohydrates, which corresponded to 60 per cent of the total carbohydrate content of the breakfast meals. The nutrient compositions of the breakfasts are shown in Table 2.

The Low GI-High DF breakfast was based on barley flakes made from a β-glucan-rich barley genotype (Prowashonupana, 17.5 % β-glucans by dry weight; Con Agra, Omaha, USA) and a sourdough-fermented bread with rye kernels, enriched with β-glucans from oat bran. The barley flakes were processed as follows: barley grains were soaked in cold water until soft and then flaked between rollers to a thickness of 0.5 mm in a full-scale plant. The barley flakes were not heat-treated. The sourdough-fermented bread was enriched with a β-glucan-rich oat bran concentrate (Liljeberg Elmståhl, Frid & Björck, unpublished data).

The High GI-Low DF breakfast was based on Cornflakes (Kellogg’s®) and a white wheat bread. The white wheat bread in the High GI-Low DF breakfast was made without monoglycerides but otherwise from the same ingredients in the same amounts and in the same way as the white wheat bread in study III. After baking, the bread was allowed to cool to room temperature and was then frozen in slices.

The standardised lunch was a typical Swedish dish composed in accordance with the present dietary recommendations [116], with a total energy content that was calculated to correspond to approximately 32 per cent of the total daily energy intake recommended for men between 31 and 60 years of age. The lunch consisted of meatballs with brown sauce, boiled potatoes, green peas, lingonberry jam, crisp bread with full-fat spread, a banana and water. The energy content was 875 kcal and the proportions of energy were 54, 16, and 30 per cent for carbohydrates, protein, and fat respectively.

In this study the participants were supplied with the breakfast meals during each dietary period and they collected the food twice a week. All ingredients were packed separately for each day and the participants received everything that they should eat for breakfast. They were asked to adhere strictly to the breakfast and to consume the whole breakfast at the same time, and not to eat anything else with their breakfast meals, but were allowed to drink coffee, tea or water. They were also asked not to prepare the ingredients in any way. If for some reason a participant was not able to eat all of the food received, he was instructed to bring it back to us.
The content of available carbohydrates in all test meals, as well as in the test diets compared, were planned to be identical within each study and the food items with differences in GI and DF were purchased in one batch and stored until used.

**Calculation and determination of glycaemic index**

The GI values for the whole meals (study I, III and IV) and the whole diets (study II) were calculated according to the method of Wolever *et al* [35], described in detail in the report from the FAO/WHO Expert Consultation [34].

In studies I and II, the GI values had been measured for most food items before the study began [51, 117-121], or derived by use of data from the literature [100, 122]. If GI data were missing, GI values were predicted by using an enzymatic in vitro procedure that has been shown to predict GI values for a large number of cereal- and legume-based foods with good accuracy, as described by Granfeldt *et al* [123].

In study III, the GI value for the wholemeal wheat bread was estimated and the GIs for the minimally processed wheat flakes and the steamed barley flakes made from a β-glucan-rich genotype were adapted from Granfeldt, Eliasson & Björck (*unpublished data*). In study IV, the GI and insulin index for the low GI barley flakes were determined, with measurement of blood glucose and insulin up to 95 minutes as proposed by Liljeberg *et al* [124]. The determinations were performed in ten healthy subjects, seven women and three men, with a mean age of 34.8 years (range 24-56). Their body weight was 71.9 ± 9.2 kg (mean ± SD), and their BMI 21.4 ± 1.9 kg/m². The GI for the low GI sourdough-fermented bread, enriched with β-glucans from oat bran, has previously been measured in healthy subjects (*Liljeberg Elmståhl, Frid & Björck, unpublished data*) and the GI values for the other included carbohydrate-rich foods were derived by using data from GI-tables [100].

**Calculations of energy and nutrient content of test meals and study diets**

In the calculations of the energy and nutrient contents and the amount of DF in the test meals (study I, III and IV) and in the standardised meals (study III and IV) and the study diets (study II), the database from the Swedish National Food Administration and a computerised calculation
programme (Dietist, Kost och näringsdata AB, Bromma, Sweden) were used. For some of the key foods included in order to obtain differences in GI and in the type and amount of DF, values for macronutrients and DF were missing in the database from the Swedish National Food Administration. In those cases the products were analysed for their macronutrient composition and DF content and the values obtained from the analyses were inserted into the computerised calculation programme.

**Chemical analyses of study meals and diets**

In order to corroborate the calculated values for energy and macronutrient composition chemical analysis of study meals and diets were performed.

Each test meal (study I) and each test diet (study II) were analysed for the amount of carbohydrates, DF, protein and fat and for the fatty acid composition. When test meals were analysed (study I and III), every single ingredient was weighed and the meals were prepared separately. When the test diets were analysed (paper II), all seven days for each diet were included and every single ingredient in every meal during a day was prepared separately. In studies III and IV, the starch-rich products included in the study breakfasts were analysed separately. Three duplicate portions from each test meal or each day of the test diets were homogenised separately together with hot water to get a temperature of 40 °C. After the addition of water, the sample was weighed again. Samples were removed and stored at -18 °C. For analyses of carbohydrates, DF and protein, the samples were freeze-dried. The contents of insoluble and soluble DF were measured by a gravimetric method according to Asp et al [125, 126]. The starch content was determined by a method described by Holm et al [127] and the other carbohydrates were measured by enzymatic methods (Boehringer-Mannheim and Roche Diagnostics, Mannheim Germany). For measurement of the total fat content and determination of the fatty acid composition (study I, II and III), 10 g of the homogenate was extracted with 50 ml methanol, 100 ml chloroform and 150 ml 0.2 mol sodium dihydrogen phosphate/l. Ten ml of the chloroform phase was evaporated to dryness and the total fat content was determined by weighing. Another aliquot of the chloroform phase was used for determination of the fatty acid composition. The fatty acids were transesterified to methyl esters and analysed by gas-liquid chromatography as described by Boberg et al [128]. In studies III (wheat- and barley-flakes) and IV, fat was measured by the Schmid-Bondzynski-Ratzlaff (SBR) method, including extraction with
ether after hydrolysis in HCl [129]. Protein was measured by wet digestion according to the method of Kjeldahl, and for calculating the protein content the factor 6.25 · nitrogen content was used.

**Dietary assessment**

Before keeping the dietary record (study II and IV) the subjects received detailed oral and written instructions from a dietitian. In study II, an estimated food record was kept for two days before entry into the study, with the recommendation that the recordings should be made on one weekday and one day at the weekend. The amounts of food eaten were reported in household measures. These measures were translated into weight in grams for each food or dish prior to nutrient calculation. The nutrient intake was calculated using the database from the Swedish National Food Administration and a computerised calculation programme (Dietist, Kost och näringsdata AB, Bromma, Sweden). These dietary records were also used for a rough calculation of the mean dietary GI from available GI tables [100].

In study IV, the weighed dietary food records were kept on two weekdays and one weekend day and each participant carried out the food recording during the same weekdays and weekend day on all three occasions. The food was weighed to the nearest gram on an electronic kitchen scale. The nutrient intake was calculated using dietary food record were analysed by using the food database from the Swedish National Food Administration (PC-Kost 1_99, SLV, Uppsala, Sweden) and the software program Stor MATs 4.05 SR-3 (Rudans Lättdata AB, Västerås, Sweden). Data on the special products included, e.g. bread and cereals, were entered into the database after analysis and used for the calculations of the breakfast meals.

**Anthropometrical measurements**

Height was measured without shoes to the nearest 0.5 cm and body weight was measured to the nearest 0.1 kg without shoes in light indoor clothing on the same electronic scale on all occasions in each study. Body mass index was calculated as the body weight (kg) divided by the height (m) squared. Waist circumference (study IV) was measured midway between the lowest rib and the iliac crest, and hip circumference at the widest part of
the hip, and the waist to hip ratio (W/H) was calculated. The waist and hip circumference were measured in the supine position.

**Blood pressure**

Blood pressure (study IV) was measured in the right arm with the subject in the supine position after a 5-minute rest, by indirect auscultation and with a mercury sphygmomanometer. Systolic and diastolic blood pressures were defined as Korotkoff phases 1 and 5, respectively.

**Biochemical measurements**

**Blood sampling**

Blood samples were drawn from an antecubital vein and all serum and plasma samples were stored at -70°C until analysed. All fasting blood samples were taken after a 12-hour overnight fast. The subjects were asked to avoid strenuous exercise the evening before the test days and in the morning before each test. During the test days the participants were sedentary.

**Insulin sensitivity**

Insulin sensitivity (study II) was evaluated by the euglycaemic-hyperinsulinaemic clamp technique according to the method of De Fronzo et al [130] with minor modifications. The insulin (Actrapid R Human; Novo, Copenhagen, Denmark) infusion rate during the clamp test was 56 mU/m²/minute, resulting in a mean plasma insulin concentration of about 100 mU/l. Euglycaemia was maintained by infusion of a 20 % glucose infusion with adjustment of the infusion rate according to the results of regular plasma glucose measurements. The target level of plasma glucose during the clamp was 5.1 mmol/l. Insulin sensitivity is expressed as the mean glucose uptake (M) and by the insulin sensitivity index (M/I₆₀-₁₂₀). The M value (mg/kg body weight/min) represents the glucose uptake during the last 60 minutes of the clamp test. The insulin sensitivity index is a measure of the tissue sensitivity to insulin expressed per unit of insulin obtained by dividing the mean glucose uptake by the mean insulin concentration (I) during the last 60 minutes of the total 120-minute clamp test (M/I, mg/kg body weight/min per mU/l multiplied by 100).
Glycaemic control
In studies I, II and III blood glucose concentrations were determined by the glucose oxidase method [131], and in study IV they were measured directly by the glucose dehydrogenase-based reaction in a HemoCue® blood-glucose photometer (HemoCue AB, Sweden). HbA1c (studies II and IV) was measured by fast performance liquid chromatography assay. Serum fructosamine (study II) was determined with the use of a Roche reagent kit (07-366694) (Roche, Basel), based on a nitro blue tetrazolium reduction in alkaline medium, in a Cobas Bio centrifugal analyser.

Insulin and insulin secretion
In study I plasma insulin assays were performed by the Phadeseph insulin test (Pharmacia, Uppsala, Sweden). In studies II and III plasma insulin assays were performed by the enzyme-immunological test for quantitative determination of human insulin in vitro (enzyme-linked immunosorbent assay/1-step sandwich assay; Boehringer Mannheim Immunodiagnostics for the ES300). In study IV an enzyme immunoassay, ELISA-kit (Mercodia AB, Uppsala, Sweden) was used for measuring plasma insulin. Plasma C-peptide was measured by the two-site enzyme immunoassay (Mercodia C-peptide ELISA Enzyme immunoassay, Mercodia AB, Uppsala, Sweden). Both plasma insulin and plasma C-peptide were determined in a Coda Automated EIA Analyzer (Bio-Rad Laboratories AB, Scandinavia). In studies I, II and III plasma C-peptide was measured according to the method of Heding [132]. In study IV plasma C-peptide was measured by the two-site enzyme immunoassay (Mercodia C-peptide ELISA Enzyme immunoassay, Mercodia AB, Uppsala, Sweden) based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the C-peptide molecule. C-peptide was determined in the Coda Automated EIA Analyzer (Bio-Rad Laboratories AB, Scandinavia).

Non-esterified fatty acids
Serum NEFAs (studies II, III and IV) were determined with a Wako NEFA C-kit (994-75409) (Wako, Neuss, Germany), modified for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, MA, USA).

Lipoproteins
In study I, TAG and cholesterol concentrations in serum were determined by enzymatic methods, using Boehringer Mannheim kits (Mannheim Germany) modified for use in a Multistat IIIF/LS apparatus (Instrumentation Laboratories, Lexington, MA, USA). In studies II, III and
IV, TAG and cholesterol concentrations were measured enzymatically in serum and in the isolated lipoprotein fractions, using the IL Test cholesterol method 181618-10 and the IL Test triglyceride enzymatic-colorimetric method 181610-60 in a Monarch apparatus (Instrumentation Laboratories). The concentrations of serum apolipoprotein (apo) A-1 and B (study II) were determined by immunochemical assay (Orion Diagnostica, Espoo, Finland) in a Monarch apparatus (Instrumentation Laboratories). Lipoprotein (a) [Lp(a)] was measured by a Pharmacia apo (a) radioimmunoassay method (Pharmacia, Uppsala, Sweden). This is based on the direct sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the apo (a) in the sample. The concentration is expressed in U/l. According to the manufacturer, 1 U of apo (a) is approximately equal to 0.7 mg Lp (a).

In studies I, III and IV, HDL-cholesterol was separated by precipitation with magnesium chloride/phosphotungstate [133]. LDL-cholesterol was calculated with Friedwald’s formula: LDL = serum cholesterol – HDL-cholesterol – (0.45 \cdot \text{serum triacylglycerol}). In study II, VLDL, LDL, and HDL-cholesterol were isolated by a combination of preparative ultracentrifugation [134] and precipitation with a sodium phosphotungstate and magnesium chloride solution [133].

**Plasminogen activator inhibitor-1 activity**
In study II, PAI-1 activity in plasma was measured with Spectrolyse/pL kits from Biopool AB (Umeå, Sweden), using polylysine as a stimulator, and in study IV, PAI-1 activity was analysed in citrate plasma using a commercially available bio immunoassay (Chromolize™ PAI-1, Biopool®, Umeå, Sweden).

**Tocopherols**
The concentrations of \( \alpha \)- and \( \gamma \)-tocopherol in serum (study II) were assayed by high performance liquid chromatography using a fluorescence detector, as described by Öhrvall *et al* [135].

**Fatty acid composition**
The fatty acid composition of the serum cholesterol esters (study II) were determined by gas-liquid chromatography as described by Boberg *et al* [128].
**Statistical analyses**

The statistical analysis was performed with the statistical program package SAS for Windows, version 6.08 (study I & II) and 6.12 (study III & IV) (SAS Institute, Cary, NC). Data are presented as means ± SD except in paper II, in which the results are presented as least-square means. Results are expressed as fasting values, AUC, and fluctuations from baseline. The AUC was calculated according to the trapezoidal model [136, 137].

In study I, the differences between the two test meals in both parts of the study were analysed by Student’s *t* test for two-paired samples.

The analysis in studies II and IV took into account the design of the experiment, the scales, and the distributions of variables [138]. For continuous variables with normal distributions (for some variables after logarithmic transformation), an analysis of variance model with factors for treatment, subject, and time was used. Comparisons were made of the two dietary periods (studies II & IV), and the results for each dietary period were also compared with the results on admission. The mean value of period one and period two minus the baseline value was used as the basis of a test of different carry-over effects between the two treatment periods. The test was performed as a *t* test between the two sequences. For continuous variables with normal distributions, a *t* test for different carry-over effects at the 10% level was used. If this test was not significant, a *t* test for different treatment effects at the 5% level was used. If the carry-over tests were significant, only data from the first dietary period were used in comparisons of treatment effects. If the usual assumptions for *t* tests did not hold, or if the data were on an ordinary scale, the *t* test was replaced by the Mann-Whitney U test. Since no carry-over effects were identified in studies II and IV, data from both periods on each diet were added irrespective of the order of treatment.

In study II an analysis of covariance with change in body weight as a covariate was carried out to separate the effects of treatment from those of changes in body weight. Some of the data were unbalanced, since a few values were missing. The results are therefore presented as least-square means, which form the basis of the statistical tests and estimates in the analyses and take the imbalance into account.

In studies III and IV, the calculated incremental AUCs after breakfast, after lunch and for the whole day were adjusted for time point zero, which meant that even when the AUC was calculated for 240 to 480 minutes the fasting
value was used as a basis. For comparisons of the mean values for AUC between the five breakfast meals, an analysis of variance model with factors for breakfast and subject was used. All breakfast comparisons were made within subjects. If the test for the breakfast factor in this model was significant at the 5 % level, all pair-wise comparisons were made.

Ethics

The studies were approved by the Ethics Committee of the Faculty of Medicine at Uppsala University, Sweden and all subjects had given their informed consent before entering the studies.


**Results**

**Study I**

*Part 1:* The AUC for both blood glucose (-35 %, \( P < 0.05 \)) and insulin (-39 %, \( P < 0.01 \)) was lower after the Low GI meal, containing pasta than after the corresponding High GI meal containing white wheat bread. The blood glucose concentrations were lower at 60, 90 and 120 minutes (Figure 3a) and plasma insulin concentrations were lower at 90, 120 and 180 minutes (Figure 3b) after the Low GI meal compared with the High GI meal.

Chapter 2 Figure 3a & b. Mean blood glucose and plasma insulin after consumption of the Low-GI meal (●) compared with the High-GI meal (▲). Areas under the curve (area units) for the Low GI and the High GI meals were 95 ± 51 and 146 ± 48 for blood glucose and 568 ± 422 and 928 ± 875 for plasma insulin, respectively. * \( P < 0.05 \), ** \( P < 0.01 \).

*Part 2:* The AUC for blood glucose (-42 %, \( P < 0.001 \)) and insulin (-39 %, \( P < 0.01 \)) was lower after the Low GI meal with parboiled rice, red kidney beans and bread made from whole-wheat grains than after the corresponding High GI meal. The blood glucose concentrations were lower at 30, 60, 90, 120 and 180 minutes (Figure 4a) and the insulin concentrations were lower at 30, 60, 90, 120 and 180 minutes (Figure 4b) after the Low GI meal compared with the High GI meal.
**Figure 4a & b.** Mean blood glucose and plasma insulin after consumption of the Low-GI meal (●) compared with the High-GI meal (▲). Areas under the curve (area units) for the Low GI and the High GI meals were 93 ± 37 and 160 ± 32 for blood glucose and 600 ± 504 and 987 ± 937 for plasma insulin, respectively *P < 0.05, **P < 0.01, ***P < 0.001.

The serum lipid levels showed similar responses after all of the tested meals.
Study II

The peripheral insulin sensitivity increased significantly and the fasting plasma glucose decreased during both treatment periods, with no difference between the two dietary periods. The changes in the serum fructosamine concentrations differed significantly between the two periods ($P < 0.05$). The incremental AUC for both blood glucose and plasma insulin was $\sim 30\%$ lower after the Low- than after the High GI diet (**Figure 5 and 6**). Total cholesterol and LDL cholesterol were markedly reduced after both diets, but the reduction was more pronounced after the Low-GI diet; total cholesterol being $5\%$ ($P < 0.01$) lower and the LDL cholesterol being $8\%$ ($P < 0.01$) lower after the Low GI diet than after the High GI diet. PAI-1 activity was normalised on the Low-GI diet, (-58 $\%$, $P < 0.001$), but remained unchanged on the High-GI diet (**Figure 7**).

**Figure 5.** Mean plasma glucose after the period with the Low GI diet (▲) compared with after the High GI diet (●). Areas under the curve (area units) for the low and the high GI diet were $1495 \pm 671.8$ and $2125 \pm 1469$ respectively. **$P < 0.01$,** ***$P < 0.001$. 

---

<table>
<thead>
<tr>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>The peripheral insulin sensitivity increased significantly and the fasting plasma glucose decreased during both treatment periods, with no difference between the two dietary periods. The changes in the serum fructosamine concentrations differed significantly between the two periods ($P &lt; 0.05$). The incremental AUC for both blood glucose and plasma insulin was $\sim 30%$ lower after the Low- than after the High GI diet (<strong>Figure 5 and 6</strong>). Total cholesterol and LDL cholesterol were markedly reduced after both diets, but the reduction was more pronounced after the Low-GI diet; total cholesterol being $5%$ ($P &lt; 0.01$) lower and the LDL cholesterol being $8%$ ($P &lt; 0.01$) lower after the Low GI diet than after the High GI diet. PAI-1 activity was normalised on the Low-GI diet, (-58 $%$, $P &lt; 0.001$), but remained unchanged on the High-GI diet (<strong>Figure 7</strong>).</td>
</tr>
</tbody>
</table>

---

**Figure 5.** Mean plasma glucose after the period with the Low GI diet (▲) compared with after the High GI diet (●). Areas under the curve (area units) for the low and the high GI diet were $1495 \pm 671.8$ and $2125 \pm 1469$ respectively. **$P < 0.01$,** ***$P < 0.001$. 

---

40
Figure 6. Mean plasma insulin after the period on the Low GI diet (▲) compared with after the High GI diet (●). The areas under the curve (area units) for the Low and the High GI diet were 12927 ± 6729 and 17724 ± 10318, respectively. * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Figure 7. Comparisons of the effects on the activity of plasminogen activator inhibitor -1 (PAI-1) after the Low- and High-GI diets. **$P < 0.01$, ***$P < 0.001$. 
The results from the 2-day estimated dietary record, kept by the subjects before entry into the study showed a mean energy intake of 1650 ± 510 kcal (6900 ± 2130 kJ). The proportions of energy derived from carbohydrates, protein and fat were 44, 18 and 34 per cent, respectively. GI data were obtained for 84 per cent of the carbohydrates included. The GI values for the remaining carbohydrate-rich foods were approximated, resulting in an estimated GI of the habitual diet of 80-90 units.

The fatty acid composition of the serum cholesterol esters did not differ between the dietary periods. The proportions of α-linolenic acid and docosahexaenoic acid had increased and the proportions of palmitoleic and stearic acid had decreased compared with those on admission, indicating good compliance in both dietary periods.
Study III

The early postprandial responses of both plasma glucose and insulin when calculated as AUC up to 90 minutes after breakfast were, as expected from the GI values for the starch-rich foods included, lower after the three low GI breakfasts than after the high GI breakfasts. When the AUC for glucose was calculated for up to 240 minutes after breakfast, the Low GI-High soluble DF breakfast showed a lower AUC after breakfast compared with all the other breakfast meals, and the AUC for plasma insulin was significantly lower after all breakfasts with low GI values than after the two high GI breakfasts. The Low GI-High insoluble DF breakfast showed a higher AUC for glucose after lunch than did the high GI breakfasts (P < 0.05), with an insulin response in parallel with the glucose response. The Low GI-High soluble DF breakfast yielded smaller fluctuations of plasma glucose from baseline compared with both high GI breakfasts, and smaller fluctuations after lunch than after the Low GI-High insoluble DF breakfast. However, none of the breakfasts could improve the glucose and insulin response after lunch. The Low GI-High insoluble DF breakfast resulted in effective suppression of NEFAs after lunch (Figure 8), and the response of TAG after lunch was lower after the low GI than after the high GI breakfasts (Figure 9).

Figure 8. Mean serum non-esterified fatty acid (s-NEFA) response throughout the day, after the High GI-Low DF (●), Low GI-Low DF (▲), Low GI-High soluble DF (■), and Low GI-High insoluble DF (♦) breakfast meals compared with the WWB reference breakfast (□).
Figure 9. Mean serum triacylglycerol response throughout the day, after the High GI-Low DF (●), Low GI-Low DF (▲), Low GI-High soluble DF (■), and Low GI-High insoluble DF (♦) breakfast meals compared with the WWB reference breakfast (□).
Study IV

No differences were seen between the dietary periods in any of the parameters measured or analysed in the fasting state. The glucose and insulin responses, calculated as the AUC up to 90 minutes after breakfast, were as expected from the GI values of the starch-rich foods included, with a significantly lower AUC for both blood glucose and plasma insulin after the Low GI-High DF than after the High GI-Low DF breakfast. Also, fluctuations from baseline after breakfast and during the day for glucose was lower after the low GI-high DF breakfast period (Figure 10). However, when the calculation of the AUC for blood glucose was lengthened up to lunch (240 minutes), no differences were found between the two breakfast periods. Neither was any differences seen in blood glucose after lunch. The AUC for plasma insulin was lower after the Low GI-High DF breakfast (also when calculated up to 240 minutes) (-32%, P = 0.0001) (Figure 11) than after the high GI-low DF breakfast period. After the low GI-high DF breakfast the AUC for TAGs after lunch was also lower (-47%, P = 0.002) compared with the period after the High GI-Low DF breakfast (Figure 12). There was no significant difference in serum NEFA response after breakfast or lunch between the two breakfast periods (Figure 13).

Figure 10. Mean blood glucose response throughout the day after the Low GI-High DF (●) and the High GI-Low DF (▲) breakfasts. The incremental areas under curve (area units) were 86.4 ± 106.9 (mean ± SD) and 130.2 ± 136.1 after breakfast (0-240 minutes) and 113.0 ± 136.6 and 91.0 ± 128.3 after the standardised lunch (240-480 minutes) for the Low GI-High DF and High GI-Low DF breakfasts, respectively.
Figure 11. Mean plasma insulin response throughout the day after the Low GI-High DF (●) and the High GI-Low DF (▲) breakfasts. The incremental areas under curve (area units) were 6105 ± 3661 (mean ± SD) and 8916 ± 4442 after breakfast (0-240 minutes) and 9535 ± 5453 and 8465 ± 4678 after the standardised lunch (240-480 minutes) for the Low GI-High DF and High GI-Low DF breakfasts, respectively.

Figure 12. Mean serum triacylglycerol (s-TAG) response throughout the day after the Low GI-High DF (●) and the High GI-Low DF (▲) breakfasts. The incremental areas under curve (area units) were 49.6 ± 40.2 (mean ± SD) and 50.1 ± 39.6 after breakfast (0-240 minutes) and 190.1 ± 99.7 and 253.5 ± 91.6 after the standardised lunch (240-480 minutes) for the Low GI-High DF and High GI-Low DF breakfasts, respectively.
Figure 13. Mean serum non-esterified fatty acid (s-NEFA) response throughout the day, after the Low GI-High DF (●) and the High GI-Low DF breakfasts (▲). The incremental areas under the curve (area units) were -61.2 ± 24.2 (mean ± SD) and -65.8 ± 24.2 after breakfast (0-240 minutes) and -67.2 ± 31.1 and -60.2 ± 24.1 and after the standardised lunch (240-480 minutes) for the Low GI-High DF and High GI-Low DF breakfasts, respectively.

There were no differences in the energy and macronutrient composition between the two dietary periods, except for the intake of dietary fibre, which was higher after the Low GI-High DF than after the High GI-Low DF period.
Discussion

Main findings

Starch-rich foods with low GI values incorporated into composite meals gave lower postprandial responses of both glucose and insulin than meals with a high GI with an identical macronutrient and DF composition in subjects with type 2 diabetes. A low GI diet taken for a period of three weeks results in a considerably improved metabolic profile with lower glucose and insulin responses throughout the day and an improved lipid profile and capacity for fibrinolysis in subjects with type 2 diabetes compared with a corresponding high GI diet. Low GI foods, especially those high in soluble DF, for breakfast tend to give a more favourable metabolic profile both when included in single meals and after inclusion in breakfast meals for a period of three weeks. A lower early glucose response and smaller glucose fluctuations from baseline after both breakfast and lunch were seen. Low GI breakfasts also resulted in lower responses of insulin and C-peptide after breakfast and lower postprandial TAG levels after a second meal. A breakfast with a low GI and a high content of insoluble DF suppressed NEFAs efficiently. However, none of the low GI breakfasts tested improved the glucose and insulin responses after lunch.

These results support the idea of a therapeutic potential of a low GI diet high in DF in the treatment of diabetes and also for prevention and treatment of several of the metabolic disturbances related to the insulin resistance syndrome. They emphasise the importance of preserving the structure of common starchy foods.

Interestingly, epidemiological studies have shown that a diet with a low glycaemic load and a high amount of cereal fibre is associated with a low relative risk of developing type 2 diabetes in both men and women [36, 139]. A low glycaemic load and a high amount of cereal fibre have also been shown to be associated with a lower relative risk of myocardial infarction in women [38], with a more pronounced risk reduction among the overweight and obese. In two large prospective cohort studies in women, whole grain consumption has been found to be inversely associated with a risk of diabetes and coronary heart disease (CHD) [140-142]. Despite its high content of insoluble DF, only cereal fibre has been
shown to be strongly associated with a reduced risk of CHD among women [143]. It has been hypothesised that the high concentrations of magnesium and antioxidant vitamins in fibre-rich cereal products might mediate the protective effect of cereal DF [144].

**Glucose and insulin responses**

In study I, both the glucose and insulin responses were considerably lower after consumption of composite lunch meals with low GIs. This is in agreement with earlier findings in healthy [40] and diabetic subjects [105, 145] given test meals with a similar macronutrient composition. The actual difference in postprandial glucose AUCs in our study was even larger than that predicted from calculations of GI values of the test meals. This study shows that the glycaemic response following a meal can actually be predicted from the calculated GI of the meal. However, in the study by Coulston *et al* [146], almost identical plasma glucose and insulin responses occurred after different mixed meals with larger differences in the calculated meal GI than in our study. This inconsistency could possibly be an effect of a rather high fat content (40 % of energy) and low carbohydrate content (40 % of energy) in their study. It might also be a result of calculating the absolute area instead of the incremental area under the curve.

After a period of 3.5 weeks on a low GI diet (study II), both the glucose and insulin responses throughout the day were lower than after the corresponding high GI diet, further supporting that the effects of low GI foods included in composite meals persist when these foods are incorporated into whole diets [76]. Since the carbohydrate-rich key foods responsible for the differences in GI were basically made from the same ingredients, this eliminates potential effects of variations in DF and nutrients. Thus, the difference between the effects of the two dietary periods can mainly be explained by the differences in GI.

In studies III and IV, the early glucose and insulin responses, calculated as the AUC for up to 90 minutes, were as expected from the determined and calculated GI values of the breakfasts compared. This was in accordance with reports from studies both in both healthy and diabetic subjects who were given breakfast meals with a high concentration of β-glucans from oat bran and high β-glucan barley [121, 147]. In study III, the low GI breakfasts produced smaller glucose fluctuations from baseline. This effect
was even more pronounced, with lower responses and smaller fluctuations from baseline also after lunch, when the low GI was combined with a high content of DF, especially of the soluble type. In agreement with this, the low GI breakfast high in soluble DF in study IV also showed smaller glucose fluctuations from baseline during the day. There were no differences in fasting levels of serum insulin between the two breakfast periods in study IV. This is in contrast to the report by Golay et al [148], who found lower fasting insulin and a lower mean day-long plasma glucose after a two-week period with muesli compared with cornflakes for breakfast.

Except for the lower AUC for plasma glucose after the Low GI-High soluble DF breakfast (in study III) compared with all other breakfasts, there were no differences in AUC for plasma glucose between the breakfasts when calculated over a longer postprandial period of 240 minutes. The reason for this was that the blood glucose had already returned to a level below baseline at 120 minutes and that negative areas are subtracted from positive ones when the AUC is calculated according to the trapezoidal model [136, 137]. In contrast to the glucose response, the insulin level remained above baseline until the next meal at 240 minutes after both the high and the low GI breakfast in studies III and IV. Different starch-rich foods result in lower glucose and insulin responses in healthy subjects than in subjects with diabetes. Glucose values usually return to baseline somewhere between 90 and 120 minutes after the beginning of the meal in healthy persons, whereas the values are still above baseline at 240 minutes in subjects with diabetes [149]. The lack of difference in AUC between the two breakfasts when the calculation was made up to 240 minutes in study IV could be expected. However, in study III the lack of difference when the AUC was calculated up to 240 minutes was unexpected, since the subjects had impaired insulin sensitivity and type 2 diabetes.

In studies II and IV there were no differences in HbA1c between the dietary periods. Compared to baseline, the low GI diet in study II resulted in significantly decreased levels of HbA1c, but a similar trend was also noted for the high GI diet. However, the changes in the serum fructosamine concentration, which may be a more appropriate indicator of glycosylation of serum protein than HbA1c during short periods of time, differed significantly between the two dietary periods. These studies were probably too short to show any significant differences in HbA1c between the diets. Since there were differences in the serum fructosamine concentrations after diets with different GIs and since fluctuations of blood glucose from
baseline during the day were smaller after the Low GI-High DF breakfast in study IV, we believe that a longer period on a diet with a low GI might result in a lower HbA1c.

**Insulin sensitivity**

In study II, the peripheral insulin sensitivity, measured by the clamp technique, increased significantly during both dietary periods. The improvement in insulin sensitivity was more accentuated after the low GI (+30 %) than after the high GI diet (+20 %), but no significant differences were seen between diets. Improvement of insulin sensitivity and of glucose tolerance (measured by OGTT and insulin-stimulated glucose uptake in isolated adipocytes) by a low GI diet has also been observed in a group of patients with advanced CHD [150]. In addition, a low GI diet has been shown to improve both adipocyte insulin sensitivity in women with a family history of CHD and in vivo insulin sensitivity measured by a short insulin tolerance test in women with and without a family history of CHD [151]. In a cross-sectional study in men and women the GI of the diet was found to be the only dietary variable significantly related to the HDL-cholesterol level [152]. It was suggested that this was an effect of improvement of insulin sensitivity by a low GI diet.

**Lipoproteins**

The extent of the total reduction of serum cholesterol and LDL cholesterol observed after both dietary periods in study II, compared with that on admission, is comparable to the reduction achieved by treatment with hydroxy-methyl-glutaryl (HMG)-CoA reductase inhibitors, such as simvastatin [153] and pravastatin [154], which lowered the plasma cholesterol by 25 and 20 per cent and LDL cholesterol by 35 and 26 per cent, respectively. An increased concentration of LDL cholesterol or total cholesterol is a major risk factor for coronary artery disease in persons with type 2 diabetes [8]. It is also a major risk factor for the general population [153-155], but because of the presence of small dense LDL particles [156] and oxidation of glycated LDL cholesterol [157] it may be more pathogenic in subjects with diabetes. Lowering of serum cholesterol has been shown to be an effective and safe method of reducing ischaemic heart disease. Results from prospective studies and clinical trials have shown that a modest reduction in serum cholesterol levels of 10 per cent in the longer
term may lower the risk by about 50 per cent (at age 40) [155, 158]. This reduction can reduce the mortality by 25-30 per cent in people aged 55-64 years. Thus, the pronounced reduction shown in study II could potentially be of great importance if maintained over a period of many years.

Inclusion of β-glucans from oat bran and oat fibre extract in a diet for a period of three weeks have been shown to lower both total and LDL cholesterol in hypercholesterolaemic subjects [159, 160]. Even though the total amount of β-glucans in the Low GI-High DF breakfast in study IV was almost twice as high as in these studies, there were no effects on fasting lipids. The key mechanism whereby β-glucans decreases the cholesterol level is through an increased viscosity of the gastrointestinal content [161]. It is therefore possible that a low viscosity might explain the lack of effect in this study.

The most interesting finding in studies III and IV was the lower response of TAG after a standardised lunch following low GI breakfasts. This is in line with the results from a single meal study [95] in which the second-meal effect was investigated after breakfast meals with low GI values. A lower response of TAG after lunch has also been observed in healthy, slightly overweight men after a period of five weeks on a carbohydrate-rich diet low in GI, compared with a high GI diet [162]. When carbohydrate absorption is prolonged, the blood glucose shows less tendency to decrease to below the basal level. This may lead to a smaller counter-regulatory response and improved glucose disposal after the next meal. A lower counter-regulatory response will in turn, result in lower levels of NEFAs [97]. Effective suppression of NEFAs as a result of a prolonged absorptive phase following breakfast could also improve the insulin sensitivity at the time of the lunch meal [41]. In studies III and IV, the lower TAG response after the breakfast with a low GI and a high content of insoluble DF might be explained by the higher insulin levels after lunch, which would result in suppressed hormone-sensitive lipase and ensure effective suppression of NEFAs, which in turn would prevent the rebound of TAG. Many of the metabolic benefits associated with a low GI carbohydrate intake can be attributed to their ability to reduce adipocyte NEFA release [163]. In study IV there was no significant difference in the post-lunch insulin and NEFA responses between the two breakfast periods, but the suppression of fat mobilisation by insulin occurs at very low insulin concentrations, ranging from around 2 mU to 20 mU/l [12, 164]. However, this cannot explain the lower TAG response after the low GI breakfast high in soluble DF in study III.
Postprandial lipaemia interacts with the process of thrombosis, in that an elevated postprandial TAG concentration has the ability to activate the coagulation factor VII and PAI-1 activity [14]. In study II we found that PAI-1 activity was normalised after a period with a low GI diet but not after a high GI diet. We found no difference in the fasting TAG response between these two dietary periods. Unfortunately, we did not measure the postprandial TAG response. A low DF intake has been shown to be associated with high PAI-1 activity [165]. It should be stressed that there were no differences in the amount and source of DF between the high and low GI diets in study II. Earlier studies have demonstrated that a reduction in fat intake and an increase in DF intake may improve the fibrinolytic activity [166, 167]. Starchy foods have also shown a more favourable impact than sucrose on factor VII coagulant activity [168]. It has been suggested that the optimal “anti-thrombotic diet” should have a low fat content and be naturally rich in DF [169].

In study II, HDL-cholesterol was lower than the baseline value after both dietary periods. This was almost certainly an effect of a lowered total dietary fat intake during both periods compared with the amount usually eaten.

**Second-meal effects**

We found no second-meal effects on glucose and insulin after any of the low GI breakfasts tested in studies III and IV. It has been suggested that the second-meal effect occurs when carbohydrates are more slowly absorbed, with a less rapid rise in glucose and a lower tendency for glucose levels to undershoot the baseline value after the first meal, leading to a smaller counter-regulatory response and improved glucose disposal after the next meal. In study III, the lack of improved second-meal carbohydrate tolerance could probably be due to a low carbohydrate content of the breakfast in comparison with that in other studies [67, 91-95]. In non-diabetic subjects, a low GI (GI = 70) was shown to be effective in suppressing NEFAs and improving second-meal carbohydrate tolerance only when present in a breakfast with a carbohydrate content almost twice as high as that in study III, while a breakfast with the same GI and a lower carbohydrate content tended to impair the second-meal carbohydrate tolerance [92]. The question has been raised whether differences in the type and amount of carbohydrate affect NEFAs in subjects with insulin resistance and diabetes, who have high fasting and postprandial NEFA
concentrations [68, 170]. The NEFA suppression achieved by the Low GI-High insoluble DF breakfast in study III suggests that this is the case. Giacco *et al* [171] found lower glucose and TAG levels after a breakfast meal rich in resistant starch, but reported that, in conformity with our low GI breakfasts (study III), this meal had no effect on the response after the subsequent meal. In spite of a higher carbohydrate content, a low GI and a high amount of soluble DF in the Low GI-High DF breakfast in study IV (compared with the breakfasts given in study III), we observed no second-meal effects on either glucose or insulin.

The effects on NEFAs noted after the low GI breakfast high in insoluble DF in study III are in conformity with the results reported after seven weeks of a high dose of uncooked cornstarch (with almost the same amount of carbohydrates as in our study) at bedtime [172]. Compared with a starch-free placebo, the nocturnal glucose and insulin responses were lower and NEFAs were suppressed. The glucose tolerance and C-peptide response were improved after breakfast the next morning. There were no improvements in insulin sensitivity or HbA1c levels. A low dose of uncooked cornstarch lowered the fasting blood glucose response after only four weeks and this effect was shown to persist after seven weeks, although no improvements in HbA1c was observed.

In a study by Liljeberg *et al* [94] a breakfast based on barley flakes made from Prowashonupana barley and a high amylose barley bread baked for a long time improved the glucose tolerance at a second meal in healthy, normal weight subjects. The metabolic response was only measured after the standardised lunch, but since the test meals resulting in improved second-meal carbohydrate tolerance showed a significantly higher blood glucose response as measured in the fasting state between meals, the glucose response after breakfast was probably above baseline for up to 240 minutes after breakfast.

The lack of a second-meal effect on glucose in studies III and IV might have been due to the fact that the glucose response returned to the baseline level as early as half time between breakfast and lunch. It may be speculated that only low GI foods, giving a more smooth glucose response, with glucose levels that remain above baseline until lunchtime, will result in improved second-meal carbohydrate tolerance.
Effects of fermentation

Resistant starch and unabsorbed carbohydrates will be fermented by the colonic microflora. It has been proposed that short-chain fatty acids, produced during fermentation, may play a role in improving postprandial glycaemia after high-fibre, high-carbohydrate diets, by inhibition of hepatic glucose production [173]. Fermentation and its products are expected to peak 12-16 hours after consumption of soluble DF and unabsorbable carbohydrates [173] and a period of three days have been shown to be sufficient to increase the bowel fermentation [174]. It is therefore unlikely that formation of metabolically active short-chain fatty acids will explain the results of study III even if the wheat flakes can be expected to contain a certain amount of resistant starch. The dietary periods in studies II and IV seem to have been sufficiently long to enable possible effects of the fermentation process to be demonstrated. Even if the test diets in study II were based on identical ingredients, the low GI diet can be expected to contain somewhat larger amounts of resistant starch [42]. However, in patients with CHD who are insulin resistant, stimulation of large bowel fermentation by lactulose has been found to have no effects on insulin, glucose, NEFAs or glucagon-like peptide 1 [175]. Battilana et al [174] investigated the effects of three days on a diet with 8.9 g of β-glucans per day as compared with a diet without β-glucans. On the third day the effects on lipid and carbohydrate metabolism were evaluated by giving the test diets every hour for 9 hours. The two diets resulted in similar lipid and carbohydrate metabolism, which does not support the hypothesis that metabolites produced during β-glucan fermentation in the large intestine exert beneficial effects on carbohydrate metabolism. The authors concluded that the effect of a meal containing β-glucans on the postprandial glucose concentration is essentially due to delayed and somewhat reduced carbohydrate absorption from the gut and is not attributable to fermentation in the colon.

Effects on body weight

In study II we did not manage to keep the subjects’ weight stable throughout the study period. The intention was to keep it stable throughout the study, but owing to the large volume of the foods included in both diets, and its effect on satiety, this was not always possible. All subjects stated that they had never eaten so much before without gaining weight. As a consequence of this there was a slight and similar reduction in body weight.
during both dietary periods. However, all results presented in paper II have been adjusted for changes in body weight. Promising results of a low GI diet on body weight have also been reported in obese children [90] and in slightly overweight men, in whom a 5-week period on a low GI diet decreased the total fat mass, mostly abdominal fat, by 500 g [162], although there was no effect on body weight. No differences in body weight or waist and hip circumference were found when only the breakfast meal was changed (paper IV). Neither were there any differences in energy and nutrient intake after the two periods with different breakfasts. A relatively long period with a low GI high DF breakfast might lead to changes in the dietary intake, which in turn might prevent weight gain and possibly reduce both the body weight and the waist and hip circumference. Thus, a low GI diet should be considered in weight loss regimes [176]. Furthermore, compliance with a prescribed low energy intake may be easier if the diet has a low GI as well, because of the low energy density of most starch-rich low GI foods and their greater effect on satiety.

Carbohydrate-rich foods in the optimal diet in the insulin resistance syndrome

The target for the treatment of the insulin resistance syndrome should be to improve insulin sensitivity and to prevent or correct associated metabolic and cardiovascular abnormalities [177]. Since obesity is the dominant factor leading to metabolic complications, the treatment in overweight subjects should primarily focus on weight reduction [3]. One target group should be first-degree relatives of subjects with type 2 diabetes [178]. The nutritional strategies for prevention and treatment of the insulin resistance syndrome are similar to those recommended for people with diabetes and those aimed at the population as a whole [17, 179]. Two basic requirements should be fulfilled, namely: reduced intake of saturated fatty acids and an increase in the intake of vegetables, legumes, fruit and low GI starchy foods [177].

In study II dramatic reductions in plasma glucose, serum cholesterol and LDL cholesterol, and improved insulin sensitivity, were also seen after the high GI diet. This indicates that both diets had a favourable nutrient composition, i.e. they had a low fat quantity, an improved fat quality and a high DF content, a composition that apparently differed from that of the habitual diet of the participants. Lowering the GI by keeping the structure of the starchy foods intact, without altering the macronutrient and DF
content, gave further improvements. In study II the subjects were provided with all the food that they should eat throughout the study period. To make all these changes in real life would probably require substantial efforts for most people. In the UK Prospective Diabetes Study (UKPDS), the effect of 3 months of diet therapy was evaluated in newly diagnosed type 2 diabetic patients [180]. Body weight was reduced by 5%, plasma glucose fell by 3 mmol/l, HbA1c decreased by 2% from 9% and TAG showed a substantial reduction by 17% in men and 10% in women. In the UKPDS study the metabolic improvements were almost certainly due to a large extent to the weight reduction. However, the dietary advice given was based on the recommendations published by the British Diabetic Association in 1982, in which information about GI and fat quality was not included. Thus, inclusion of recommendations regarding low GI starchy foods and an improved fat quality would most likely have yielded further improvements, perhaps somewhere between the results of study II and those of the UKPDS diet therapy study.

A reduction of the GI of carbohydrate rich foods at several meals will require substantial changes in dietary habits. It is probably not enough merely to incorporate low GI foods in the breakfast meal if one wants to achieve major changes in the metabolic profile. Also, the present study (paper IV) might have been too short to show major effects on the measured parameters after changing the composition of only one meal. However, a change in the breakfast meal is probably quite easy to accomplish and if improvements could be shown this could be of practical clinical importance for the motivation to make further changes in the dietary habits.

An alternative approach to achieve an extended blood glucose response to low GI and high DF foods could be to eat more frequently, with some small meals between the large meals or several small meals during a longer period of the day, also known as nibbling. An increased meal frequency has shown cholesterol-lowering effects [181, 182] and advantages for carbohydrate tolerance in type 2 diabetes [183, 184]. However, in the only long-term study that has addressed the effects of an increased meal frequency in subjects with diabetes, no change in either carbohydrate or lipid metabolism was found (Arnold, Ball & Mann, unpublished data) [185].
In study II we found improvements of many of the components of the insulin resistance syndrome. To treat these metabolic abnormalities the “traditional way” of using drugs would require multipharmaceutic measures. Many of the drugs will alleviate symptoms, but will not really affect the cause of the disease.

**What is considered to be a low glycaemic index?**

In free-living subjects with type 2 diabetes it has been shown that the GI of the diet ranges from 70 to 97.8 units, with a mean of 85 ± 5 (mean ± SD) [186]. This is comparable to the GI calculated for the high GI diet in study II and to the calculations based on the 2-day dietary record kept by the participants before entering the study. In a prospective study in elderly men, similar GI values were found when calculated from dietary history records [187]. When the GI is calculated from dietary records, only a rough estimation can be obtained, since defined GI values are still lacking for many carbohydrate-rich foods. In one study concerning the effect of a low GI in the management of hyperlipidaemia, GI values similar to those in our Low GI diet in study II were achieved [78]. In subjects with type 2 diabetes who were given dietary instructions weekly by a dietitian during a period of 12 weeks, the dietary GI was found to be 77 in comparison with 91 when advice about a high GI diet was given [188]. In another study, lasting 12 weeks, in subjects with type 2 diabetes, a decrease in the GI from 82 to 77 units was found [77]. A dietary GI of about 60 must be considered quite low. However, every reduction of the dietary GI, even if it does not reach such a low level, is probably to be considered beneficial.

**The glycaemic index concept revisited**

When the GI is determined during time periods of up to 90 and 120 minutes, the extended metabolic response after that time point and up to the next meal is not known. The GI value alone does not give any information about the length of the response and the shape of the glucose curve. Foods with similar GI values can yield very divergent shapes of the glucose response curve. Hypothetically the glucose response can be very high and short, and may therefore go below the baseline levels even during the GI determination (early postprandial response); or it can be low and extended through the late postprandial period (2-3 h). Carbohydrate-rich foods can
have the same GI but result in large differences in glucose fluctuations from baseline when studied for an extended period after the meal.

Carbohydrate-rich foods with low GI values that give a prolonged glucose and insulin response with small intra-day excursions are probably the most favourable. This usually means a low early response and a prolonged absorption phase. An extended glycaemic response may promote a second-meal effect. The shape of the curve is indicative of the late glucose response. It is suggested that a GI value published in a GI table could be supplemented with a glucose response curve. To evaluate the possible second-meal effect of a low GI food, the determination of the glucose response should preferably be extended until it is time for the next meal. When the GI is determined for separate foods, the glucose response is calculated up to 90 or 120 minutes. Extension of measurements after fasting levels have been re-established may lead to smaller differences in GI between foods, and if the area calculated includes the negative area, a very low response (below baseline) could be evaluated as a better response than it actually is. In addition to the AUC calculation, differences in fluctuations from baseline can be calculated as a measure of intra-day excursions. It has been suggested that the peak blood glucose value may give a better indication of the “true” glycaemic effect [189]. Since high GI foods result in relatively fast glucose responses with high peaks, there is also a tendency to undershoot baseline levels. The length of the early glucose response up to the time when the value goes beneath the baseline level could also give additional information.

**Methodological concerns, strengths and limitations of the studies**

In studies I and II the carbohydrate-rich key foods responsible for the differences in GI were basically made from the same ingredients. This eliminates potential effects of variations in DF and nutrients and makes it possible to evaluate the effects of differences in GI only. Compared to that in many other investigations of the second-meal effect of different carbohydrate-rich foods, the amount of carbohydrates in study III was smaller and corresponded to what could be considered realistic for a breakfast. However, the amount of soluble DF in the low GI breakfast meals is probably higher than one can expect to have in a standard breakfast. In study IV, the amount of breakfast cereals in the low GI breakfast was quite large.
The last sample for measurement of serum TAG in studies III and IV was obtained 4 hours after the standardised lunch. Since the plasma TAG concentration reaches a peak between 4 and 6 hours after a carbohydrate-rich meal [190], this period might have been too short for an optimal evaluation of the differences in AUC for TAG. We did not measure the quality of the postprandial TAG response, and therefore we do not know whether the TAGs are exogenously or endogenously synthesised. The higher TAG level seen after the high GI, low DF breakfasts could be due to a prolonged elimination of exogenous TAG-rich chylomicrons, chylomicron remnants, or increased endogenous lipid synthesis. It is probably a combination of both.

The treatment periods in studies II and IV were relatively short. In study II it was not possible to elongate the study period, mainly because of inconvenience for the study subjects. However, to examine the effects of a change in the breakfast only (study IV) would have been possible, even in the longer term.

The studies were performed in different categories of subjects, probably with differences in their severity of glucose intolerance. The effect of a diet high in carbohydrates and low in DF on the blood glucose control has been shown to vary according to severity of glucose intolerance [191]. Thus the results from studies III and IV may not be relevant in all respects for subjects with type 2 diabetes.
Conclusions

As discussed above, there is much evidence supporting the benefit of including low GI foods in the diet for people with the insulin resistance syndrome – with or without diabetes mellitus.

Main conclusions:

- Differences in postprandial responses to starchy foods persist even when the foods are incorporated into mixed meals composed in accordance with dietary recommendations, and with identical nutrient compositions and DF contents (paper I).

- A diet characterised by low GI starchy foods lowers the glucose and insulin responses throughout the day and improves the lipid profile and capacity for fibrinolysis. A low GI diet thus has several advantages in the treatment of type 2 diabetes and the insulin resistance syndrome (paper II).

- Even a moderate change of the diet, such as selecting low GI foods high in soluble DF for breakfast, gives a more favourable metabolic profile, with smaller excursions of glucose during the day, with lower peaks and less undershoot below baseline after the meal (papers III and IV).

- Low GI breakfasts give lower insulin and C-peptide responses and lower the TAG level after a standardised lunch. These effects were even more pronounced when the low GI was combined with a large amount of soluble DF (papers III and IV).

- A low GI breakfast high in insoluble DF suppresses the NEFA levels efficiently throughout the day (paper III).
Future perspectives

The result of many studies support the inclusion of low GI foods in the diet for people with diabetes and the insulin resistance syndrome. However, most of these investigations have been conducted during rather short time periods. There is a need for a large well-planned investigation of the long-term effects of a diet with a low GI. This should preferably be performed in subjects with insulin resistance and overweight persons. Specific aims should be to establish whether a change towards a diet with a lower GI is feasible in the long term and whether the metabolic effects seen in short-term studies persist or can even be reinforced. Long-term effects on weight control are another specific question to be addressed.

The GI tables need to be further developed and native foods and traditional dishes should be tested for their GI values. With GI values of additional foods included in the tables, it could be possible, in addition to calculating the energy, nutrient and DF contents, to calculate the GI by computerised programs. Low GI foods should be regarded as a part of a healthy diet and the concept should be made more “user-friendly”, with practical advice about foods low in GI in educational materials.

There is a need for more low GI products, especially breakfast cereals and bread. Today there is much knowledge regarding ways of optimising the GI of foods through the choice of raw material and changes in processing conditions, sometimes even by modest modifications [41]. Low GI foods are valuable especially for those with diabetes, hyperlipidaemia and obesity, but it should be stressed that such foods can probably be regarded as healthy for most people.

As for dietary fat, dietary carbohydrates are not homogeneous with respect to chemical structure and biological functions. Thus all carbohydrates should not be regarded as equal, and the type of carbohydrate-rich foods included in the diet should be considered when investigating the effects of other nutrients. Information about the content of DF is sometimes given but there is rarely any information about the GI of the carbohydrate-rich foods.

Diets high in monounsaturated fatty acids are sometimes considered to be more healthy for people with diabetes [19] than are diets with a high carbohydrate content. A high-carbohydrate diet has been regarded as
detrimental for people with diabetes, as it may induce hyper-
triglyceridaemia and lower the HDL cholesterol as well as increasing the
blood glucose level [192]. It is important to emphasise that these effects
have mainly been observed in isoenergetic weight-maintaining diets, often
without a definition of the type of carbohydrate-rich foods included.
Studies on the long-term effects on the metabolic control and body weight
after advice about a diet high in monounsaturated fatty acids in
comparison with a diet high in low GI carbohydrate-rich foods would be
desirable.
The work was carried out at the Department of Public Health and Caring Sciences, Section for Geriatrics/Clinical Nutrition Research at Uppsala University, Sweden. I wish to express my sincere gratitude and appreciation to everyone who have been involved in the work underlying this thesis, with special thanks to:

Bengt Vessby, my supervisor, who encouraged me to perform this work, for your never ending enthusiasm, for expert scientific support, great knowledge in nutrition research, constructive criticism and for never ending patience and encouragement.

Brita Karlström, my second supervisor, co-worker and friend for introducing me into clinical nutrition research, teaching me all about the practicalities in nutrition research, for great support and optimism and for showing me how to enjoy the good things in life.

Hans Lithell, the head of the department for providing me the opportunity to carry out my project at the department and for creating a productive working atmosphere.

Marina Spoverud-Älvebratt, the chef in the metabolic kitchen for preparing all the different (and sometimes strange) diets and enthusiastically encouraging the subjects to participate.

The entire staff at the metabolic ward, for good collaboration throughout the years both at the clinic and with the research work. Especially thanks to those in charge of the blood sampling in the studies for taking excellent care of the study participants, Gun-Britt Ångman, Bitte Söderling and Birgitta Hyvänén.

Barbro Simu, Eva Sejby and Siv Tengblad, for excellent laboratory work

Lars Berglund and Rawya Mohsen, for skilled and invaluable help with the statistics.

My friends and colleagues in Bengt’s group at the section for Clinical Nutrition Research: Agneta Andersson, Achraf Daryani, Johanna Helmersson, Marie Lemcke-Noroljärvi, Margaretha Nydahl, Cecilia Nålsén, Ulf Risérus, Annika Smedman and Eva Södergren for interesting discussions at the nutrition seminars and for sharing the joy of being a PhD student in nutrition research. A special thanks to Agneta, for being a good friend and the perfect coach, trying to teach me that winning isn’t everything – it’s the only thing!

All dietitian colleagues at “Länsgeriatriska kliniken” and “Medicinkliniken”, Uppsala University Hospital for being great colleagues and friends. A special thanks to Susanne Fredén, Agneta Nilsson and Erika Olsson for happy workdays at the clinic during many years.

Åsa Andersson, for introducing me to Brita Karlström, nearly 12 years ago.
Karin Andersson and Boel Sollenberg for helping me with energy and nutrient calculations.

Jan Hall, for preparation of figures and illustrations.

My co-workers at the Department of Applied Nutrition and Food Chemistry, Chemical Centre, Lund University: Inger Björck, Yvonne Granfeldt, Helena Liljeborg Elmstahl and Nils-Georg Asp, for fruitful collaboration between our research groups.


Maud Marsden, for excellent linguistic revision of the manuscripts, making them readable.

Study participants, for your enthusiastic participation in our studies.

My parents and my brother and sister with their families for always supporting me.

Fredrik, for patience and never ending support.

This work was supported by grants from the Swedish Medical Research Council, The Cerealia Foundation for Research and Development, The Swedish Nutrition Foundation, The Swedish Diabetes Association, Albert Påhlsson’s Foundation, the Swedish Council for Forestry and Agricultural Research, the Ernfors Foundation, the School of Household Management Fund and Birger Giertz foundation.
References


